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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver in elderly: a cohort study

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Abstracts

Objective: Nonalcoholic fatty liver diseases (NAFLD) is being one of the major causes of liver-related disease. While the relationship between triglyceride glucose (TyG) and NAFLD in elderly is not reported yet. In this study, we aimed to investigate the role of TyG index for predicting the incidence of NAFLD in elderly.

Design and setting：This is a prospective cohort study in Henan, China, from 2011-2018

Participants and Methods：In total, 46693 elderly who participated in a routine physical examination programme from 2011 to 2018 were included in this study. TyG index was calculated as ln [fasting triglyceride (mg/dl)×fasting plasma glucose (mg/dl)/2], while NAFLD was defined as hepatic steatosis after excluding other causes based on the results of abdominal ultrasonography; Cox regression model were performed to explore the relationship between TyG index and NAFLD; Mediate effect was used to analyze the role of the TyG index in WHtR and NAFLD.

Results: During the 149041.50 person-years follow-up, a total of 5660 NAFLD events occurred (3.80/100 pearson-years). After adjusting for potential confounder factors, quartiles 4 of TyG index significantly increased the risk of NAFLD compared with quartile 1 on NAFLD, the hazard ratios (HRs) and 95% confidence intervals (CI) were 1.314(1.234,1.457). In addition, TyG index played a partial mediating role in the relationship between WHtR and NAFLD, and indirect effect was 1.009(1.006,1.011).

Conclusion: Higher TyG index was associated with higher risk of NAFLD in the older, so TyG index may be a novel predictor for NAFLD, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD.

Key words: Nonalcoholic fatty liver disease; Triglyceride glucose index; Waist-to-height ratio; Mediation effect.

Strengths and limitations of this study：

Data for this cohort study were retrieved from a large regular physical examination in

Henan, China.

Restricted cubic spline and Mediation effect were used in this study, which can reflect the relationship between TyG index and NAFLD more realistically

The sample size and statistical power were sufficient in this study.

The severity of disease in this cohort study was not available when data were retrieved.

Part of participants were excluded in this study because they did not have abdominal ultrasound testing.

Introduction

Nonalcoholic fatty liver disease(NAFLD) encompasses a series of spectrum of liver diseases, ranged from simple steatosis, nonalcoholic steatohepatitis, cirrhosis to hepatocellular carcinoma[1]. With the change of lifestyle and increase of obesity, NAFLD is exceeding viral hepatitis and becoming the most common chronic liver diseases, about a quarter of common population were affected by NAFLD in the world[2, 3]. NAFLD is being one of the major causes of liver-related disease such as cirrhosis, hepatocellular carcinoma and liver transplantation. Moreover, NAFLD is also associated with higher prevalence and incidence of cardiovascular disease such as coronary, cerebrovascular and peripheral vascular disease [4, 5]. In the next decade, NAFLD is expected to become the leading cause for liver transplantation in the United States instead of hepatitis C[6].

The development of NAFLD is a complex process involving genetic and environmental factors. As an organ of metabolism, the disorder of glucose and lipid metabolism plays an important role in the progress of NAFLD[7]. Several studies found that IR could increase the risk of NAFLD even without the existence of T2DM, and IR may be a start factor of steatosis[7-10].Furthermore, as an early marker of IR, TyG has been proposed to be an inexpensive and reliable surrogate to IR[11, 12].While researches about the relationship between TyG index and the risk of NAFLD were still limited [9, 13]. What's more, as far as we know, the association between TyG index and the incidence of NAFLD in the older has not been reported.

WHtR is a maker of abdominal obesity, studies also found WHtR related to IR[14, 15] as well as the higher risk of NAFLD [16-22]. Whether there is an existing effect of TyG index on the relationship between WHtR and NAFLD and how it affects NAFLD is still unclear. This retrospective cohort study was aimed to explore relationship between baseline level of TyG index triglyceride glucose index and the incidence of NAFLD.

1.Participants and methods

1.1 Subjects:

We retrospectively analyzed the 99997 subjects who had the data of liver ultrasonography, fasting triglyceride as well as fasting plasma glucose in physical examination programme in Xinzheng, Henan Province, in Central China City from January 2011 to December 2018. This physical examination programme was for the local residents over 60 years old and was supported by the government of Xinzheng, Henan Province in China. Individuals with the any of the following criteria were excluded:1) missing follow-up; 2) subjects with NAFLD at baseline;3) subjects with hepatitis B or C virus or had the history of excess alcohol intaking (the threshold for women <20 g/ d and <30 g/d for men). A total of 46693 eligible participants were included in this cohort study (**Figure 1**). The datasets generated and/or analysed during the current study are available upon request. Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China.

Figure 1 Flow chart for participants exclusions performed

1.2 Data collection

Demographic data and clinical information of the subjects were collected when they underwent health check-up. Demographic data including age, sex, excess alcohol intake (yes/no), current smoking status (yes/no), lives alone(yes/no), exercises regularly(yes/no). The definition of current smoking was that subjects used to smoke

100 cigarettes or above in their lifetime, and now is still smoking regularly. Excessive drinking was defined as that drinking more than 30g /d for men and 20g/d for women. Exercise regularly was defined as more than 3 times a week, and 30 minutes moderate intensity exercise each time. The clinic records including anthropometric measurements (such as height, weight, blood pressure, waist circumference) and laboratory data. Height measurement required the subjects without shoes, stand straight on the ground, and their hips and heels against the wall, to measure the weight, the participants were without shoes and wore light clothing. Blood pressure of the subjects in a sitting position after 5 minutes of rest was measured twice by an electronic sphygmomanometer (Omron HEM-7125, Kyoto, Japan), and the mean value of the two measurements was recorded. Waist circumference was measured at the midpoint of the distance between the lowest costal ridge and the upper border of the iliac crest. After fasting for 8 hours, the blood samples of subjects were collected to detect the level of fasting plasma glucose (FPG), total cholesterol (TC), and triglyceride (TG) by a biochemical detector (DIRUI CS380, Changchun, China). Alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (TB) was detected by (DIRUI CS380, Changchun, China). The TyG index were calculated as the ln [fasting TG (mg/dl) × FPG (mg/dl)/2]. WHtR was defined waist circumference(cm)/height(cm).

1.3 NAFLD definition

All participants included underwent liver ultrasonography (SIUI CZXL-38G, Shantou, China). The results of ultrasound prompted the existence of steatohepatitis: enhanced liver echogenicity, echogenicity greater in liver than kidneys, deep attenuation and vascular blurring[23], and after excluding the steatohepatitis caused by alcohol, viruses and drugs was defined as NAFLD. All ultrasound examinations were performed by an experienced professional radiologist.

1.4 Statistics

Categorical variables were showed as proportions while continuous variables were presented as means ± standard deviation (SD) or median (interquartile range) (IQR). ANOVA analysis /two-paired sample t tests (continuous variables, normal distribution)

and chi-square tests (categorical variables) were used to compare the difference in different groups. Logistic regression for categorical variables and linear regression for continuous variables were used to obtain the *P* value for trend.

Cox regression models were used to explore the relationship between TyG index and the incidence of NAFLD, the lowest quartiles of TyG index was defined as the reference. Hazards ratio (*HR*) and confidence interval (*CI*) of NAFLD in quartiles and continuous were expressed in separate models. to assess the relationship across increasing quartiles, *P* value for trend tests were used by entering median value in each quartile in Cox regression models.

AUCs were used to evaluate the ability of baseline TyG index to predict the risk of NAFLD. Restricted cubic spline models were used to explore whether there was a nonlinear relationship between continuous and occurrence of NAFLD [24]. Mediation analysis used a Cox regression to study the mediate effect of TyG index between WHtR and NAFLD.

Mediation analysis was conducted using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria), and Restricted cubic spline were performed in Stata v 12.0 (Stata Corp, College Station, TX, USA). The other analyses were performed by SPSS software, version 21.0 (SPSS Inc, Chicago). Probability values for statistical tests, where two-tailed *P*-values<0.05 were regarded as significant.

2. Result

2.1 Baseline Characteristics of included subjects

A total of 46693 subjects were included in this study. The baseline characteristics of included participants based on follow-up were presented in **Table 1**. The mean age of subjects was 68.91 (7.17), and 48.89% were men. The average follow-up was 3.19(1.52) years, during the 149041 person-years follow-up, 5660 subjects occurred NAFLD (3.80/100 pearson-years). Subjects with NAFLD with higher BMI, WHtR, TC, FBG, DBP, SBP, ALT, and TB, and the incidence of NAFLD was higher in younger, current smoking, regular exercise, with diabetes, hypertension, while the baseline level of AST had no association with the incident of NAFLD.

Table1 baseline characteristics of included subjects according to the follow-up outcome

variables	No NAFLD (n=41033)	NAFLD (n=5660)	*P* value
Men, n (%) †	20799(50.69)	2033(35.92)	<0.001
Age (years) ‡	69.18±7.27	66.93±6.00	<0.001
Current smoking, n (%) †	6846 (16.70)	594(10.51)	<0.001
Exercise, n (%) †	7865(19.22)	1131(20.02)	<0.001
Live alone, n (%) †	8772(21.38)	1015(17.93)	<0.001
Diabetes, n (%) †	5554(13.54)	1344(23.75)	<0.001
Hypertension, n (%) †	15945 (38.86)	2952(52.16)	<0.001
WHtR‡	0.51±0.06	0.54±0.07	<0.001
BMI‡	23.62±2.85	26.02±3.10	<0.001
SBP (mmHg) ‡	132.62±19.38	135.32±19.76	<0.001
DBP (mmHg) ‡	78.92±10.34	80.91±10.33	<0.001
FPG (mmol/L) §	5.20(4.70-5.70)	5.30(4.80-5.93)	<0.001
TC (mmol/L) §	4.59(4.01-5.21)	4.69(4.09-5.38)	<0.001
TG (mmol/L) §	1.10(0.81-1.47)	1.28(0.90-1.77)	<0.001
TB (μmol/L) §	11.51(8.60-14.10)	11.30(8.30-13.80)	<0.001
ALT(U/L) §	18.00(13.00-24.50)	18.60(14.00-25.00)	<0.001
AST(U/L) §	21.80(16.89-26.90)	21.90(16.89-27.00)	0.892
TyG‡	8.43±0.54	8.61±0.59	<0.001

†: n (%); ‡: mean (SD); §: median (IQR)

Abbreviations: BMI, body mass index; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, triglycerides; TG, triglyceride; TB, total bilirubin; ALT, alanine aminotransferase, AST, aspartate aminotransferase; TyG, triglyceride glucose.

2.2 Association between TyG index and NAFLD

The relationship between TyG index and the incidence of NAFLD were showed in **Table 2**. Compared with quartile 1 of TyG index, the hazard ratio (HR) and its corresponding 95% confidence interval (CI) for quartile2, quartile3, and quartile 4 were 1.056(0.971,1.149), 1.300(1.200,1.408) and 1.972(1.832,2.123), respectively, risk for NAFLD significantly higher with increasing quartiles of TyG for the *P* value of trend of linearity <0.001. What's more, even after adjusted possible confounding factors, the risk of quartile 3 of TyG index (1.314(1.234,1.457)) on the incident of NAFLD still existed. In order to further verify the relationship between the TyG index and the incidence of NAFLD, TyG index was used as continuous for the above analysis, the results also proved that higher level of TyG increased the risk of NAFLD

(1.265(1.200,1.334)), even after adjusted possible confounding factors. These results proved that the baseline level of TyG was associated with the risk of NAFLD, the higher of TyG, the higher incidence of NAFLD.

Table 2 relationship between TyG index and the risk of NAFLD

		TyG index		
	n	unadjusted	Model 1	Model 2
As continuous	50037	1.697(1.619,1.779)	1.577(1.504,1.653)	1.265(1.200,1.334)
Quartile 1	12556	Reference	Reference	Reference
Quartile 2	12458	1.056(0.971,1.149)	1.035 (0.951,1.126)	0.980(0.895,1.073)
Quartile 3	12522	1.300(1.200,1.408)	1.259(1.162,1.364)	1.090(0.999,1.190)
Quartile 4	12501	1.972(1.832,2.123)	1.783(1.655,1.920)	1.314(1.234,1.457)
P for trend		<0.001	<0.001	<0.001

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol and diabetes.

2.3 The ability of TyG to predict NAFLD

The receiver operating characteristic curves (ROC) were used to evaluate the ability of baseline level of TyG to predict the development of NAFLD. The best cutoff value for TyG index to diagnosis NAFLD was 8.63 (sensitivity: 0.48, specificity: 0.67), and its corresponding area under the receiver operating characteristic curves (AUCs) was 0.60 (95%CI:0.58,0.61)，showed in **Figure 2**.

Figure 2 ROC curve of TyG on NAFLD

Receiver operating characteristics (ROC) curves for baseline TyG to predict NAFLD among 60 years old or more. The ROC area was 0.594(0.590 - 0.599). TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases.

2.4 Restricted cubic spline analysis to evaluate the relationship between TyG and NAFLD

In order to further explore the relationship between TyG and the risk of NAFLD, restricted cubic spline graph was used to analyze the dose-response relationship between TyG and the incidence of NAFLD. As showed in **Figure 3**, there was a nonlinear relationship between level of TyG index and the risk of NAFLD based on the adjusted Cox regression model. TyG index significantly increased the risk of NAFLD

when it was 8.63 and above. This result indicated that higher level of TyG was associated with higher incidence of NAFLD.

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD. Multivariate adjusted hazard ratios of NAFLD increasing during follow-up when the baseline level of TyG index 8.63 and above. Adjusted variables including age, sex, living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases; CI, confidence interval.

2.5 Mediation effects

In previous analysis we found that WHtR was associated with the incidence of NAFLD as well as the relationship between WHtR and TyG index ($P<0.05$, and the data wasn't showed). Based on WHtR would lead to IR, and TyG index was a reliable and cheap surrogate indicate for IR, so there might be a mediate effect of TyG index between WHtR and NAFLD. Then mediation analysis was used to certificated whether there was a mediating effect and how TyG index affect the relationship between WHtR and NAFLD. Results (showed in **Table 3 and Figure 4**) of mediation analysis showed that the total effect of WHtR on NAFLD was 1.476 (1.437,1.517), and the direct effect was 1.463(1.842,1.950), TyG index play a partial role and the indirect effect was 1.019 (1.006,1.011).This result may indicate that abdominal obesity may lead to disorders of glycolipid dyslipidemia, and then lead to an increase of the TyG index.

