# ADVANCES IN RESEARCH OF THE CARDIOVASCULAR DISEASE CONTINUUM: ENDOCRINE ASPECTS OF DISEASE PATHOPHYSIOLOGY, RISK PREDICTORS, THERAPEUTICS, AND MANAGEMENT OF DIABETES AND HYPERTENSION

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# ADVANCES IN RESEARCH OF THE CARDIOVASCULAR DISEASE CONTINUUM: ENDOCRINE ASPECTS OF DISEASE PATHOPHYSIOLOGY, RISK PREDICTORS, THERAPEUTICS, AND MANAGEMENT OF DIABETES AND HYPERTENSION

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# Myocardial Infarction and Coronary Artery Disease in Menopausal Women With Type 2 Diabetes Mellitus Negatively Correlate With Total Serum Bile Acids

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**Background:** As metabolic molecules, bile acids (BAs) not only promote the absorption of fat-soluble nutrients, but they also regulate many metabolic processes, including the homeostasis of glucose and lipids. Although total serum BA (TBA) measurement is a readily available clinical test related to coronary artery disease (CAD), myocardial infarction (MI), and type 2 diabetes mellitus (T2DM), the relationship between TBA and these pathological conditions remain unclear, and research on this topic is inconclusive.

**Methods:** This study enrolled 20,255 menopausal women aged over 50 years, including 6,421 T2DM patients. The study population was divided into different groups according to the median TBA level in order to explore the clinical characteristics of menopausal women with different TBA levels. Spline analyses, generalized additive model (GAM) model and regression analyses based on TBA level were used to explore the relationship between TBA and different diseases independently, including CAD and MI, or in combination with T2DM.

**Results:** Both in the general population and in the T2DM subgroup, the TBA level was significantly lower in CAD patients than in non-CAD patients. Spline analyses indicated that within normal clinical range of TBA concentration (0–10 μmol/L), the presence of CAD and MI showed similar trends in total and T2DM population. Similarly, the GAM model indicated that within the 0–10 μmol/L clinical range, the predicted probability for CAD and MI alone and in combination with T2DM was negatively correlated with TBA concentration. Multivariate regression analysis suggested that low TBA level was positively associated with the occurrence of CAD combined with T2DM (OR: 1.451; 95%CI: 1.141–1.847).

**Conclusions:** In menopausal women, TBA may represent a valuable clinical serum marker with negative correlation for CAD and MI in patients with T2DM.

**Keywords: total serum bile acids, myocardial infarction, coronary artery disease, menopausal women, type 2 diabetes mellitus**

## BACKGROUND

Bile acids (BAs) are endogenous metabolites synthesized from cholesterols in the liver and can be modified by intestinal microbes. Since they are important metabolic and signal transducers in the body, they may play an important role in regulating lipid and carbohydrate metabolism, as well as in shaping the composition of intestinal microbiota (1). Previous studies have emphasized potentially harmful effects of BAs in cardiovascular diseases (CVDs). For example, elevated level of BAs may have a toxic effect on the heart, and secondary BAs may promote arrhythmia (2). Recent studies have reported an important link between BAs in cholesterol metabolism and autophagic activity, suggesting that BAs may be closely related to atherosclerosis (3). Coronary artery disease (CAD) may involve general metabolic disorders. During CAD progression, phospholipid catabolism and tricarboxylic acid cycle decrease, amino acid metabolism and short-chain carnitine increase, and primary BA biosynthesis decreases. Therefore, serum metabonomics, which describes metabolic disorders, is very valuable. Differences in small molecule metabolites may reflect underlying CAD and serve as biomarkers during CAD progression (4).