Table 3 Mediation analysis of the relationship between TyG index and NAFLD by WHtR

Effect	HR (95%CI)
Total effect	1.476 (1.437,1.517)
Direct effect	1.463 (1.842,1.950)
Indirect effect	1.009(1.006,1.011)

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

The effect of WHtR on NAFLD was mediated by baseline level TyG index by adjusted potential confounders age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic

blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

3. Discussion

With the acceleration of the aging process, the health in the elderly has gradually become an important issue in society. NAFLD, a common liver disease in China, also has become one of the main health problems of the elderly. Although there were some researches about TyG index and NAFLD, research about TyG index and the incidence of NAFLD in the older has not been reported.

In this retrospective cohort study, we found the incidence of NAFLD was 3.80/100 pearson-years among the elderly over 60, which was lower than a cohort study of Korea (the incidence of NAFLD was 4.84/100 pearson-years)[25]. This might be due to the age of the subjects in Korean cohort study(mean age are 36.1) was younger than ours, and age could affect the prevalent and severity of NAFLD[26]. What's more, the incidence of NAFLD decreased with the age increased in this study, and the $P_{\text{value for trend}}$ <0.001(the data did not show in this research) A retrospective cohort study on TyG index and NAFLD in Japan also found that higher TyG was associated with higher risk of NAFLD, which was consistent with our results[13]. While, as far as we know, this research is the first study to focus on the effect of TyG index on the incidence of NAFLD in the elderly. Our study also indicated relationship between TyG index was nonlinear, the incidence of NAFLD would increase significantly when TyG is above 8.63. What's more, the results of the mediation effect suggested that TyG index was not an independent factor for the development of NAFLD, but a partial mediator between WHtR and NAFLD.

The results of ROC suggested that 8.63 was the best cutoff value of TyG index for predicting the prevalent of NAFLD, which was close to the research of L. Fedchuk et al[9]. TyG index as an inexpensive and reliable surrogate index for IR, and IR plays an important role in the development and progression of NAFLD, even in the absence of diabetes, IR can lead to changes from normal liver to NAFL to NASH[10]. Large

numbers of studies have confirmed that IR is closely related to the occurrence and development of NAFLD[27-30].

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, IR participates in the occurrence and development of NAFLD by disturbing the glucose and lipid metabolism disorder of the liver[27]. Insulin resistance reduces glucose uptake in adipose tissue and muscle and hydrolysis of triglycerides in adipose tissue as well as increases the conversion of glucose to fatty acids in the liver. Furthermore, IR could also increase de novo lipogenesis by activating sterol regulatory element binding protein (SREBP1),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 be a start of steatosis and then lead to the occurrence of NAFLD[7, 31, 32]. On the other hand, IR is always linked to chronic mild inflammation, by releasing inflammatory factors such as TNFα, IL-6, IL-1 and monocyte chemoattractant protein-1, immune cells or adipocytes can in turn promote IR and participate the developing and progressing of NAFLD[33, 34].

Obesity is a key factor in development and progress of NAFLD. Studies also have found the subjects with NAFLD have the higher level of BMI, and higher BMI was associated with the risk of NAFLD[35, 36].Overweight/obesity participates in the development of NAFLD through insulin resistance. While growing evidence suggested that the determinant of insulin resistance is not the degree of obesity, but the distribution of fat, abdominal fat accumulation is related to insulin resistance[37, 38]. Studies also shown that 2-h glucose and insulin resistance are significantly increased with higher visceral fat than those with lower abdominal fat in obese adolescents [39, 40]. Central obesity can lead to inflammatory, oxidative stress and metabolic disorders, which are related to the development of insulin resistance[41, 42]. In this study, WHtR, as an indicator of central obesity, was associated with NAFLD, and the effect was mediated by TyG index in this study. Which could be explained by the central obesity leads to the TyG index increasing, and thus leads to the incidence of NAFLD.

The major strength of this study was a large sample size retrospective cohort study which included 50037 aged more than 60 years old, and cohort study could better reflect the real relationship between TyG index and NAFLD. Moreover, nonlinear relationship between TyG index and NAFLD was found by cubic spline graph and the mediation effect of TyG index between WHtR and NAFLD in this study. However, there were several limitations in this study. First, the diagnosis of NAFLD was based on the results of abdominal ultrasound instead of liver biopsy and only provide information on whether there was the presence of NAFLD. However, it is difficult to find mild steatosis by ultrasonography, the incidence of NAFLD could be underestimated[31, 43]. Then the subjects could not respond their history of lipid-lowering therapy or antidiabetic drugs as they were elder; So information about therapy was missing in the data. Additionally, we could not evaluate the relationship between TyG index and different NAFLD severity. In addition, we lack other more accurately index which can reflect the abdominal obesity status, even though we used WHtR to indicate the abdominal obesity we were unable to assess the more accurately visceral fat index and the prevalent of NAFLD.

In conclusion, high baseline level of TyG index is significantly associated with the higher risk of NAFLD. In addition, TyG index play a partial mediating role in the relationship between WHtR and NAFLD, our results also indicated that TyG index may be a potential marker for NAFLD. So measured the TyG index to assess the risk of NAFLD routinely in clinical practice is useful.

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Author Contributions:

H.C. and Q.L. designed the study. H.C., S.L., X.C., Z.S., G.W and J.H. participated in the data collection and analysis. H.C. and A.A. drafted this manuscript, H.C. S.L., and S.S. interpreted the data. S.S and Q.L. reviewed and revised this manuscript. All authors have approved the final manuscript.

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Conflict of Interest:

All authors have no conflicts of interest.

Patient and public involvement: Participants were not involved in the recruitment and conduct of the study

Patient consent for publication: Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China.

Ethics approval:

The study protocol was approved by ethics committee of Zhengzhou University in China (approval number: ZZURIB202004). Given the retrospective nature of the research, the requirement for informed consent was waived.

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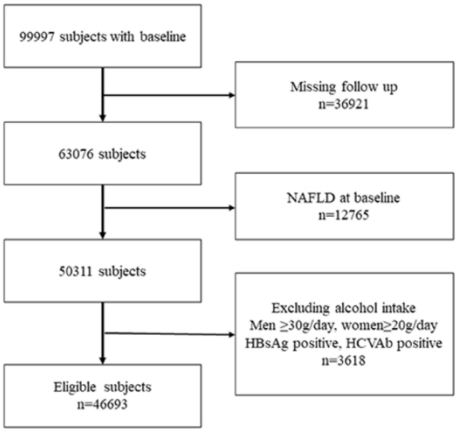

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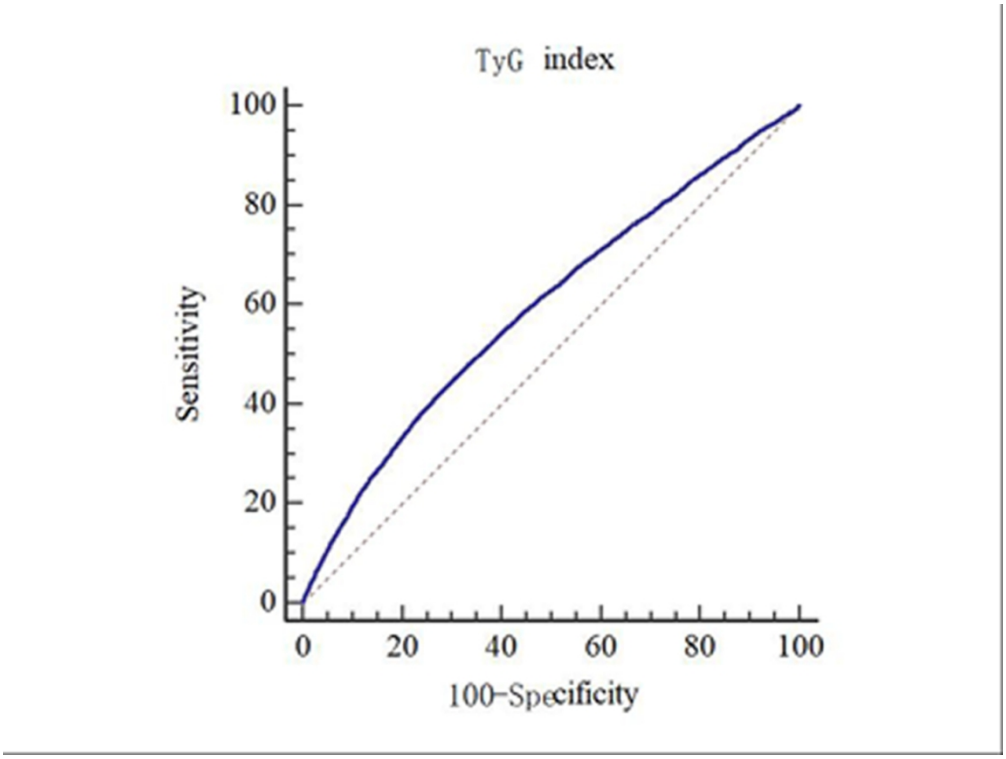

Receiver operating characteristics (ROC) curves for baseline TyG to predict NAFLD among 60 years old or more. The ROC area was 0.594(0.590 - 0.599). TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases.

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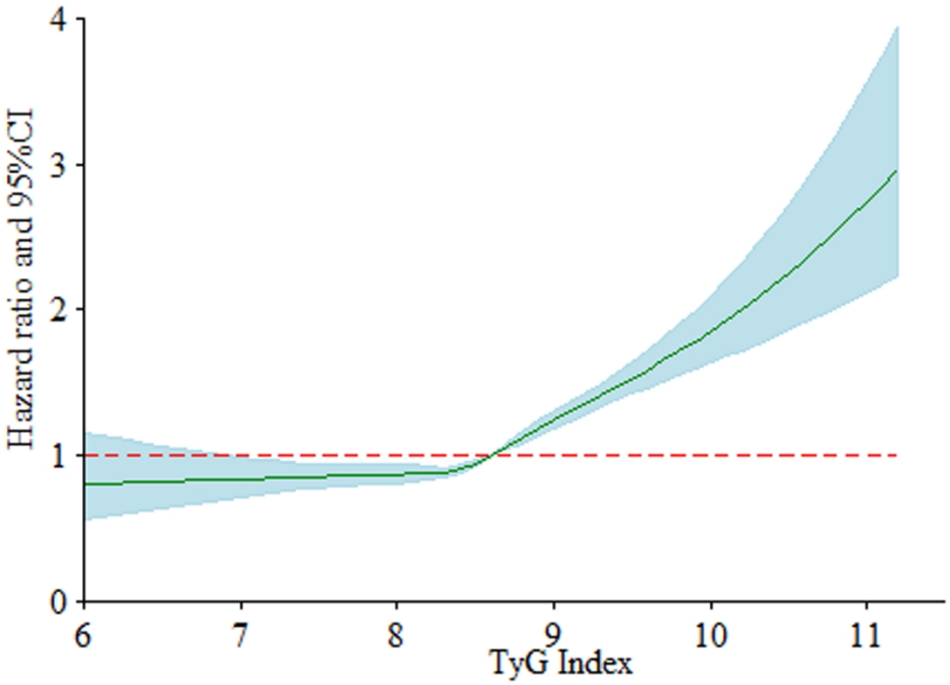

Multivariate adjusted hazard ratios of NAFLD increasing during follow-up when the baseline level of TyG index 8.63 and above. Adjusted variables including age, sex, living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases; CI, confidence interval.

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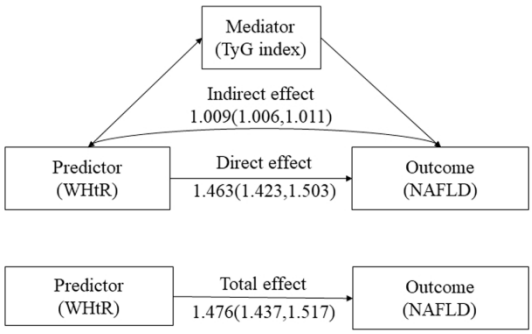

The effect of WHtR on NAFLD was mediated by baseline level TyG index by adjusted potential confounders age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,5
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			6,7,8,9
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6,7
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7,8 7,8 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China

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Abstracts

Objective: Nonalcoholic fatty liver disease (NAFLD) is one of the major causes of liver-related diseases but relationship between triglyceride glucose (TyG) and NAFLD in the elderly is not reported yet. In this study, we investigated the role of TyG index for predicting the incidence of NAFLD in the elderly.

Design and setting：This is a prospective cohort study in Henan, China, from 2011-2018.

Participants and Methods：In total, 46,693 elderly who participated in a routine physical examination programme from 2011 to 2018 were included in this study. TyG index was calculated as ln [fasting triglyceride (mg/dl) ×fasting plasma glucose (mg/dl)/2], while NAFLD was defined as hepatic steatosis after excluding other causes based on the results of abdominal ultrasonography; Cox regression model was performed to explore the relationship between TyG index and NAFLD. Also, mediation effect was used to analyze the role of the TyG index in WHtR and NAFLD.

Results: During the 149041.50 person-years follow-up, a total of 5660 NAFLD events occurred (3.80/100 person-years). After adjusting for potential confounding factors, quartiles 4 of TyG index significantly increased the incidence of NAFLD compared with quartile 1, the hazard ratios (HRs) and 95% confidence intervals (CI) were 1.314(1.234,1.457). In addition, TyG index played a partial mediating role in the relationship between WHtR and NAFLD and indirect effect was 1.009(1.006,1.011).

Conclusion: Higher TyG index was associated with higher risk of NAFLD in the aged and therefore, TyG index may be a novel predictor for incidence of NAFLD. Further, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD.

Key words: Nonalcoholic fatty liver disease; Triglyceride glucose index; Waist-to-height ratio; Mediation effect.

Strengths and limitations of this study：

Data for this cohort study were retrieved from a large regular physical examination in Henan, China.

Restricted cubic spline analysis and mediation effect were used in this study, which can reflect the relationship between TyG index and NAFLD more realistically.

The sample size and statistical power were sufficient in this study.

The severity of disease in this cohort study was not available when data were retrieved.

Some participants were excluded in this study because they did not have abdominal ultrasound testing.

Introduction

Nonalcoholic fatty liver disease(NAFLD) encompasses a series of spectrum of liver diseases, ranging from simple steatosis, nonalcoholic steatohepatitis, cirrhosis to hepatocellular carcinoma[1]. With the change of lifestyle and increase of obesity, NAFLD currently exceeds viral hepatitis and is becoming the most common chronic liver disease, affecting about a quarter of the common population in the world[2, 3]. NAFLD is one of the major causes of liver-related disease such as cirrhosis, hepatocellular carcinoma and liver transplantation. Moreover, NAFLD is also associated with higher prevalence and incidence of cardiovascular disease such as coronary, cerebrovascular and peripheral vascular disease [4, 5]. In the next decade, NAFLD is expected to become the leading cause for liver transplantation in the United States instead of hepatitis C[6].

The development of NAFLD is a complex process involving genetic and environmental factors. As an organ of metabolism, the disorder of glucose and lipid metabolism plays an important role in the progress of NAFLD[7]. Several studies found that IR could increase the risk of NAFLD even without the existence of T2DM and IR may be the genesis of steatosis[7-10].Furthermore, as an early marker of IR, TyG has been proposed to be an inexpensive and reliable surrogate to IR[11, 12] Yet researches about the relationship between TyG index and the risk of NAFLD are limited [9, 13]. Additionally, as far as we know, the association between TyG index and the incidence

of NAFLD in the older has not been reported.

WHtR is a maker of abdominal obesity and studies have also reported that WHtR is related to IR[14, 15] and also the higher risk of NAFLD [16-22]. Whether there is an existing effect of TyG index on the relationship between WHtR and NAFLD and how it affects NAFLD is still unclear. This retrospective cohort study therefore sought to explore the relationship between baseline level of TyG index and the incidence of NAFLD.

1.Participants and methods

1.1 Subjects:

We retrospectively analyzed the 99,997 subjects who had the data of liver ultrasonography, fasting triglyceride as well as fasting plasma glucose in physical examination programme in Xinzheng, Henan Province, in Central China City from January 2011 to December 2018. This physical examination programme was for the local residents over 60 years old and was supported by the government of Xinzheng, Henan Province in China. Individuals with any of the following criteria were excluded:1) missing follow-up; 2) subjects with NAFLD at baseline;3) subjects with hepatitis B or C virus or had the history of excess alcohol intake (the threshold for women <20 g/d and <30 g/d for men). A total of 46,693 eligible participants were included in this cohort study (**Figure 1**). The datasets generated and/or analyzed during the current study are available upon request. Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China (Approve number: ZZURIB202004).

Figure 1 Flow chart for participants exclusions performed

1.2 Data collection

Demographic data and clinical information of the subjects were collected when they underwent health check-up. Demographic data included age, sex, excess alcohol intake

(yes/no), current smoking status (yes/no), lives alone(yes/no) and exercises regularly(yes/no). The definition of current smoking was that subjects used to smoke 100 cigarettes or above in their lifetime, and now is still smoking regularly. Excessive drinking was defined as drinking more than 30g /d for men and 20g/d for women. Exercising regularly was defined as more than 3 times a week, and 30 minutes moderate intensity exercise each time. The clinic records included anthropometric measurements (such as height, weight, blood pressure, waist circumference) and laboratory data. Height measurement required the subjects without shoes, stand straight on the ground, and their hips and heels against the wall, to measure the weight, the participants were without shoes and wore light clothing. Blood pressure of the subjects in a sitting position after 5 minutes of rest was measured twice by an electronic sphygmomanometer (Omron HEM-7125, Kyoto, Japan), and the mean value of the two measurements was recorded. Waist circumference was measured at the midpoint of the distance between the lowest costal ridge and the upper border of the iliac crest. After fasting for 8 hours, the blood samples of subjects were collected to determine the level of fasting plasma glucose (FPG), total cholesterol (TC) and triglyceride (TG) using a biochemical detector (DIRUI CS380, Changchun, China). Alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (TB) was detected by (DIRUI CS380, Changchun, China). The TyG index were calculated as the ln [fasting TG (mg/dl) × FPG (mg/dl)/2]. WHtR was defined as waist circumference(cm)/height(cm).

1.3 NAFLD definition

All participants of this study underwent liver ultrasonography (SIUI CZXL-38G, Shantou, China). The results of ultrasound prompted the existence of steatohepatitis: enhanced liver echogenicity, echogenicity greater in liver than kidneys, deep attenuation and vascular blurring[23] and after excluding the steatohepatitis caused by alcohol, viruses and drugs was defined as NAFLD. All ultrasound examinations were performed by an experienced professional radiologist.

1.4 Statistics

Categorical variables were showed as proportions while continuous variables were

presented as means ± standard deviation (SD) or median (interquartile range) (IQR). ANOVA/two - paired sample t tests (continuous variables, normal distribution) and chi-square tests (categorical variables) were used to compare the difference in different groups. Logistic regression for categorical variables and linear regression for continuous variables were used to obtain the *P* value for trend.

Cox regression models were used to explore the relationship between TyG index and the incidence of NAFLD, the lowest quartiles of TyG index was defined as the reference. Hazards ratio (*HR*) and confidence interval (*CI*) of NAFLD in quartiles and continuous were expressed in separate models. To assess the relationship across increasing quartiles, *P* value for trend tests were used by entering median value in each quartile in Cox regression models.

AUCs were used to evaluate the ability of the baseline TyG index to predict the risk of NAFLD. Restricted cubic spline models were used to explore whether there was a nonlinear relationship between continuous and occurrence of NAFLD [24]. Mediation analysis used a Cox regression to study the mediate effect of TyG index between WHtR and NAFLD.

Mediation analysis was conducted using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria), and Restricted cubic spline were performed in Stata v 12.0 (Stata Corp, College Station, TX, USA). The other analyses were performed by SPSS software, version 21.0 (SPSS Inc, Chicago). Probability values for statistical tests, where two-tailed *P*-values<0.05 were regarded as significant.

2. Result

2.1 Baseline Characteristics of included subjects

A total of 46,693 subjects were included in this study. The baseline characteristics of included participants based on follow-up were presented in **Table 1**. The mean age of subjects was 68.91 (7.17) and 48.89% were men. The average follow-up was 3.19(1.52) years, during the 149041 person-years follow-up, 5660 subjects occurred NAFLD (3.80/100 person-years). Subjects with NAFLD with higher BMI, WHtR, TC, FBG, DBP, SBP, ALT, and TB and the incidence of NAFLD was higher in younger, current

smoking, regular exercise, with diabetes and hypertension but the baseline level of AST had no association with the incidence of NAFLD.

Table1 baseline characteristics of included subjects according to the follow-up outcome

variables	No NAFLD (n=41033)	NAFLD (n=5660)	*P* value
Men, n (%) †	20799(50.69)	2033(35.92)	<0.001
Age (years) ‡	69.18±7.27	66.93±6.00	<0.001
Current smoking, n (%) †	6846 (16.70)	594(10.51)	<0.001
Exercise, n (%) †	7865(19.22)	1131(20.02)	<0.001
Live alone, n (%) †	8772(21.38)	1015(17.93)	<0.001
Diabetes, n (%) †	5554(13.54)	1344(23.75)	<0.001
Hypertension, n (%) †	15945 (38.86)	2952(52.16)	<0.001
WHtR‡	0.51±0.06	0.54±0.07	<0.001
BMI‡	23.62±2.85	26.02±3.10	<0.001
SBP (mmHg) ‡	132.62±19.38	135.32±19.76	<0.001
DBP (mmHg) ‡	78.92±10.34	80.91±10.33	<0.001
FPG (mmol/L) §	5.20(4.70-5.70)	5.30(4.80-5.93)	<0.001
TC (mmol/L) §	4.59(4.01-5.21)	4.69(4.09-5.38)	<0.001
TG (mmol/L) §	1.10(0.81-1.47)	1.28(0.90-1.77)	<0.001
TB (μmol/L) §	11.51(8.60-14.10)	11.30(8.30-13.80)	<0.001
ALT(U/L) §	18.00(13.00-24.50)	18.60(14.00-25.00)	<0.001
AST(U/L) §	21.80(16.89-26.90)	21.90(16.89-27.00)	0.892
TyG‡	8.43±0.54	8.61±0.59	<0.001

†: n (%); ‡: mean (SD); §: median (IQR)

Abbreviations: BMI, body mass index; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, triglycerides; TG, triglyceride; TB, total bilirubin; ALT, alanine aminotransferase, AST, aspartate aminotransferase; TyG, triglyceride glucose.

2.2 Association between TyG index and NAFLD

The relationship between TyG index and the incidence of NAFLD were showed in **Table 2**. Compared with quartile 1 of TyG index, the hazard ratio (HR) and its corresponding 95% confidence interval (CI) for quartile2, quartile3, and quartile 4 were 1.056(0.971,1.149), 1.300(1.200,1.408) and 1.972(1.832,2.123), respectively. Risk for NAFLD was significantly higher with increasing quartiles of TyG for the *P* value of trend of linearity <0.001. In addition, even after adjusted possible confounding factors, the risk of quartile 3 of TyG index (1.314(1.234,1.457)) on the incidence of NAFLD still existed. In order to further verify the relationship between the TyG index and the

incidence of NAFLD, TyG index was used as continuous various for the above analysis and the results also proved that higher level of TyG increased the risk of NAFLD (1.265(1.200,1.334)), even after adjusted possible confounding factors. These results proved that the baseline level of TyG was associated with the risk of NAFLD, thus the higher the TyG level, the higher incidence of NAFLD.

Table 2 relationship between TyG index and the risk of NAFLD

		TyG index		
	N	unadjusted	Model 1	Model 2
As continuous	50037	1.697(1.619,1.779)	1.577(1.504,1.653)	1.265(1.200,1.334)
Quartile 1	12556	Reference	Reference	Reference
Quartile 2	12458	1.056(0.971,1.149)	1.035 (0.951,1.126)	0.980(0.895,1.073)
Quartile 3	12522	1.300(1.200,1.408)	1.259(1.162,1.364)	1.090(0.999,1.190)
Quartile 4	12501	1.972(1.832,2.123)	1.783(1.655,1.920)	1.314(1.234,1.457)
P for trend		<0.001	<0.001	<0.001

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol and diabetes.

2.3 The ability of TyG to predict NAFLD

The receiver operating characteristic curves (ROC) were used to evaluate the ability of baseline level of TyG to predict the development of NAFLD. The best cutoff value for TyG index to diagnosis NAFLD was 8.63, and its corresponding area under the receiver operating characteristic curves (AUCs) was 0.60 (95%CI:0.58,0.61), showed in **Figure 2**.

Figure 2 ROC curve of TyG on NAFLD

Receiver operating characteristics (ROC) curves for baseline TyG to predict NAFLD among 60 years old or more. The ROC area was 0.594(0.590 - 0.599). TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases.

2.4 Restricted cubic spline analysis to evaluate the relationship between TyG and NAFLD

In order to further explore the relationship between TyG and the risk of NAFLD, restricted cubic spline graph was used to analyze the dose-response relationship between TyG and the incidence of NAFLD. As shown in **Figure 3**, there was a nonlinear relationship between level of TyG index and the risk of NAFLD based on the

adjusted Cox regression model. TyG index significantly increased the risk of NAFLD when it was 8.63 and above. This result indicated that higher level of TyG was associated with higher incidence of NAFLD.

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD. Multivariate adjusted hazard ratios of NAFLD increased during follow-up when the baseline level of TyG index was 8.63 and above. Adjusted variables including age, sex, living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases; CI, confidence interval.

2.5 Mediation effects

In previous analysis we found that WHtR was associated with the incidence of NAFLD, what's more, WHtR was also associated with TyG index ($P<0.05$, and the data wasn't shown). If WHtR would lead to IR and TyG index was a reliable and cheap surrogate indicate for IR, then there might be a mediating effect of TyG index between WHtR and NAFLD. Therefore, mediation analysis was used to confirm whether there was a mediating effect and to what extent TyG index affect the relationship between WHtR and NAFLD. Results (presented in **Table 3 and Figure 4**) of mediation analysis revealed that the total effect of WHtR on NAFLD was 1.476 (1.437,1.517) and the direct effect was 1.463(1.842,1.950). Therefore, TyG index played a partial role and the indirect effect was 1.019 (1.006,1.011). This result may indicate that abdominal obesity may lead to disorders of glycolipid dyslipidemia which can lead to an increase of the TyG index.

Table 3 Mediation analysis of the relationship between TyG index and NAFLD by WHtR

Effect	HR (95%CI)
Total effect	1.476 (1.437,1.517)
Direct effect	1.463 (1.842,1.950)
Indirect effect	1.009(1.006,1.011)

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

The effect of WHtR on NAFLD was mediated by baseline level TyG index by adjusted potential confounders namely age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

3. Discussion

With the acceleration of the aging process, the health in the elderly has gradually become an important issue in the society. NAFLD, a common liver disease in China, also has become one of the main health problems of the elderly. Although there were some studies about TyG index and NAFLD, research about TyG index and the incidence of NAFLD in the older adults has not been reported.

In this retrospective cohort study, we found the incidence of NAFLD was 3.80/100 person-years among the elderly over 60, which was lower than a cohort study of Korea (the incidence of NAFLD was 4.84/100 person-years)[25]. Probably, the age of the subjects in the Korean cohort study(mean age was 36.1) was younger than ours and age could affect the prevalence and severity of NAFLD[26]. Additionally, the incidence of NAFLD decreased as the age increased in this study and the $P_{\text{value for trend}}$ <0.001(data not presented in this research). A retrospective cohort study on TyG index and NAFLD in Japan also found that higher TyG was associated with higher risk of NAFLD, which was consistent with our results[13]. As far as we know, this research is the first study to focus on the effect of TyG index on the incidence of NAFLD in the elderly in China. Our study also reported the relationship between TyG index was nonlinear hence the incidence of NAFLD would increase significantly when TyG is above 8.63. Furthermore, the results of the mediation effect suggested that TyG index was not an independent factor for the development of NAFLD, but a partial mediator between WHtR and NAFLD.

The results of ROC suggested that 8.63 was the best cutoff value of TyG index for predicting the incidence of NAFLD, which is consistent with a study by Fedchuk et al[9]. TyG index is an inexpensive and reliable surrogate index for IR and plays an

important role in the development and progression of NAFLD. Consequently, even in the absence of diabetes, IR can lead to changes from normal liver to NAFL to NASH[10]. Various studies have confirmed that IR is closely related to the occurrence and development of NAFLD[27-30].