From a global perspective, CVDs affect nearly 30% of patients with the type 2 diabetes mellitus (T2DM). For the past 20 years, CVDs have remained the main cause of morbidity and mortality in patients with T2DM (5, 6). In these patients, metabolomics also provides unique snapshots of biochemical changes, and may reflect unique metabolic signatures under different pathophysiological conditions. Thus, broad-spectrum metabolic changes may emphasize complex abnormalities in complications associated with elevated level of blood glucose. Hence, measuring metabolite biomarkers in blood has become a new strategy to stratify patients with T2DM complications (7). Interestingly, BAs play certain roles in glucose homeostasis, energy expenditure, and weight control, through receptor-dependent and -independent mechanisms, thereby regulating and maintaining the metabolism of lipids, glucose, and other energy sources, as well as protecting the heart from inflammation, and preventing T2DM and obesity (8–10). When BA metabolism is altered, imbalanced in lipids, glucose, and energy metabolism may lead to inflammatory metabolic diseases, including T2DM, non-alcoholic fatty liver, and CVDs (11). Moreover, serum total bile acid (TBA) is a candidate marker to predict the risk of T2DM. Previous studies have found that changes in TBA levels precede the occurrence of T2DM, supporting the potential role of TBA metabolism in T2DM pathogenesis (12). However, the specificity of TBA involvement in different populations and its exact link with the disease are still unclear.

To date, in patients with T2DM combined with CAD and myocardial infarction (MI), the correlation between TBA and disease, or the influence of TBA changes on the predicted probability of disease have not been confirmed. Besides these questions, considering potential gender differences in TBA and specific metabolic changes in menopausal women, this study aimed to explore the relationship between TBA and MI or CAD, combined or not with T2DM, based on clinical data in the population of menopausal women. The relationship between TBA and these diseases might provide helpful reference for clinicians.

**Abbreviations:** BAs, Bile acids; CVDs, cardiovascular diseases; CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; TBA, total bile acid; MI, myocardial infarction; LVEF, left ventricular ejection fraction; Cr, creatinine; ALT, alanine amino transferase; AST, aspartate amino transferase; γ-GGT, gamma-glutamyl transpeptidase; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TBIL, total bilirubin; DBIL, direct bilirubin; ALB, albumin; hs-TNI, hypersensitive troponin I; BNP, brain natriuretic peptide; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin A1c; RCS, restricted cube splines; GAM, generalized additive model; OR, odds ratios; CI, confidence intervals; DBP, diastolic blood pressure; BMI, body mass index; SBP, systolic blood pressure; HT, hypertension; eGFR, estimated glomerular filtration rate; ARB, angiotensin receptor blockers; ACEI, angiotensin converting enzyme inhibitor; β-blockers, beta blockers; CTA, computed tomography angiography; IR, insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; FXR, farnesoid X receptor; TGR5, Takeda G-protein receptor 5; GLP-1, glycogen-like peptide 1.

## METHODS

### Patient Cohort and Data Collection

This study included 29,553 female patients who had undergone coronary angiography between 2016 and 2019, from the electronic database of 99,300 patients in Anzhen Hospital. According to the collected medical history and the confirmation of menstrual and childbearing history, the patients were divided into different groups as follows: menopausal women aged 50 years or more (n = 28,039) and women under 50 years or non-menopausal women (n = 1,514). Then, the data were screened through the exclusion criteria, which were as follows: cardiac insufficiency (left ventricular ejection fraction [LVEF < 50%] or a history of cardiac insufficiency, n = 893); abnormal renal function (creatinine [Cr] higher than the normal range in women from 41 to 81umol/L according to Central Laboratory of Beijing Anzhen Hospital or a history of abnormal renal function, n = 1,005); and abnormal liver function (abnormal level of alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyl transpeptidase [γ-GGT], or a previous history of liver function abnormalities, n = 2,603). Moreover, patients with incomplete clinical data (n = 3,282) were excluded (**Figure 1**). After joint confirmation of the coronary angiography results by three experienced interventional surgeons, any coronary artery presenting with lesions narrowing more than 50% was defined as CAD. Patients with CAD were diagnosed as MI based on medical history and related examinations. For the diagnosis of MI, patients had to meet at least two of the following criteria: (1) typical chest pain; (2) elevated serum cardiac biomarkers including hypersensitive troponin I (hs-TNI), myohemoglobin and creatine kinase isoenzyme, but mainly according to hs-TNI; and (3) typical features of MI on the electrocardiogram (13).