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 participates in the occurrence and development of NAFLD by disturbing the glucose and lipid metabolism disorder of the liver[27]. Insulin resistance reduces glucose uptake in the adipose tissues and muscles while the hydrolysis of triglycerides in adipose tissue as well increases the conversion of glucose to fatty acids in the liver. Moreover, IR could also increase de novo lipogenesis by activating sterol regulatory element binding protein (SREBP1). 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, 31, 32]. On the other hand, IR is always linked to chronic mild inflammation by releasing inflammatory factors such as TNFα, IL-6, IL-1 and monocyte chemoattractant protein-1, immune cells or adipocytes which can in turn promote IR and participate in the development and progression of NAFLD[33, 34].

Obesity is a key factor in development and progress of NAFLD. It has been documented that subjects with NAFLD have a e higher level of BMI, and this is associated with the risk of NAFLD[35, 36]. While growing evidence suggested that the determinant of insulin resistance is not the degree of obesity, but the distribution of fat, abdominal fat accumulation is related to insulin resistance[37, 38]. Studies have also shown that in obese adolescents 2-h glucose and insulin resistance are significantly increased with higher visceral fat than those with lower abdominal fat [39, 40]. Central obesity can lead to inflammatory, oxidative stress and metabolic disorders, which are related to the development of insulin resistance[41, 42]. 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.

The major strength of this study was a large sample size retrospective cohort study which included 46,693 aged more than 60 years old. it is believed a cohort study could better reflect the real relationship between TyG index and NAFLD. Moreover, we established a nonlinear relationship between TyG index and NAFLD using cubic spline graph analysis, furthermore, TyG index played a mediation effect between WHtR and NAFLD in this study. However, there were several limitations in this study. First, the diagnosis of NAFLD was based on the results of abdominal ultrasound instead of liver biopsy which only provided information on whether there was the presence of NAFLD. Since it is difficult to find mild steatosis by ultrasonography, the incidence of NAFLD could be underestimated[31, 43]. Further, the subjects could not provide their history of lipid-lowering therapy or antidiabetic drugs as they were elder therefore, information about therapy was missing in the data. Additionally, we could not evaluate the relationship between TyG index and different NAFLD severity. Also, we lacked other more accurately index which can reflect the abdominal obesity status. Even though we used WHtR to indicate the abdominal obesity, we were unable to assess a more accurately visceral fat index and the prevalence of NAFLD.

In conclusion, high baseline level of TyG index is significantly associated with a higher risk of NAFLD. In addition, TyG index plays a partial mediating role in the relationship between WHtR and NAFLD. Our results suggest that it has important clinical meanings for monitoring TyG index to prevent NAFLD.

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Author Contributions:

H.C. and Q.L. designed the study. H.C., S.L., B.Y., X.C., Z.S., W.G and J.H. participated in the data collection and analysis. H.C. and A.A. drafted this manuscript, H.C. S.L. and S.S. interpreted the data. S.S and Q.L. reviewed and revised this manuscript. All authors approved the final manuscript.

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Conflict of Interest:

All authors have no conflicts of interest.

Patient and public involvement: Participants were not involved in the recruitment and conduct of the study

Patient consent for publication: Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China.

Ethics approval:

The study protocol was approved by ethics committee of Zhengzhou University in China (approval number: ZZURIB202004). Given the retrospective nature of the research, the requirement for informed consent was waived.

Provenance and peer review: Not commissioned; externally peer reviewed

Data availability statement: The datasets generated and/or analyzed during the current study are available upon request.

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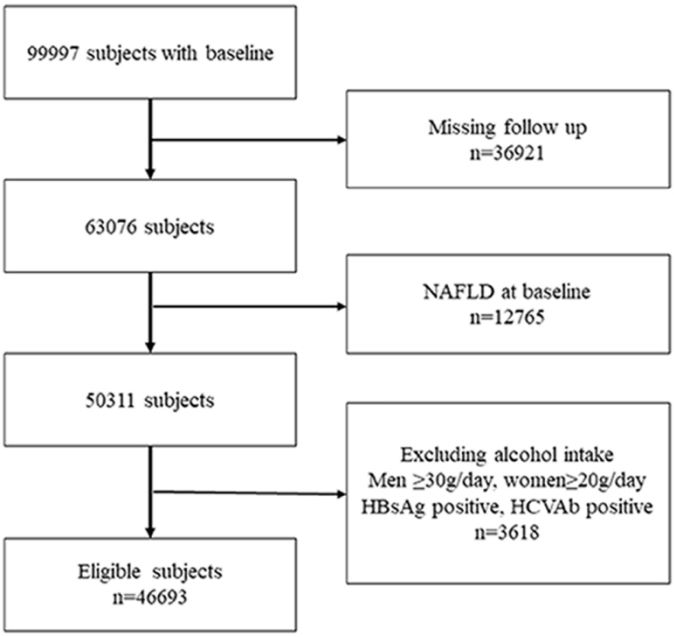

Figure 1 ROC curve of TyG on NAFLD

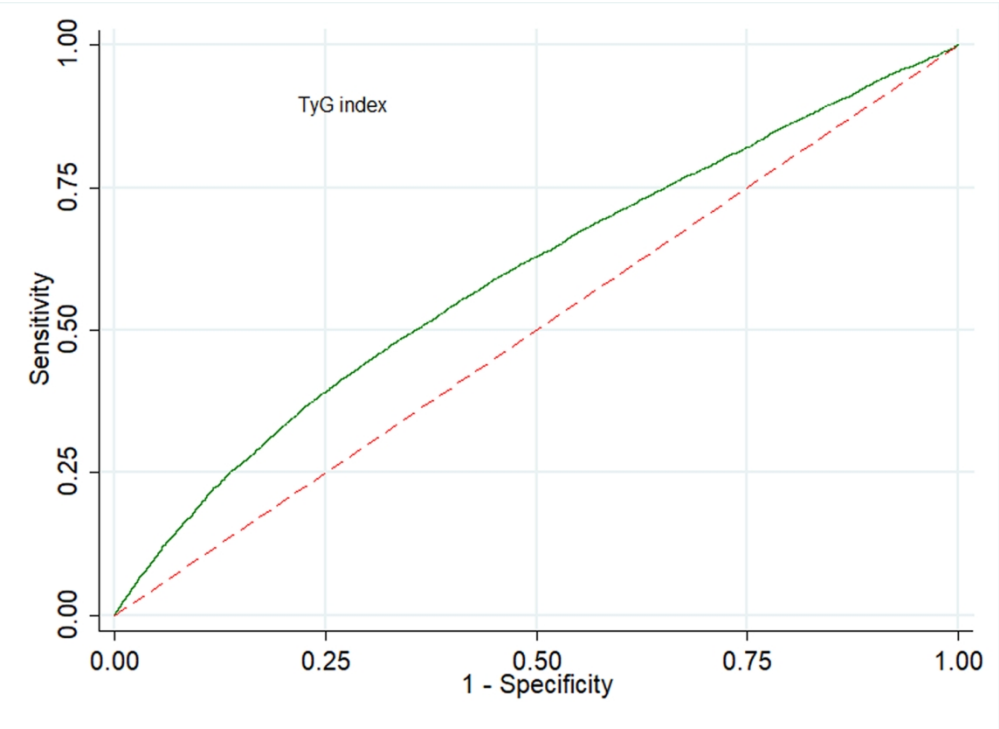

Figure 2 ROC curve of TyG on NAFLD

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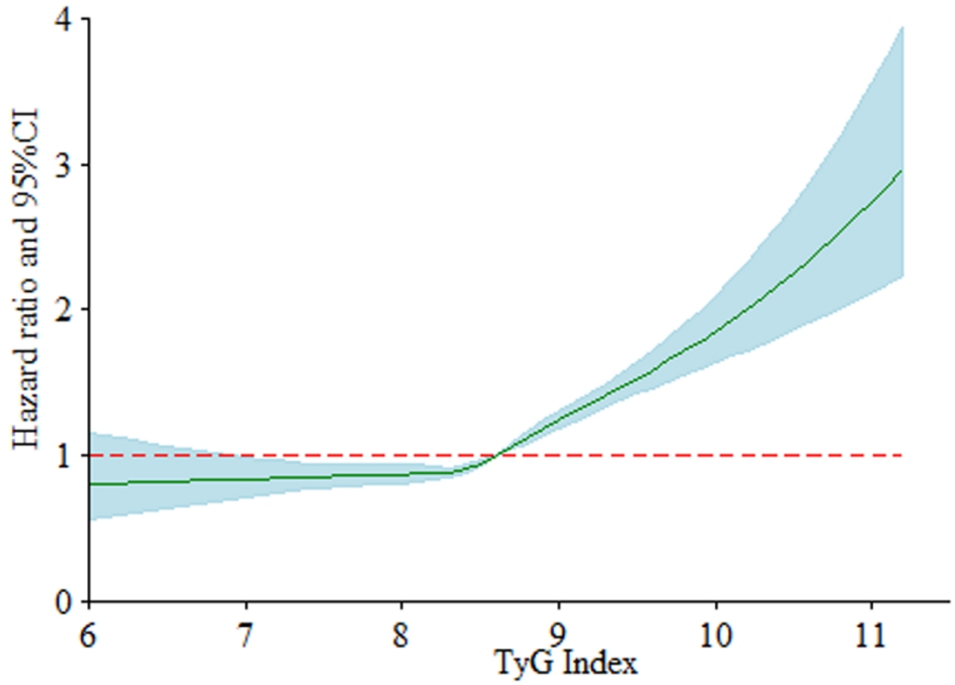

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

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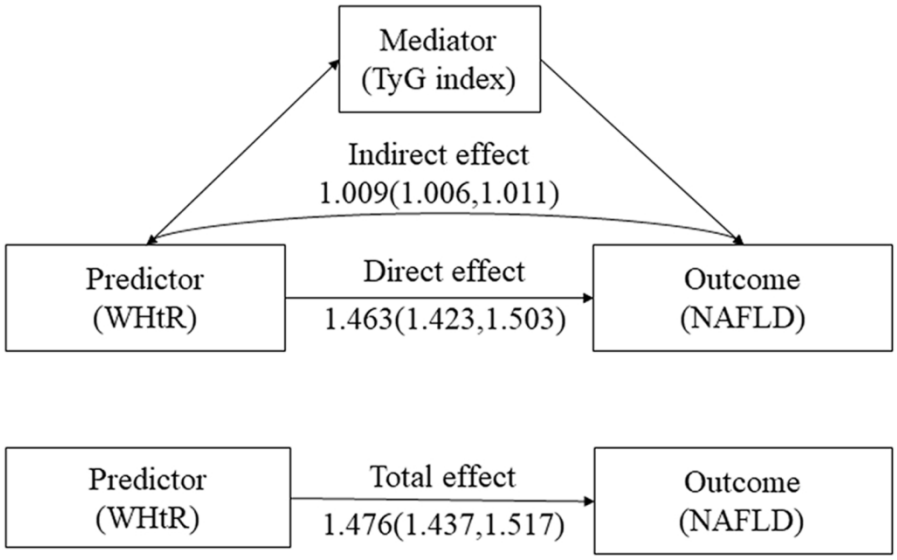

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,5
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			6,7,8,9
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6,7
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7,8 7,8 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China

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Secondary Subject Heading:	Gastroenterology and hepatology
Keywords:	Hepatobiliary disease < GASTROENTEROLOGY, Lipid disorders < DIABETES & ENDOCRINOLOGY, PUBLIC HEALTH

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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China

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Abstracts

Objective: Nonalcoholic fatty liver disease (NAFLD) is one of the major causes of liver-related diseases but relationship between triglyceride glucose (TyG) and NAFLD in the elderly is not reported yet. In this study, we investigated the role of TyG index for predicting the incidence of NAFLD in the elderly.

Design and setting：This is a prospective cohort study in Henan, China, from 2011-2018.

Participants and Methods：In total, 46,693 elderly who participated in a routine physical examination programme from 2011 to 2018 were included in this study. TyG index was calculated as ln [fasting triglyceride (mg/dl) ×fasting plasma glucose (mg/dl)/2], while NAFLD was defined as hepatic steatosis after excluding other causes based on the results of abdominal ultrasonography; Cox regression model was performed to explore the relationship between TyG index and NAFLD. Also, mediation effect was used to analyze the role of the TyG index in WHtR and NAFLD.

Results: During the 149041.50 person-years follow-up, a total of 5660 NAFLD events occurred (3.80/100 person-years). After adjusting for potential confounding factors, quartiles 4 of TyG index significantly increased the incidence of NAFLD compared with quartile 1, the hazard ratios (HRs) and 95% confidence intervals (CI) were 1.314(1.234,1.457). In addition, TyG index played a partial mediating role in the relationship between WHtR and NAFLD and indirect effect was 1.009(1.006,1.011).

Conclusion: Higher TyG index was associated with higher risk of NAFLD in the aged and therefore, TyG index may be a novel predictor for incidence of NAFLD. Further, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD.

Key words: Nonalcoholic fatty liver disease; Triglyceride glucose index; Waist-to-height ratio; Mediation effect.

Strengths and limitations of this study：

Data for this cohort study were retrieved from a large regular physical examination in Henan, China.

Restricted cubic spline analysis and mediation effect were used in this study, which can reflect the relationship between TyG index and NAFLD more realistically.

The sample size and statistical power were sufficient in this study.

The severity of disease in this cohort study was not available when data were retrieved.

Some participants were excluded in this study because they did not have abdominal ultrasound testing.

Introduction

Nonalcoholic fatty liver disease(NAFLD) encompasses a series of spectrum of liver diseases, ranging from simple steatosis, nonalcoholic steatohepatitis, cirrhosis to hepatocellular carcinoma[1]. With the change of lifestyle and increase of obesity, NAFLD currently exceeds viral hepatitis and is becoming the most common chronic liver disease, affecting about a quarter of the common population in the world[2, 3]. NAFLD is one of the major causes of liver-related disease such as cirrhosis, hepatocellular carcinoma and liver transplantation. Moreover, NAFLD is also associated with higher prevalence and incidence of cardiovascular disease such as coronary, cerebrovascular and peripheral vascular disease [4, 5]. In the next decade, NAFLD is expected to become the leading cause for liver transplantation in the United States instead of hepatitis C[6].

The development of NAFLD is a complex process involving genetic and environmental factors. As an organ of metabolism, the disorder of glucose and lipid metabolism plays an important role in the progress of NAFLD[7]. Several studies found that IR could increase the risk of NAFLD even without the existence of T2DM and IR may be the genesis of steatosis[7-10].Furthermore, as an early marker of IR, TyG has been proposed to be an inexpensive and reliable surrogate to IR[11, 12] Yet researches about the relationship between TyG index and the risk of NAFLD are limited [9, 13]. Additionally, as far as we know, the association between TyG index and the incidence

of NAFLD in the older has not been reported.

WHtR is a maker of abdominal obesity and studies have also reported that WHtR is related to IR[14, 15] and also the higher risk of NAFLD [16-22]. Whether there is an existing effect of TyG index on the relationship between WHtR and NAFLD and how it affects NAFLD is still unclear. This retrospective cohort study therefore sought to explore the relationship between baseline level of TyG index and the incidence of NAFLD.

1.Participants and methods

1.1 Subjects:

We retrospectively analyzed the 99,997 subjects who had the data of liver ultrasonography, fasting triglyceride as well as fasting plasma glucose in physical examination programme in Xinzheng, Henan Province, in Central China City from January 2011 to December 2018. This physical examination programme was for the local residents over 60 years old and was supported by the government of Xinzheng, Henan Province in China. Individuals with any of the following criteria were excluded:1) missing follow-up; 2) subjects with NAFLD at baseline;3) subjects with hepatitis B or C virus or had the history of excess alcohol intake (the threshold for women <20 g/d and <30 g/d for men). A total of 46,693 eligible participants were included in this cohort study (**Figure 1**). The datasets generated and/or analyzed during the current study are available upon request. Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China (Approve number: ZZURIB202004).

Figure 1 Flow chart for participants exclusions performed

1.2 Data collection

Demographic data and clinical information of the subjects were collected when they underwent health check-up. Demographic data included age, sex, excess alcohol intake

(yes/no), current smoking status (yes/no), lives alone(yes/no) and exercises regularly(yes/no). The definition of current smoking was that subjects used to smoke 100 cigarettes or above in their lifetime, and now is still smoking regularly. Excessive drinking was defined as drinking more than 30g /d for men and 20g/d for women. Exercising regularly was defined as more than 3 times a week, and 30 minutes moderate intensity exercise each time. The clinic records included anthropometric measurements (such as height, weight, blood pressure, waist circumference) and laboratory data. Height measurement required the subjects without shoes, stand straight on the ground, and their hips and heels against the wall, to measure the weight, the participants were without shoes and wore light clothing. Blood pressure of the subjects in a sitting position after 5 minutes of rest was measured twice by an electronic sphygmomanometer (Omron HEM-7125, Kyoto, Japan), and the mean value of the two measurements was recorded. Waist circumference was measured at the midpoint of the distance between the lowest costal ridge and the upper border of the iliac crest. After fasting for 8 hours, the blood samples of subjects were collected to determine the level of fasting plasma glucose (FPG), total cholesterol (TC) and triglyceride (TG) using a biochemical detector (DIRUI CS380, Changchun, China). Alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (TB) was detected by (DIRUI CS380, Changchun, China). The TyG index were calculated as the ln [fasting TG (mg/dl) × FPG (mg/dl)/2]. WHtR was defined as waist circumference(cm)/height(cm).

1.3 NAFLD definition

All participants of this study underwent liver ultrasonography (SIUI CZXL-38G, Shantou, China). The results of ultrasound prompted the existence of steatohepatitis: enhanced liver echogenicity, echogenicity greater in liver than kidneys, deep attenuation and vascular blurring[23] and after excluding the steatohepatitis caused by alcohol, viruses and drugs was defined as NAFLD. All ultrasound examinations were performed by an experienced professional radiologist.

1.4 Statistics

Categorical variables were showed as proportions while continuous variables were

presented as means ± standard deviation (SD) or median (interquartile range) (IQR). ANOVA/two - paired sample t tests (continuous variables, normal distribution) and chi-square tests (categorical variables) were used to compare the difference in different groups. Logistic regression for categorical variables and linear regression for continuous variables were used to obtain the *P* value for trend.

Cox regression models were used to explore the relationship between TyG index and the incidence of NAFLD, the lowest quartiles of TyG index was defined as the reference. Hazards ratio (*HR*) and confidence interval (*CI*) of NAFLD in quartiles and continuous were expressed in separate models. To assess the relationship across increasing quartiles, *P* value for trend tests were used by entering median value in each quartile in Cox regression models.

AUCs were used to evaluate the ability of the baseline TyG index to predict the risk of NAFLD. Restricted cubic spline models were used to explore whether there was a nonlinear relationship between continuous and occurrence of NAFLD [24]. Mediation analysis used a Cox regression to study the mediate effect of TyG index between WHtR and NAFLD.

Mediation analysis was conducted using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria), and Restricted cubic spline were performed in Stata v 12.0 (Stata Corp, College Station, TX, USA). The other analyses were performed by SPSS software, version 21.0 (SPSS Inc, Chicago). Probability values for statistical tests, where two-tailed *P*-values<0.05 were regarded as significant.

2. Result

2.1 Baseline Characteristics of included subjects

A total of 46,693 subjects were included in this study. The baseline characteristics of included participants based on follow-up were presented in **Table 1**. The mean age of subjects was 68.91 (7.17) and 48.89% were men. The average follow-up was 3.19(1.52) years, during the 149041 person-years follow-up, 5660 subjects occurred NAFLD (3.80/100 person-years). Subjects with NAFLD with higher BMI, WHtR, TC, FBG, DBP, SBP, ALT, and TB and the incidence of NAFLD was higher in younger, current

smoking, regular exercise, with diabetes and hypertension but the baseline level of AST had no association with the incidence of NAFLD.

Table1 baseline characteristics of included subjects according to the follow-up outcome

variables	No NAFLD (n=41033)	NAFLD (n=5660)	*P* value
Men, n (%) †	20799(50.69)	2033(35.92)	<0.001
Age (years) ‡	69.18±7.27	66.93±6.00	<0.001
Current smoking, n (%) †	6846 (16.70)	594(10.51)	<0.001
Exercise, n (%) †	7865(19.22)	1131(20.02)	<0.001
Live alone, n (%) †	8772(21.38)	1015(17.93)	<0.001
Diabetes, n (%) †	5554(13.54)	1344(23.75)	<0.001
Hypertension, n (%) †	15945 (38.86)	2952(52.16)	<0.001
WHtR‡	0.51±0.06	0.54±0.07	<0.001
BMI‡	23.62±2.85	26.02±3.10	<0.001
SBP (mmHg) ‡	132.62±19.38	135.32±19.76	<0.001
DBP (mmHg) ‡	78.92±10.34	80.91±10.33	<0.001
FPG (mmol/L) §	5.20(4.70-5.70)	5.30(4.80-5.93)	<0.001
TC (mmol/L) §	4.59(4.01-5.21)	4.69(4.09-5.38)	<0.001
TG (mmol/L) §	1.10(0.81-1.47)	1.28(0.90-1.77)	<0.001
TB (μmol/L) §	11.51(8.60-14.10)	11.30(8.30-13.80)	<0.001
ALT(U/L) §	18.00(13.00-24.50)	18.60(14.00-25.00)	<0.001
AST(U/L) §	21.80(16.89-26.90)	21.90(16.89-27.00)	0.892
TyG‡	8.43±0.54	8.61±0.59	<0.001

†: n (%); ‡: mean (SD); §: median (IQR)

Abbreviations: BMI, body mass index; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, triglycerides; TG, triglyceride; TB, total bilirubin; ALT, alanine aminotransferase, AST, aspartate aminotransferase; TyG, triglyceride glucose.

2.2 Association between TyG index and NAFLD

The relationship between TyG index and the incidence of NAFLD were showed in **Table 2**. Compared with quartile 1 of TyG index, the hazard ratio (HR) and its corresponding 95% confidence interval (CI) for quartile2, quartile3, and quartile 4 were 1.056(0.971,1.149), 1.300(1.200,1.408) and 1.972(1.832,2.123), respectively. Risk for NAFLD was significantly higher with increasing quartiles of TyG for the *P* value of trend of linearity <0.001. In addition, even after adjusted possible confounding factors, the risk of quartile 3 of TyG index (1.314(1.234,1.457)) on the incidence of NAFLD still existed. In order to further verify the relationship between the TyG index and the

incidence of NAFLD, TyG index was used as continuous various for the above analysis and the results also proved that higher level of TyG increased the risk of NAFLD (1.265(1.200,1.334)), even after adjusted possible confounding factors. These results proved that the baseline level of TyG was associated with the risk of NAFLD, thus the higher the TyG level, the higher incidence of NAFLD.

Since the majority of the subjects could not respond on their history of lipid-lowering therapy or antidiabetic drugs, we failed to access information about therapy. Sensitivity analysis (n=37428) was executed after excluding subjects with diabetes, CVD and stroke, who might take medicine that affects level of FPG and TG. The results of sensitivity analysis (Suppl table 1) also suggested that higher level of TyG significantly increased the incidence of NAFLD, which were similar with the findings in total population.

Table 2 relationship between TyG index and the risk of NAFLD

		TyG index		
	N	unadjusted	Model 1	Model 2
As continuous	50037	1.697(1.619,1.779)	1.577(1.504,1.653)	1.265(1.200,1.334)
Quartile 1	12556	Reference	Reference	Reference
Quartile 2	12458	1.056(0.971,1.149)	1.035 (0.951,1.126)	0.980(0.895,1.073)
Quartile 3	12522	1.300(1.200,1.408)	1.259(1.162,1.364)	1.090(0.999,1.190)
Quartile 4	12501	1.972(1.832,2.123)	1.783(1.655,1.920)	1.314(1.234,1.457)
P for trend		<0.001	<0.001	<0.001

Quartile 1≤8.11; 8.12<Quartile 2≤8.44; 8.45<Quartile 3≤8.78; 8.79<Quartile 4

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol and diabetes.

2.3 The ability of TyG to predict NAFLD

The receiver operating characteristic curves (ROC) were used to evaluate the ability of baseline level of TyG to predict the development of NAFLD. The best cutoff value for TyG index to diagnosis NAFLD was 8.63, and its corresponding area under the receiver operating characteristic curves (AUCs) was 0.60 (95%CI:0.58,0.61), showed in **Figure 2A**. Subgroup analysis was performed to further analyze the influence of sex on ability

of TyG index to predict incident NAFLD, the best cutoff value for men (8.68) was lower in men than women (8.75), and corresponding AUCs was 0.587 (95%CI:0.573-0.600) for men and 0.584 (95%CI:0.573-0.594) for men, respectively(**Figure 2B and 2C**).

Figure 2 ROC curve of TyG on NAFLD

Receiver operating characteristics (ROC) curves for baseline TyG to predict incident NAFLD among 60 years old or older. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases.

2.4 Restricted cubic spline analysis to evaluate the relationship between TyG and NAFLD

In order to further explore the relationship between TyG and the risk of NAFLD, restricted cubic spline graph was used to analyze the dose-response relationship between TyG and the incidence of NAFLD. As shown in **Figure 3A**, there was a nonlinear relationship between level of TyG index and the risk of NAFLD based on the adjusted Cox regression model. This result proved again that higher level of TyG was associated with higher incidence of NAFLD. Subgroup analyses also found that there was a nonlinear relationship between TyG index and incident NAFLD both in men and women (**Figure 3B and C**).

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

Multivariate adjusted hazard ratios of NAFLD increased during follow-up when the baseline level of TyG index was 8.63 and above. Adjusted variables including age, sex (not for sex subgroup analysis), living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases; CI, confidence interval.

2.5 Mediation effects

In previous analysis we found that WHtR was associated with the incidence of NAFLD, what's more, WHtR was also associated with TyG index (P<0.05, and the data wasn't

shown). If WHtR would lead to IR and TyG index was a reliable and cheap surrogate indicate for IR, then there might be a mediating effect of TyG index between WHtR and NAFLD. Therefore, mediation analysis was used to confirm whether there was a mediating effect and to what extent TyG index affect the relationship between WHtR and NAFLD. Results (presented in **Table 3 and Figure 4**) of mediation analysis revealed that the total effect of WHtR on NAFLD was 1.476 (1.437,1.517) and the direct effect was 1.463(1.842,1.950). Therefore, TyG index played a partial role and the indirect effect was 1.019 (1.006,1.011). This result may indicate that abdominal obesity may lead to disorders of glycolipid dyslipidemia which can lead to an increase of the TyG index.

Table 3 Mediation analysis of the relationship between TyG index and NAFLD by WHtR

Effect	HR (95%CI)
Total effect	1.476 (1.437,1.517)
Direct effect	1.463 (1.842,1.950)
Indirect effect	1.009(1.006,1.011)

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

The effect of WHtR on NAFLD was mediated by baseline level TyG index by adjusted potential confounders namely age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

3. Discussion

With the acceleration of the aging process, the health in the elderly has gradually become an important issue in the society. NAFLD, a common liver disease in China, also has become one of the main health problems of the elderly. Although there were some studies about TyG index and NAFLD, research about TyG index and the incidence of NAFLD in the older adults has not been reported.

In this retrospective cohort study, we found the incidence of NAFLD was 3.80/100 person-years among the elderly over 60, which was lower than a cohort study of Korea

(the incidence of NAFLD was 4.84/100 person-years)[25]. Probably, the age of the subjects in the Korean cohort study(mean age was 36.1) was younger than ours and age could affect the prevalence and severity of NAFLD[26]. Additionally, the incidence of NAFLD decreased as the age increased in this study and the $P_{\text{value for trend}}$ <0.001(data not presented in this research). A retrospective cohort study on TyG index and NAFLD in Japan also found that higher TyG was associated with higher risk of NAFLD, which was consistent with our results[13]. As far as we know, this research is the first study to focus on the effect of TyG index on the incidence of NAFLD in the elderly in China. Our study also reported the relationship between TyG index was nonlinear hence the incidence of NAFLD would increase significantly when TyG is above 8.63. Furthermore, the results of the mediation effect suggested that TyG index was not an independent factor for the development of NAFLD, but a partial mediator between WHtR and NAFLD.

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[27],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[13, 28, 29]; 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. TyG index is an inexpensive and reliable surrogate index for IR and plays an important role in the development and progression of NAFLD. Consequently, even in the absence of diabetes, IR can lead to changes from normal liver to NAFL to NASH[10]. Various studies have confirmed that IR is closely related to the occurrence and development of NAFLD[30-33].