### Laboratory Analysis

After admission, after overnight fasting, the venous blood of the participants was collected. Levels of AST, γ-GGT, albumin (ALB), triglyceride (TG), serum uric acid (SUA), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C),

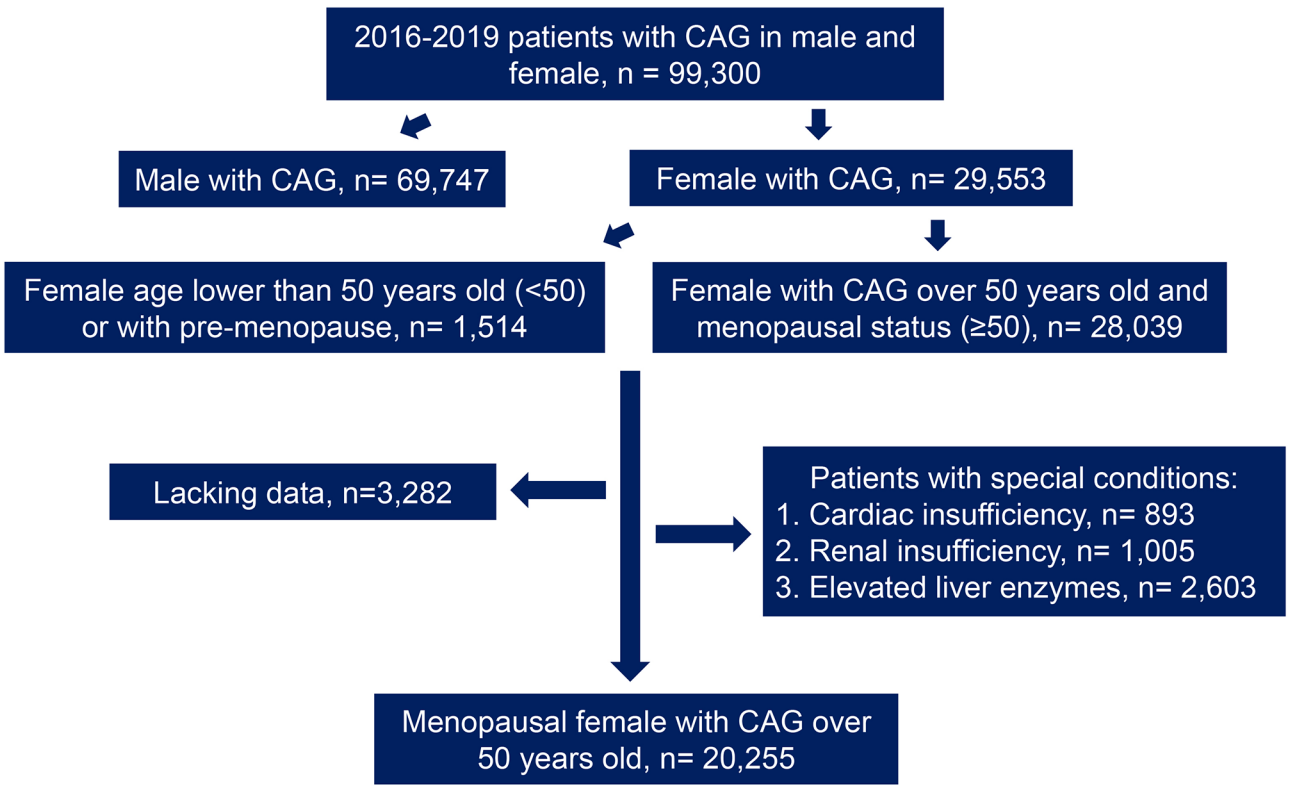


**FIGURE 1** | Flow chart. CAG, coronary angiography.

and high-density lipoprotein cholesterol (HDL-C) were tested using colorimetric method; Cr was detected by Jaffe's assay; fasting blood glucose (FBG) was measured by hexose kinase method; fasting insulin and brain natriuretic peptide (BNP) were tested using chemiluminescence; ALT was tested using International Federation of Clinical Chemistry method; and total bilirubin (TBIL)/direct bilirubin (DBIL) was test using diazo method. All the abovementioned tests used in-vitro diagnostic assay kits (Roche Diagnostics, Mannheim, Germany). In addition, ion exchange high-performance liquid chromatography (HPLC) method was used to test the level of HbA1c. TBA was determined using TBA assay kit by enzyme cycle (Maccura, Chengdu, China). hs-TNI was tested by chemiluminescence using Access hs-TNI (Beckman, Minnesota, USA).

## Statistical Analysis

Data were summarized as mean standard deviation (SD), median (interquartile range), and percentage, as appropriate for continuous and categorical variables. Differences between groups were calculated using one-way analysis of variance or the Kruskal-Wallis test for continuous variables, and the chi-square test for categorical variables representing counts (percentages). Correlations between TBA and lipids characteristics were assessed using the Spearman correlation test. We evaluated the association between the level of TBA and odds ratios (ORs) of diseases with restricted cube splines (RCS), using the package of 'RMS' in R for the shape visualization. Besides, the generalized additive model (GAM) was applied using the package of 'VAGM' in R to represent the smoothing splines for disease risk prediction of changing levels of TBA (14). ORs with 95% confidence intervals (CIs) were used to present results of the logistic regression including univariate regression and multivariate regression, after adjusting the traditional influential factors, such as age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-C and medical history of T2DM, hypertension (HT), hyperlipidemia, smoking, and familial CVD. All of the analyses were performed in R software (version 4.0.0).

# RESULTS

## Baseline Clinical Characteristics

In the entire study population, the CAD group had significantly higher age, BMI, and SBP, and more frequent medical history of HT, hyperlipidemia, smoking, family CVD, and T2DMthan the non-CAD group, while DBP and alcohol history were similar between these two groups. In the T2DM subgroup, only age, SBP, medical history of hyperlipidemia, smoking history, and family CVD were significantly higher in the CAD group than in the non-CAD group. In terms of lipid composition, both in the general and in the T2DM population, the TC, HDL-C, and LDL-C levels were significantly lower in the CAD group than in the

non-CAD group ($P < 0.05$). As for the levels of LDL-C and TC, we assumed that the lower levels in the CAD group were influenced by statins because the use of statin therapy in the CAD group was significantly more common. In contrast, the TG level was significantly higher in the CAD group than in the non-CAD group ($P < 0.05$). In the total population and in the T2DM subgroup, ALT and AST levels were significantly lower in the non-CAD group than in the CAD group, whereas TBIL was significantly higher in the CAD group. In terms of cardiac indicators, the level of BNP was significantly higher in the CAD group than in the non-CAD group, but there was no significant difference in TNI between the two groups. In terms of renal function, in the total population, the Cr level was significantly higher, and the estimated glomerular filtration rate (eGFR) was significantly lower in the CAD group than in the non-CAD group. Similarly, in the T2DM subgroup, the Cr level was significantly higher in the CAD group, but there was no significant difference in eGFR ($P = 0.145$) between the two groups. Remarkably, the TBA levels in the non-CAD group were significantly higher than those in the CAD group (median 3.70 μmol/L [IQR: 1.90-13.00 μmol/L] *vs.* 3.50 μmol/L [IQR: 1.80-11.00 μmol/L]; $p < 0.001$). Likewise, in the T2DM subgroup, the TBA levels in the non-CAD group were significantly higher than those in the CAD group. In terms of medications, drug use frequency in the CAD group of the total population was significantly higher than that of the non-CAD group. In the T2DM subgroup, there was no significant difference in the usage of angiotensin receptor blockers/angiotensin converting enzyme inhibitor (ARB/ACEI) between the two groups, while the frequency of usage about other drugs was significantly higher in the CAD group (**Table 1** and **Table 2**).