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 participates in the occurrence and development of NAFLD by disturbing the glucose and lipid metabolism disorder of the liver[30]. Insulin resistance reduces glucose uptake in the adipose tissues and muscles while the hydrolysis of triglycerides in adipose tissue as well increases the conversion of glucose to fatty acids in the liver. Moreover, IR could also increase de novo lipogenesis by activating sterol regulatory element binding protein (SREBP1). 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 by releasing inflammatory factors such as TNFα, IL-6, IL-1 and monocyte chemoattractant protein-1, immune cells or adipocytes 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 progress of NAFLD. It has been documented that subjects with NAFLD have a e higher level of BMI, and this is associated with the risk of NAFLD[38, 39]. While growing evidence suggested that the determinant of insulin resistance is not the degree of obesity, but the distribution of fat, abdominal fat accumulation is related to insulin resistance[40, 41]. Studies have also shown that in obese adolescents 2-h glucose and insulin resistance are significantly increased with higher visceral fat than those with lower abdominal fat [42, 43]. Central obesity can lead to inflammatory, oxidative stress and metabolic disorders, which are related to the development of insulin resistance[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.

The major strength of this study was a large sample size retrospective cohort study which included 46,693 aged more than 60 years old. it is believed a cohort study could better reflect the real relationship between TyG index and NAFLD. Moreover, we established a nonlinear relationship between TyG index and NAFLD using cubic spline

graph analysis, furthermore, TyG index played a mediation effect between WHtR and NAFLD in this study. However, there were several limitations in this study. First, 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[46]. Furthermore, the subjects could not provide their history of lipid-lowering therapy or antidiabetic drugs as they were elder therefore, information about therapy was missing in the data. Additionally, we could not evaluate the relationship between TyG index and different NAFLD severity. Also, we lacked other more accurately index which can reflect the abdominal obesity status. Even though we used WHtR to indicate the abdominal obesity, we were unable to assess a more accurately visceral fat index and the prevalence of NAFLD.

In conclusion, high baseline level of TyG index is significantly associated with a higher risk of NAFLD. In addition, TyG index plays a partial mediating role in the relationship between WHtR and NAFLD. Our results suggest that it has important clinical meanings for monitoring TyG index to prevent NAFLD.

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Author Contributions:

H.C. and Q.L. designed the study. H.C., S.L., B.Y., X.C., Z.S., W.G and J.H. participated in the data collection and analysis. H.C. and A.A. drafted this manuscript,

H.C. S.L. and S.S. interpreted the data. S.S and Q.L. reviewed and revised this manuscript. All authors approved the final manuscript.

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Conflict of Interest:

All authors have no conflicts of interest.

Patient and public involvement: Participants were not involved in the recruitment and conduct of the study

Patient consent for publication: Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China.

Ethics approval:

The study protocol was approved by ethics committee of Zhengzhou University in China (approval number: ZZURIB202004). Given the retrospective nature of the research, the requirement for informed consent was waived.

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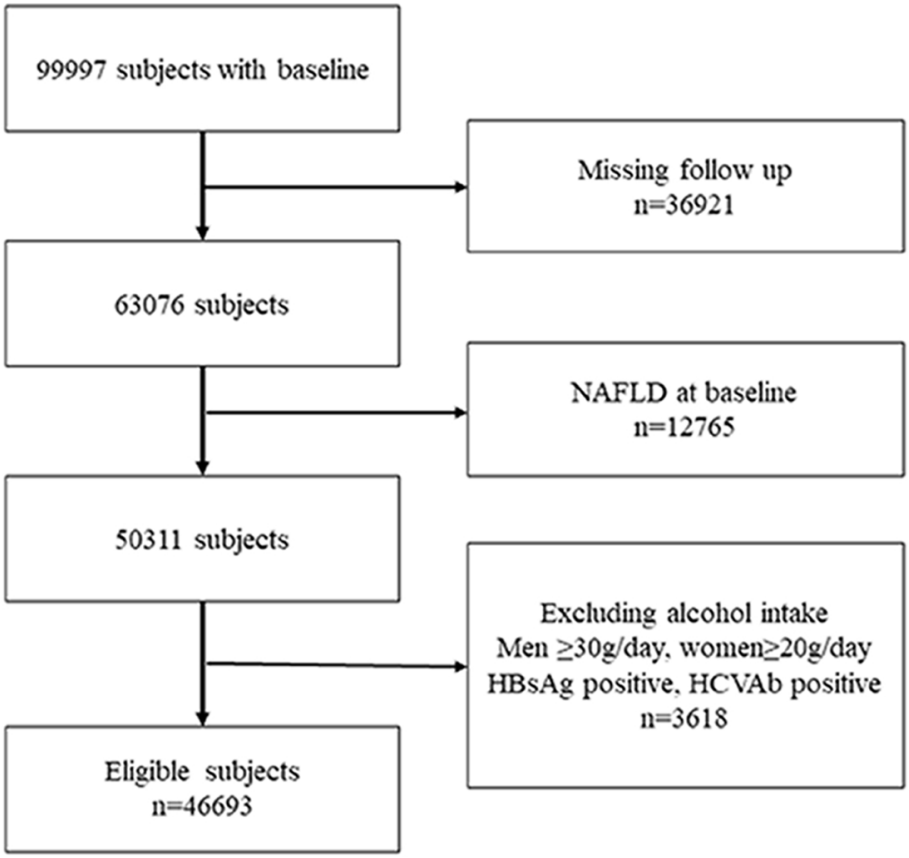

Figure 1 Flow chart for participants exclusions performed

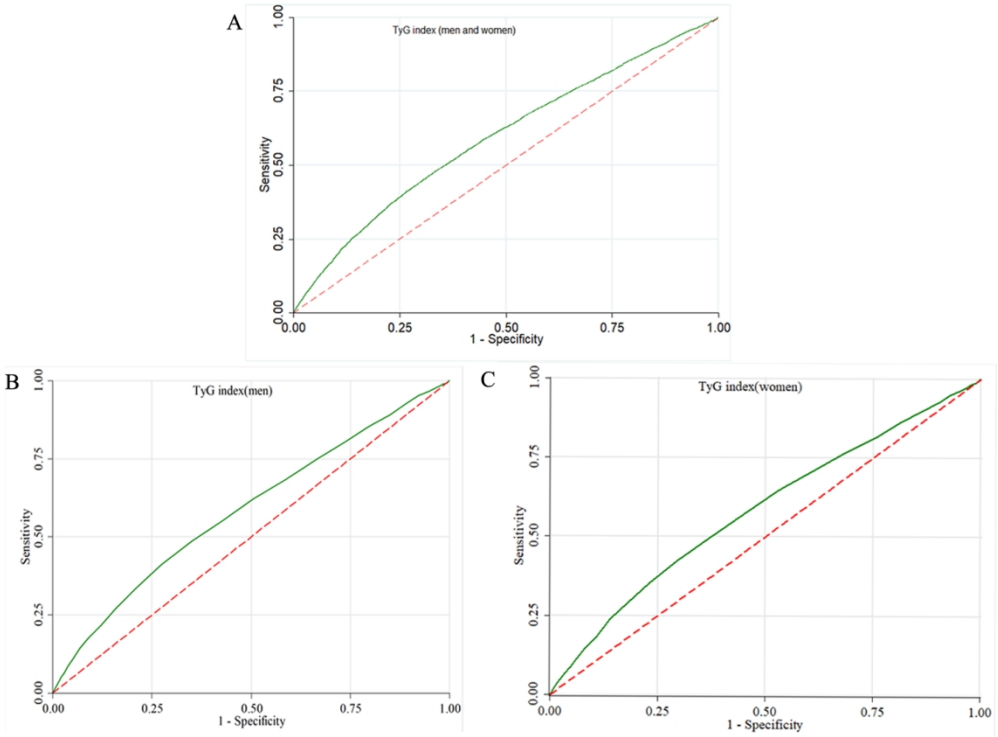

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

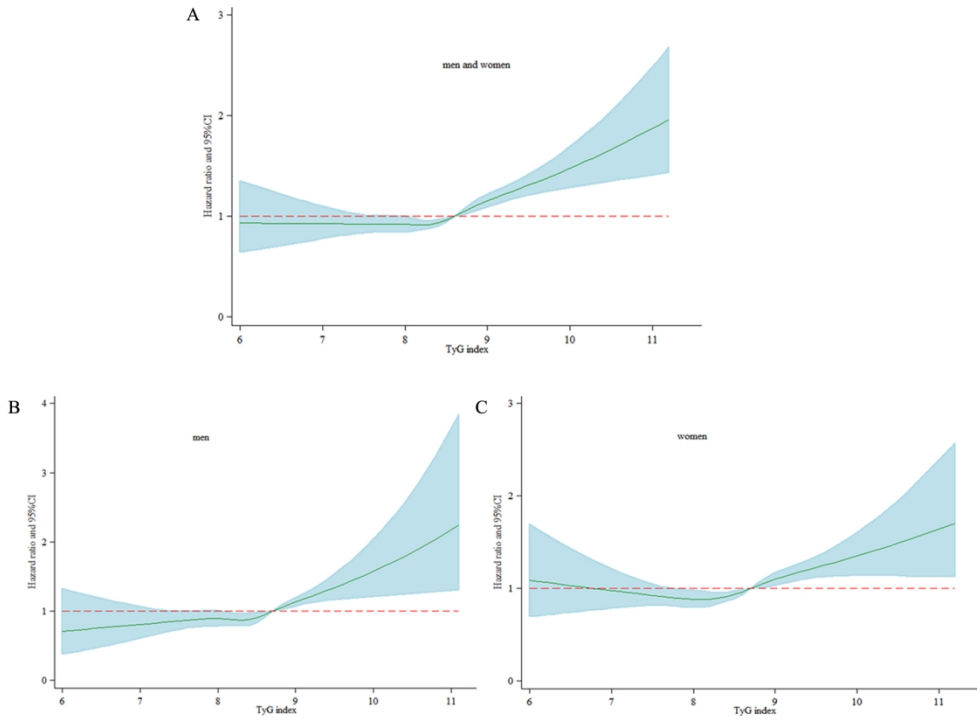

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

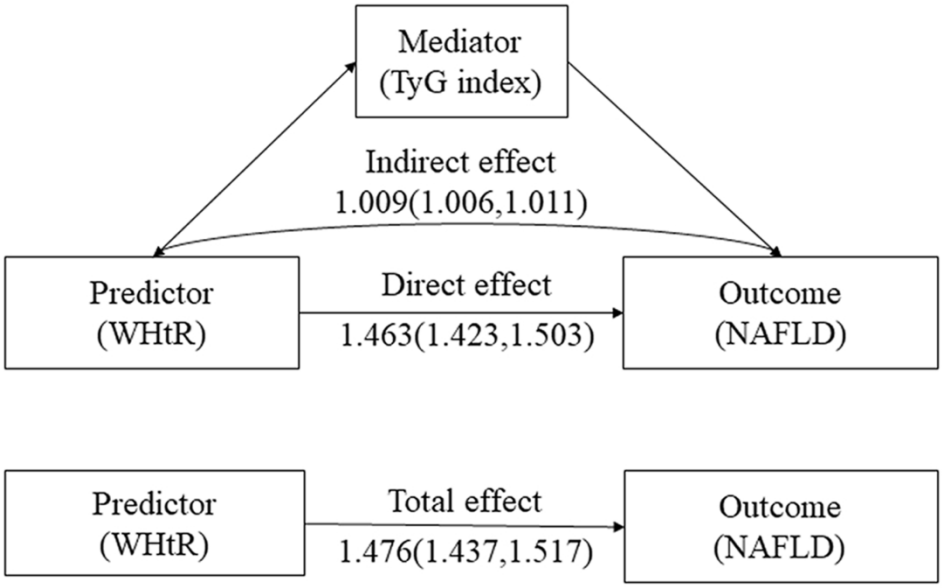

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

Supplementary Table 1 relationship between TyG index and the risk of NAFLD excluded subjects with diabetes, CVD and stroke

		TyG index		
	n	unadjusted	Model 1	Model 2
As continuous	37428	1.687(1.589,1.792)	1.571(1.480,1.668)	1.342(1.257,1.433)
Quartile 1	9536	Reference	Reference	Reference
Quartile 2	9368	1.036(0.938,1.144)	1.019 (0.923,1.125)	0.986(0.887,1.097)
Quartile 3	9350	1.239(1.127,1.362)	1.209(1.099,1.330)	1.076(0.970,1.193)
Quartile 4	9354	1.846(1.691,2.014)	1.681(1.540,1.836)	1.389(1.261,1.530)
P for trend		<0.001	<0.001	<0.001

Quartile 1≤8.08; 8.09<Quartile 2≤8.40; 8.41<Quartile 3≤8.72; 8.73< Quartile 4

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,5
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			6,7,8,9
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6,7
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7-10 7,8 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12,13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China

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Relationship between triglyceride glucose index and the incidence of nonalcoholic fatty liver disease in the elderly: a retrospective cohort study in China

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Abstracts

Objective: Nonalcoholic fatty liver disease (NAFLD) is one of the major causes of liver-related diseases but relationship between triglyceride glucose (TyG) and NAFLD in the elderly is not reported yet. In this study, we investigated the role of TyG index for predicting the incidence of NAFLD in the elderly.

Design and setting：This is a prospective cohort study in Henan, China, from 2011-2018.

Participants and Methods：In total, 46,693 elderly who participated in a routine physical examination programme from 2011 to 2018 were included in this study. TyG index was calculated as ln [fasting triglyceride (mg/dl) ×fasting plasma glucose (mg/dl)/2], while NAFLD was defined as hepatic steatosis after excluding other causes based on the results of abdominal ultrasonography; Cox regression model was performed to explore the relationship between TyG index and NAFLD. Also, mediation effect was used to analyze the role of the TyG index in WHtR and NAFLD.

Results: During the 149041.50 person-years follow-up, a total of 5660 NAFLD events occurred (3.80/100 person-years). After adjusting for potential confounding factors, quartiles 4 of TyG index significantly increased the incidence of NAFLD compared with quartile 1, the hazard ratios (HRs) and 95% confidence intervals (CI) were 1.314(1.234,1.457). In addition, TyG index played a partial mediating role in the relationship between WHtR and NAFLD and indirect effect was 1.009(1.006,1.011).