## Characteristics of the Groups With Different TBA Levels

Based on the median level of TBA, three groups with gradual increase in TBA level, including low, medium, and high, were formed. In the overall population, medical history of hyperlipidemia, BNP, FBG and HbA1c level showed a gradually increasing trend mirroring the increase in TBA level in the three groups. However, medical history of familial CVD, CAD, and MI and the levels of TC, LDL-C, ALT, DBIL, and ALB showed a gradually decreasing trend. In the T2DM subgroup, medical history of HT or hyperlipidemia, and BNP level showed a gradually increasing trend, whereas medical history of family CVD, CAD, and MI and the levels of TC, DBIL, and ALB showed a gradually decreasing trend. In terms of medications, the proportions of individuals using aspirin, clopidogrel, nitrates, and beta blockers (β-blockers) in both the total and the T2DM populations declined gradually, whereas ARB/ACEI usage in the total population rose gradually (**Table 3** and **Table 4**).

## Relationship Between TBA and Lipids

Next, we explored the relationship between TBA and lipid composition in blood among the subgroups with different median TBA levels. In the CAD population (median TBA = 3.5 μmol/L), the TC level correlated negatively with low-level TBA ($P < 0.05$) and positively with high-level TBA ($P < 0.05$) (**Figure 2A**). The LDL-C level also correlated negatively with low TBA level ($P = 0.008$), and positively with high TBA level ($P < 0.001$) (**Figure 2B**). In contrary, the TG level positively correlated with both low- and high-level TBA ($P < 0.001$), while HDL-C negatively correlated with both low- and high-level TBA ($P < 0.001$). In patients with CAD combined with T2DM (median TBA = 3.5 μmol/L), the HDL-C level negatively correlated with low-level TBA ($P = 0.007$), while the LDL-C, TC, and TG levels positively correlated with high-level TBA ($P < 0.05$). In the MI population (median TBA = 3.5 μmol/L), the TG level positively correlated with low and high-level TBA ($P < 0.05$) (**Figure 2C**). The HDL-C level negatively correlated with low- and high-level TBA ($P < 0.001$) (**Figure 2D**). There was no significant correlation between TBA and lipid composition in patients with MI combined with T2DM (median TBA = 3.4 μmol/L). In the T2DM subgroup (median TBA = 3.8 μmol/L), the LDL-C and TC levels correlated positively with high-level TBA ($P < 0.001$). TG correlated positively with low- and high-level TBA ($P < 0.05$) (**Figure 2E**), while HDL-C correlated negatively with low- and high-level TBA ($P < 0.05$) (**Figure 2F**).

## Relationship Between TBA and Disease Entities

We used the spline analysis, with three as number of selected nodes, and after adjusted for typical influencing factors (including age, BMI, SBP, DBP and medical history of HT, hyperlipidemia, smoking, and drinking). We showed that as the level of TBA increased in CAD, MI, and CAD or MI combined with T2DM subgroups, the corresponding OR values showed an 'L'-shaped trend curve (**Figure 3**). Furthermore, at low TBA levels, the OR values of TBA for CAD were all greater than 1. Within the normal clinical range of TBA concentrations (0–10 μmol/L), the OR value decreased as the level of TBA increased. Higher levels of TBA corresponded to OR values for CAD that showed a horizontal or slightly upward trend (**Figures 3A, B, D**) in the subgroups CAD, and CAD or MI combined with T2DM, and a large increase in the MI subgroup (**Figure 3C**). As for T2DM, in both the total and CAD populations, the OR value first showed a slight increase at first, with the increase of TBA level and then a decreasing trend (**Figures 4A, C**). In the MI subgroup, as the TBA level increased, the OR value showed a downward trend, but without reaching any significance (**Figure 4E**).