Conclusion: Higher TyG index was associated with higher risk of NAFLD in the aged and therefore, TyG index may be a novel predictor for incidence of NAFLD. Further, regular examination and evaluation of the TyG index might be useful for controlling the occurrence of NAFLD.

Key words: Nonalcoholic fatty liver disease; Triglyceride glucose index; Waist-to-height ratio; Mediation effect.

Strengths and limitations of this study：

Data for this cohort study were retrieved from a large regular physical examination in Henan, China.

Restricted cubic spline analysis and mediation effect were used in this study, which can reflect the relationship between TyG index and NAFLD more realistically.

The sample size and statistical power were sufficient in this study.

The severity of disease in this cohort study was not available when data were retrieved.

Some participants were excluded in this study because they did not have abdominal ultrasound testing.

Introduction

Nonalcoholic fatty liver disease(NAFLD) encompasses a series of spectrum of liver diseases, ranging from simple steatosis, nonalcoholic steatohepatitis, cirrhosis to hepatocellular carcinoma[1]. With the change of lifestyle and increase of obesity, NAFLD currently exceeds viral hepatitis and is becoming the most common chronic liver disease, affecting about a quarter of the common population in the world[2, 3]. NAFLD is one of the major causes of liver-related disease such as cirrhosis, hepatocellular carcinoma and liver transplantation. Moreover, NAFLD is also associated with higher prevalence and incidence of cardiovascular disease such as coronary, cerebrovascular and peripheral vascular disease [4, 5]. In the next decade, NAFLD is expected to become the leading cause for liver transplantation in the United States instead of hepatitis C[6].

The development of NAFLD is a complex process involving genetic and environmental factors. As an organ of metabolism, the disorder of glucose and lipid metabolism plays an important role in the progress of NAFLD[7]. Several studies found that IR could increase the risk of NAFLD even without the existence of T2DM and IR may be the genesis of steatosis[7-10].Furthermore, as an early marker of IR, TyG has been proposed to be an inexpensive and reliable surrogate to IR[11, 12] Yet researches about the relationship between TyG index and the risk of NAFLD are limited [9, 13]. Additionally, as far as we know, the association between TyG index and the incidence

of NAFLD in the older has not been reported.

WHtR is a maker of abdominal obesity and studies have also reported that WHtR is related to IR[14, 15] and also the higher risk of NAFLD [16-22]. Whether there is an existing effect of TyG index on the relationship between WHtR and NAFLD and how it affects NAFLD is still unclear. This retrospective cohort study therefore sought to explore the relationship between baseline level of TyG index and the incidence of NAFLD.

1.Participants and methods

1.1 Subjects:

We retrospectively analyzed the 99,997 subjects who had the data of liver ultrasonography, fasting triglyceride as well as fasting plasma glucose in physical examination programme in Xinzheng, Henan Province, in Central China City from January 2011 to December 2018. This physical examination programme was for the local residents over 60 years old and was supported by the government of Xinzheng, Henan Province in China. Individuals with any of the following criteria were excluded:1) missing follow-up; 2) subjects with NAFLD at baseline;3) subjects with hepatitis B or C virus or had the history of excess alcohol intake (the threshold for women <20 g/d and <30 g/d for men). A total of 46,693 eligible participants were included in this cohort study (**Figure 1**). The datasets generated and/or analyzed during the current study are available upon request. Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China (Approve number: ZZURIB202004).

Figure 1 Flow chart for participants exclusions performed

1.2 Data collection

Demographic data and clinical information of the subjects were collected when they underwent health check-up. Demographic data included age, sex, excess alcohol intake

(yes/no), current smoking status (yes/no), lives alone(yes/no) and exercises regularly(yes/no). The definition of current smoking was that subjects used to smoke 100 cigarettes or above in their lifetime, and now is still smoking regularly. Excessive drinking was defined as drinking more than 30g /d for men and 20g/d for women. Exercising regularly was defined as more than 3 times a week, and 30 minutes moderate intensity exercise each time. The clinic records included anthropometric measurements (such as height, weight, blood pressure, waist circumference) and laboratory data. Height measurement required the subjects without shoes, stand straight on the ground, and their hips and heels against the wall, to measure the weight, the participants were without shoes and wore light clothing. Blood pressure of the subjects in a sitting position after 5 minutes of rest was measured twice by an electronic sphygmomanometer (Omron HEM-7125, Kyoto, Japan), and the mean value of the two measurements was recorded. Waist circumference was measured at the midpoint of the distance between the lowest costal ridge and the upper border of the iliac crest. After fasting for 8 hours, the blood samples of subjects were collected to determine the level of fasting plasma glucose (FPG), total cholesterol (TC) and triglyceride (TG) using a biochemical detector (DIRUI CS380, Changchun, China). Alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (TB) was detected by (DIRUI CS380, Changchun, China). The TyG index were calculated as the ln [fasting TG (mg/dl) × FPG (mg/dl)/2]. WHtR was defined as waist circumference(cm)/height(cm).

1.3 NAFLD definition

All participants of this study underwent liver ultrasonography (SIUI CZXL-38G, Shantou, China). The results of ultrasound prompted the existence of steatohepatitis: enhanced liver echogenicity, echogenicity greater in liver than kidneys, deep attenuation and vascular blurring[23] and after excluding the steatohepatitis caused by alcohol, viruses and drugs was defined as NAFLD. All ultrasound examinations were performed by an experienced professional radiologist.

1.4 Statistics

Categorical variables were showed as proportions while continuous variables were

presented as means ± standard deviation (SD) or median (interquartile range) (IQR). ANOVA/two - paired sample t tests (continuous variables, normal distribution) and chi-square tests (categorical variables) were used to compare the difference in different groups. Logistic regression for categorical variables and linear regression for continuous variables were used to obtain the *P* value for trend.

Cox regression models were used to explore the relationship between TyG index and the incidence of NAFLD, the lowest quartiles of TyG index was defined as the reference. Hazards ratio (*HR*) and confidence interval (*CI*) of NAFLD in quartiles and continuous were expressed in separate models. To assess the relationship across increasing quartiles, *P* value for trend tests were used by entering median value in each quartile in Cox regression models.

AUCs were used to evaluate the ability of the baseline TyG index to predict the risk of NAFLD. Restricted cubic spline models were used to explore whether there was a nonlinear relationship between continuous and occurrence of NAFLD [24]. Mediation analysis used a Cox regression to study the mediate effect of TyG index between WHtR and NAFLD.

Mediation analysis was conducted using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria), and Restricted cubic spline were performed in Stata v 12.0 (Stata Corp, College Station, TX, USA). The other analyses were performed by SPSS software, version 21.0 (SPSS Inc, Chicago). Probability values for statistical tests, where two-tailed *P*-values<0.05 were regarded as significant.

2. Result

2.1 Baseline Characteristics of included subjects

A total of 46,693 subjects were included in this study. The baseline characteristics of included participants based on follow-up were presented in **Table 1**. The mean age of subjects was 68.91 (7.17) and 48.89% were men. The average follow-up was 3.19(1.52) years, during the 149041 person-years follow-up, 5660 subjects occurred NAFLD (3.80/100 person-years). Subjects with NAFLD with higher BMI, WHtR, TC, FBG, DBP, SBP, ALT, and TB and the incidence of NAFLD was higher in younger, current

smoking, regular exercise, with diabetes and hypertension but the baseline level of AST had no association with the incidence of NAFLD.

Table1 baseline characteristics of included subjects according to the follow-up outcome

variables	No NAFLD (n=41033)	NAFLD (n=5660)	*P* value
Men, n (%) †	20799(50.69)	2033(35.92)	<0.001
Age (years) ‡	69.18±7.27	66.93±6.00	<0.001
Current smoking, n (%) †	6846 (16.70)	594(10.51)	<0.001
Exercise, n (%) †	7865(19.22)	1131(20.02)	<0.001
Live alone, n (%) †	8772(21.38)	1015(17.93)	<0.001
Diabetes, n (%) †	5554(13.54)	1344(23.75)	<0.001
Hypertension, n (%) †	15945 (38.86)	2952(52.16)	<0.001
WHtR‡	0.51±0.06	0.54±0.07	<0.001
BMI‡	23.62±2.85	26.02±3.10	<0.001
SBP (mmHg) ‡	132.62±19.38	135.32±19.76	<0.001
DBP (mmHg) ‡	78.92±10.34	80.91±10.33	<0.001
FPG (mmol/L) §	5.20(4.70-5.70)	5.30(4.80-5.93)	<0.001
TC (mmol/L) §	4.59(4.01-5.21)	4.69(4.09-5.38)	<0.001
TG (mmol/L) §	1.10(0.81-1.47)	1.28(0.90-1.77)	<0.001
TB (μmol/L) §	11.51(8.60-14.10)	11.30(8.30-13.80)	<0.001
ALT(U/L) §	18.00(13.00-24.50)	18.60(14.00-25.00)	<0.001
AST(U/L) §	21.80(16.89-26.90)	21.90(16.89-27.00)	0.892
TyG‡	8.43±0.54	8.61±0.59	<0.001

†: n (%); ‡: mean (SD); §: median (IQR)

Abbreviations: BMI, body mass index; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, triglycerides; TG, triglyceride; TB, total bilirubin; ALT, alanine aminotransferase, AST, aspartate aminotransferase; TyG, triglyceride glucose.

2.2 Association between TyG index and NAFLD

The relationship between TyG index and the incidence of NAFLD were showed in **Table 2**. Compared with quartile 1 of TyG index, the hazard ratio (HR) and its corresponding 95% confidence interval (CI) for quartile2, quartile3, and quartile 4 were 1.056(0.971,1.149), 1.300(1.200,1.408) and 1.972(1.832,2.123), respectively. Risk for NAFLD was significantly higher with increasing quartiles of TyG for the *P* value of trend of linearity <0.001. In addition, even after adjusted possible confounding factors, the risk of quartile 3 of TyG index (1.314(1.234,1.457)) on the incidence of NAFLD still existed. In order to further verify the relationship between the TyG index and the

incidence of NAFLD, TyG index was used as continuous various for the above analysis and the results also proved that higher level of TyG increased the risk of NAFLD (1.265(1.200,1.334)), even after adjusted possible confounding factors. These results proved that the baseline level of TyG was associated with the risk of NAFLD, thus the higher the TyG level, the higher incidence of NAFLD.

Since the majority of the subjects could not respond on their history of lipid-lowering therapy or antidiabetic drugs, we failed to access information about therapy. Sensitivity analysis (n=37428) was executed after excluding subjects with diabetes, CVD and stroke, who might take medicine that affects level of FPG and TG. The results of sensitivity analysis (Suppl table 1) also suggested that higher level of TyG significantly increased the incidence of NAFLD, which were similar with the findings in total population.

Table 2 relationship between TyG index and the risk of NAFLD

		TyG index		
	N	unadjusted	Model 1	Model 2
As continuous	50037	1.697(1.619,1.779)	1.577(1.504,1.653)	1.265(1.200,1.334)
Quartile 1	12556	Reference	Reference	Reference
Quartile 2	12458	1.056(0.971,1.149)	1.035 (0.951,1.126)	0.980(0.895,1.073)
Quartile 3	12522	1.300(1.200,1.408)	1.259(1.162,1.364)	1.090(0.999,1.190)
Quartile 4	12501	1.972(1.832,2.123)	1.783(1.655,1.920)	1.314(1.234,1.457)
P for trend		<0.001	<0.001	<0.001

Quartile 1≤8.11; 8.12<Quartile 2≤8.44; 8.45<Quartile 3≤8.78; 8.79<Quartile 4

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol and diabetes.

2.3 The ability of TyG to predict NAFLD

The receiver operating characteristic curves (ROC) were used to evaluate the ability of baseline level of TyG to predict the development of NAFLD. The best cutoff value for TyG index to diagnosis NAFLD was 8.63, and its corresponding area under the receiver operating characteristic curves (AUCs) was 0.60 (95%CI:0.58,0.61), showed in **Figure 2A**. Subgroup analysis was performed to further analyze the influence of sex on ability

of TyG index to predict incident NAFLD, the best cutoff value for men (8.68) was lower in men than women (8.75), and corresponding AUCs was 0.587 (95%CI:0.573-0.600) for men and 0.584 (95%CI:0.573-0.594) for men, respectively(**Figure 2B and 2C**).

Figure 2 ROC curve of TyG on NAFLD

Receiver operating characteristics (ROC) curves for baseline TyG to predict incident NAFLD among 60 years old or older. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases.

2.4 Restricted cubic spline analysis to evaluate the relationship between TyG and NAFLD

In order to further explore the relationship between TyG and the risk of NAFLD, restricted cubic spline graph was used to analyze the dose-response relationship between TyG and the incidence of NAFLD. As shown in **Figure 3A**, there was a nonlinear relationship between level of TyG index and the risk of NAFLD based on the adjusted Cox regression model. This result proved again that higher level of TyG was associated with higher incidence of NAFLD. Subgroup analyses also found that there was a nonlinear relationship between TyG index and incident NAFLD both in men and women (**Figure 3B and C**).

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

Multivariate adjusted hazard ratios of NAFLD increased during follow-up when the baseline level of TyG index was 8.63 and above. Adjusted variables including age, sex (not for sex subgroup analysis), living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol. TyG, triglyceride glucose; NAFLD, nonalcoholic fatty liver diseases; CI, confidence interval.

2.5 Mediation effects

In previous analysis we found that WHtR was associated with the incidence of NAFLD, what's more, WHtR was also associated with TyG index (P<0.05, and the data wasn't

shown). If WHtR would lead to IR and TyG index was a reliable and cheap surrogate indicate for IR, then there might be a mediating effect of TyG index between WHtR and NAFLD. Therefore, mediation analysis was used to confirm whether there was a mediating effect and to what extent TyG index affect the relationship between WHtR and NAFLD. Results (presented in **Table 3 and Figure 4**) of mediation analysis revealed that the total effect of WHtR on NAFLD was 1.476 (1.437,1.517) and the direct effect was 1.463(1.842,1.950). Therefore, TyG index played a partial role and the indirect effect was 1.019 (1.006,1.011). This result may indicate that abdominal obesity may lead to disorders of glycolipid dyslipidemia which can lead to an increase of the TyG index.

Table 3 Mediation analysis of the relationship between TyG index and NAFLD by WHtR

Effect	HR (95%CI)
Total effect	1.476 (1.437,1.517)
Direct effect	1.463 (1.842,1.950)
Indirect effect	1.009(1.006,1.011)

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

The effect of WHtR on NAFLD was mediated by baseline level TyG index by adjusted potential confounders namely age, sex, living alone, current smoking, exercise, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

3. Discussion

With the acceleration of the aging process, the health in the elderly has gradually become an important issue in the society. NAFLD, a common liver disease in China, also has become one of the main health problems of the elderly. Although there were some studies about TyG index and NAFLD, research about TyG index and the incidence of NAFLD in the older adults has not been reported.

In this retrospective cohort study, we found the incidence of NAFLD was 3.80/100 person-years among the elderly over 60, which was lower than a cohort study of Korea

(the incidence of NAFLD was 4.84/100 person-years)[25]. Probably, the age of the subjects in the Korean cohort study(mean age was 36.1) was younger than ours and age could affect the prevalence and severity of NAFLD[26]. Additionally, the incidence of NAFLD decreased as the age increased in this study and the $P_{\text{value for trend}}$ <0.001(data not presented in this research). A retrospective cohort study on TyG index and NAFLD in Japan also found that higher TyG was associated with higher risk of NAFLD, which was consistent with our results[13]. As far as we know, this research is the first study to focus on the effect of TyG index on the incidence of NAFLD in the elderly in China. Our study also reported the relationship between TyG index was nonlinear hence the incidence of NAFLD would increase significantly when TyG is above 8.63. Furthermore, the results of the mediation effect suggested that TyG index was not an independent factor for the development of NAFLD, but a partial mediator between WHtR and NAFLD.

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[27],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[13, 28, 29]; 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. TyG index is an inexpensive and reliable surrogate index for IR and plays an important role in the development and progression of NAFLD. Consequently, even in the absence of diabetes, IR can lead to changes from normal liver to NAFL to NASH[10]. Various studies have confirmed that IR is closely related to the occurrence and development of NAFLD[30-33].

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.

The major strength of this study was a large sample size retrospective cohort study which included 46,693 aged more than 60 years old. it is believed a cohort study could better reflect the real relationship between TyG index and NAFLD. Moreover, we established a nonlinear relationship between TyG index and NAFLD using cubic spline graph analysis, furthermore, TyG index played a mediation effect between WHtR and NAFLD in this study. However, there were several limitations in this study. First, 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[46]. Furthermore, the subjects could not provide their history of lipid-lowering therapy or antidiabetic drugs as they were elder therefore, information about therapy was missing in the data. Additionally, we could not evaluate the relationship between TyG index and different NAFLD severity. Also, we lacked other more accurately index which can reflect the abdominal obesity status. Even though we used WHtR to indicate the abdominal obesity, we were unable to assess a more accurately visceral fat index and the prevalence of NAFLD.

In conclusion, high baseline level of TyG index is significantly associated with a higher risk of NAFLD. In addition, TyG index plays a partial mediating role in the relationship between WHtR and NAFLD. Our results suggest that it has important clinical meanings for monitoring TyG index to prevent NAFLD.

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Author Contributions:

H.C. and Q.L. designed the study. H.C., S.L., B.Y., X.C., Z.S., W.G and J.H. participated in the data collection and analysis. H.C. and A.A. drafted this manuscript, H.C. S.L. and S.S. interpreted the data. S.S and Q.L. reviewed and revised this manuscript. All authors approved the final manuscript.

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Conflict of Interest:

All authors have no conflicts of interest.

Patient and public involvement: Participants were not involved in the recruitment and conduct of the study

Patient consent for publication: Given the retrospective nature of the research, the requirement for informed consent was waived. Patients were not involved in the recruitment and conduct of the study. The study protocol was approved by ethics committee of Zhengzhou University in China.

Ethics approval:

The study protocol was approved by ethics committee of Zhengzhou University in China (approval number: ZZURIB202004). Given the retrospective nature of the research, the requirement for informed consent was waived.

Provenance and peer review: Not commissioned; externally peer reviewed

Data availability statement: The datasets generated and/or analyzed during the current study are available upon request.

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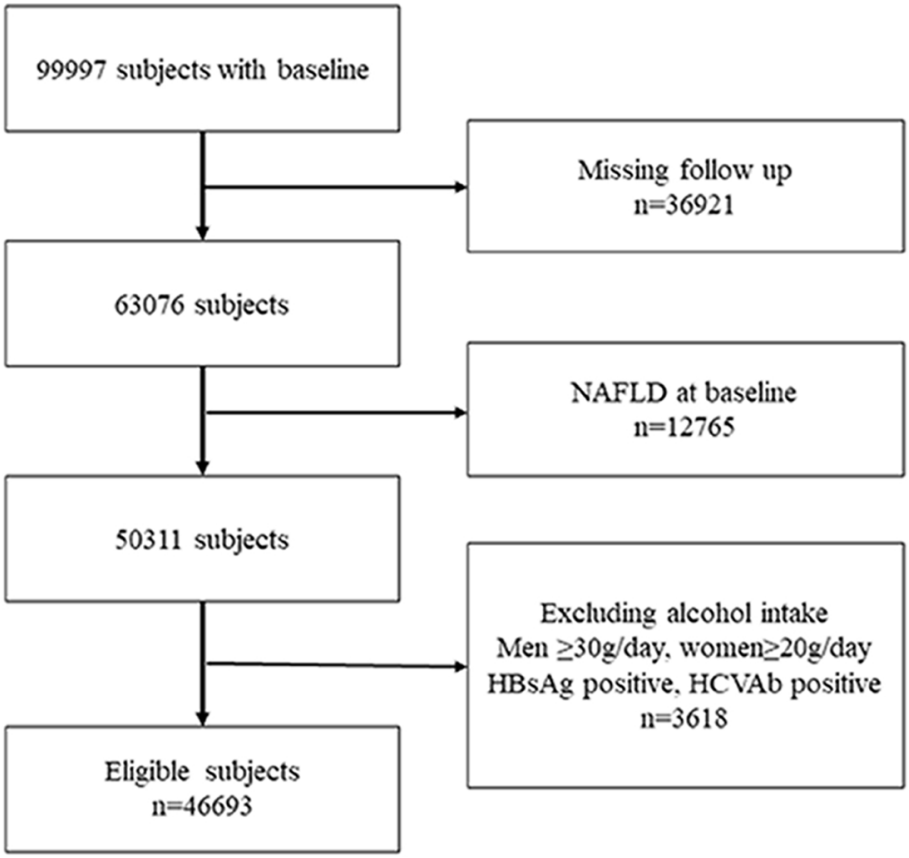

Figure 1 Flow chart for participants exclusions performed

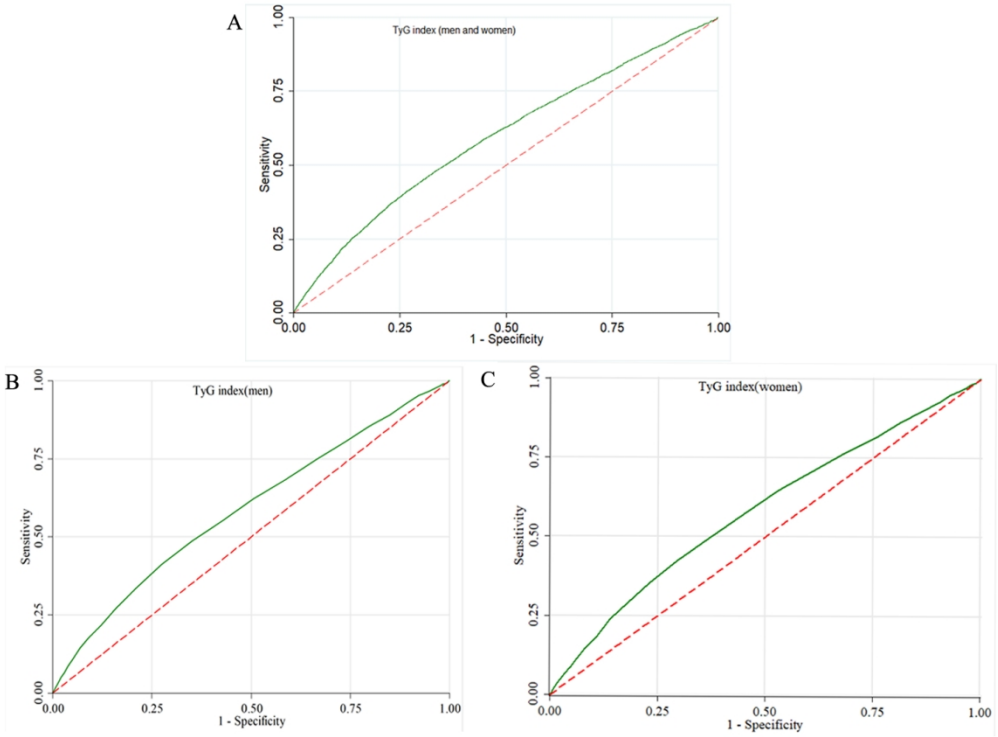

Figure 2 ROC curve of TyG on NAFLD

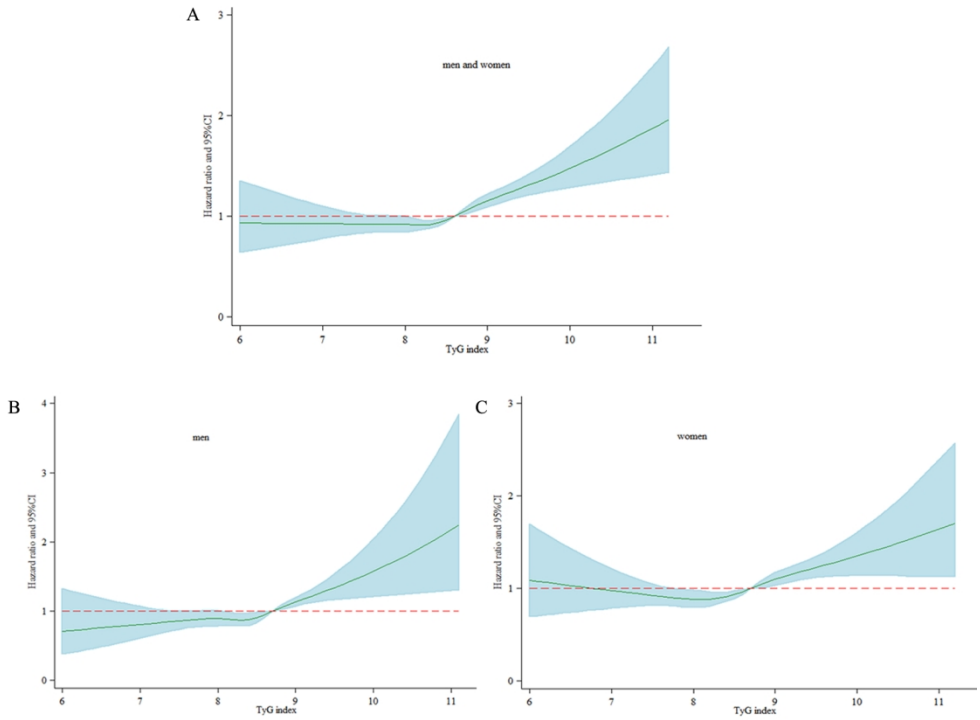

Figure 3 Restricted cubic spline graph of risk of TyG index and the development of NAFLD.

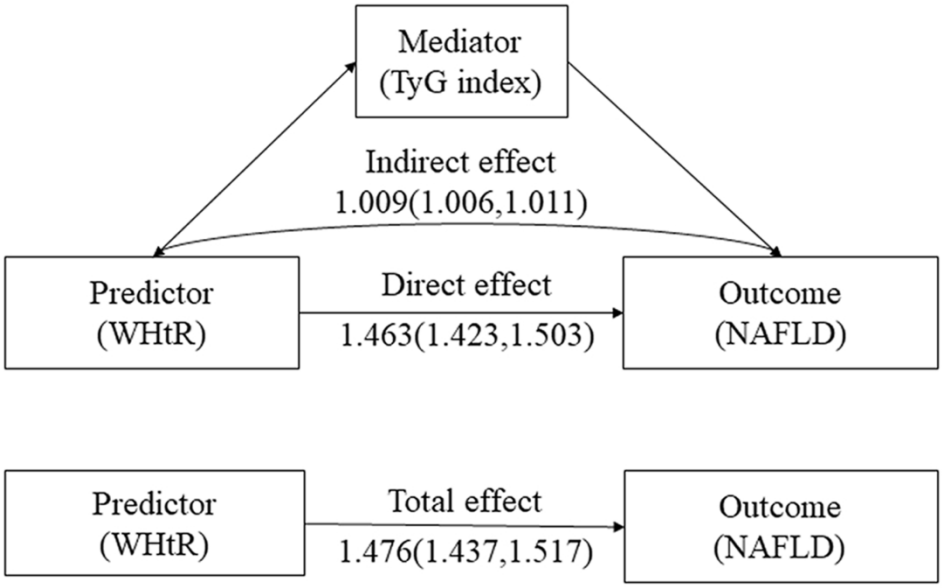

Figure 4 The mediate effect of TyG index on WHtR and NAFLD.

Supplementary Table 1 relationship between TyG index and the risk of NAFLD excluded subjects with diabetes, CVD and stroke

		TyG index		
	n	unadjusted	Model 1	Model 2
As continuous	37428	1.687(1.589,1.792)	1.571(1.480,1.668)	1.342(1.257,1.433)
Quartile 1	9536	Reference	Reference	Reference
Quartile 2	9368	1.036(0.938,1.144)	1.019 (0.923,1.125)	0.986(0.887,1.097)
Quartile 3	9350	1.239(1.127,1.362)	1.209(1.099,1.330)	1.076(0.970,1.193)
Quartile 4	9354	1.846(1.691,2.014)	1.681(1.540,1.836)	1.389(1.261,1.530)
P for trend		<0.001	<0.001	<0.001

Quartile 1≤8.08; 8.09<Quartile 2≤8.40; 8.41<Quartile 3≤8.72; 8.73< Quartile 4

Model 1: adjusted age and sex;

Model 2: model 1 plus living alone, current smoking, exercise, waist-to-height ratio, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total cholesterol.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4,5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,5
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			6,7,8,9
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6,7
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7-10 7,8 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12,13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10,11,12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.