To discuss the GAM of binary variables, we used CAD, MI, T2DM, and CAD or MI combined with T2DM as the outcome variables and the TBA concentration as the predictor. The results showed that when TBA decreased, the disease state changed (**Figures 4** and **5**). The predicted probability for MI changed the most in the T2DM population, with a decrease of about 5%. However, when TBA level exceeded the normal clinical range, the characteristics of the disease varied greatly between the different populations. In the CAD population, for a TBA level higher than 10 μmol/L, the predicted probability of the disease would probably reach a peak again at 30 μmol/L (**Figure 5A**), while in the CAD population combined with T2DM, above 30 μmol/L, it is likely that the predicted probability curve conserved

**TABLE 1** | Baseline characteristics in overall population.

| Characteristics | All (n=20,255) | non-CAD (7,616) | CAD (12,639) | P value |
|---|---|---|---|---|
| **Age, year** | 64.00 [59.00-70.00] | 63.00 [58.00-68.00] | 65.00 [60.00-70.00] | <0.001 |
| **BMI, kg/m$^2$** | 25.51 ± 4.88 | 25.36 ± 3.57 | 25.61 ± 5.52 | 0.012 |
| **SBP, mmHg** | 132.51 ± 16.97 | 130.77 ± 16.48 | 133.55 ± 17.18 | <0.001 |
| **DBP, mmHg** | 75.21 ± 10.39 | 75.40 ± 10.26 | 75.09 ± 10.46 | 0.152 |
| **Smoking** | 1,123 (5.5) | 326 (4.3) | 797 (6.3) | <0.001 |
| **Drinking** | 293 (1.4) | 117 (1.5) | 176 (1.4) | 0.442 |
| **Medical history, n (%)** | | | | |
| **HT** | 13,577 (67.0) | 4,484 (58.9) | 9,093 (71.9) | <0.001 |
| **Hyperlipidemia** | 11,439 (56.5) | 4,127 (54.2) | 7,312 (57.9) | <0.001 |
| **Family CVD** | 1,593 (7.9) | 527 (6.9) | 1,066 (8.4) | <0.001 |
| **T2DM** | 6,421 (31.7) | 1,720 (22.6) | 4,701 (37.2) | <0.001 |
| **Laboratory test** | | | | |
| **TC, mmol/L** | 4.23 [3.63-4.95] | 4.39 [3.76-5.09] | 4.14 [3.57-4.86] | <0.001 |
| **TG, mmol/L** | 1.35 [0.99-1.86] | 1.30 [0.96-1.79] | 1.38 [1.01-1.90] | <0.001 |
| **HDL-C, mmol/L** | 1.19 [1.03-1.39] | 1.23 [1.06-1.43] | 1.17 [1.02-1.36] | <0.001 |
| **LDL -C, mmol/L** | 2.40 [1.89-3.04] | 2.54 [1.98-3.17] | 2.32 [1.84-2.92] | <0.001 |
| **ALT, U/L** | 15.00 [9.00-21.00] | 15.00 [9.00-20.00] | 15.00 [9.60-21.00] | <0.001 |
| **AST, U/L** | 18.00 [12.00-23.00] | 18.00 [10.00-22.00] | 19.00 [13.00-23.00] | <0.001 |
| **TBIL, μmol/L** | 23.00 [11.8-42.90] | 36.70 [12.30-43.50] | 19.90 [11.50-42.60] | <0.001 |
| **DBIL, μmol/L** | 2.04 [1.61-2.75] | 2.03 [1.60-2.76] | 2.04 [1.61-2.74] | 0.797 |
| **γ-GGT, U/L** | 18.00 [8.13-18.60] | 17.00 [5.73-25.00] | 18.00 [10.00-26.00] | <0.001 |
| **ALB, g/L** | 40.80 [35.6-43.90] | 40.60 [33.00-43.90] | 40.90 [36.20-43.90] | 0.002 |
| **TBA, μmol/L** | 3.60 [1.80-12.00] | 3.70 [1.90-13.00] | 3.50 [1.80-11.00] | <0.001 |
| **hs-TNI, pg/mL** | 0.01 [0.00-7.90] | 0.01 [0.00-8.90] | 0.01 [0.00-7.13] | 0.076 |
| **BNP, pg/mL** | 55.00 [35.00-71.10] | 54.70 [35.00-69.35] | 55.10 [35.70-72.00] | 0.025 |
| **FBG, mmol/L** | 6.18 [5.20-9.50] | 5.95 [5.13-9.03] | 6.37 [5.24-9.80] | <0.001 |
| **HbA1c, %** | 6.20 [5.70-7.10] | 6.00 [5.60-6.60] | 6.30 [5.80-7.30] | <0.001 |
| **eGFR, mL/min/1.73m$^2$** | 91.64 ± 11.15 | 92.55 ± 11.01 | 91.11 ± 11.21 | <0.001 |
| **Cr, μmol/L** | 44.70 ± 25.59 | 43.47 ± 25.73 | 45.45 ± 25.47 | <0.001 |
| **LVEF%** | 64.79 ± 5.09 | 64.86 ± 5.02 | 64.75 ± 5.13 | 0.216 |
| **Medical treatment, n (%)** | | | | |
| **Aspirin** | 17,344 (85.8) | 5,130 (67.4) | 12,214 (96.6) | <0.001 |
| **P2Y12 inhibitors** | 13,030 (64.4) | 1,869 (24.5) | 11,161 (88.3) | <0.001 |
| **Statins** | 16,863 (83.3) | 5,309 (69.7) | 11,554 (91.4) | <0.001 |
| **Nitrate** | 8,078 (40.0) | 1,773 (23.3) | 6,305 (49.9) | <0.001 |
| **β-blockers** | 10,673 (52.8) | 3,115 (40.9) | 7,558 (61.4) | <0.001 |
| **Insulin** | 1,532 (7.6) | 342 (4.5) | 1,190 (9.4) | <0.001 |
| **ARB/ACEI** | 3,722 (18.4) | 1,177 (15.5) | 2,545 (20.1) | <0.001 |

*CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate amino transferase; TBIL, total bilirubin; DBIL, direct bilirubin; γ-GGT, gamma-glutamyl transpeptidase; ALB, albumin; TBA, total bile acid; hs-TNI, hypersensitive troponin I; BNP, brain natriuretic peptide; HbA1c, glycosylated hemoglobin A1c; eGFR, estimate glomerular filtration rate; Cr, creatinine; LVEF, left ventricular ejection fraction; β-blockers, beta-blockers; ARB, angiotensin receptor blockers; ACEI, angiotensin converting enzyme inhibitors.*

a U-shape (**Figure 5B**). In the MI population, the predicted probability for MI reached a peak when the TBA level reached 50–60 μmol/L (**Figure 5C**). In the MI combined with T2DM population, when the TBA level reached about 40 μmol/L, the predicted probability for MI in the T2DM population reached a peak again (**Figure 5D**). In addition, in the total and the CAD populations, the predicted probability for T2DM was substantially different from the variations observed for CVDs. At low TBA levels (approximately TBA < 5 μmol/L), the predicted probability of T2DM presented an upward trend (**Figures 4B, D**). At high concentrations of TBA (> 10 μmol/L), the predicted probability for T2DM first presented a decreasing trend and then an increasing trend. In the MI population, the predicted probability for T2DM did not vary significantly at extremely low TBA levels. When considering the range 5–15 μmol/L, the predicted probability for T2DM showed a downward trend, which transformed into an increasing trend when reaching the range of 20–35 μmol/L. At high levels of TBA (> 35 μmol/L), the predicted probability for T2DM gradually decreased (**Figure 4F**).

## Regression Analyses

Taking medium-level TBA as a reference, the univariate regression analysis indicated that in the total population, low-level TBA may be related to CAD and MI, while high-level TBA may be a protective factor against MI and T2DM. After adjusting for confounding factors, the multivariate regression analysis indicated that low-level TBA was independently associated with CAD. In contrast, high levels of TBA may be a protective factor against T2DM. Further regression analysis in the T2DM subgroup showed that higher levels of TBA may be protective against MI. After adjusting for confounding factors, including age, BMI, SBP, DBP, LDL-C and medical history of HT, hyperlipidemia, smoking, and familial CVD, the multivariate regression analysis suggested that low-level TBA was

**TABLE 2** | Baseline characteristics in T2DM subgroup.

| Characteristics | All (n=6,421) | non-CAD (n=1,720) | CAD (n=4,701) | P value |
|---|---|---|---|---|
| **Age, year** | 65.51 [61.00-70.00] | 65.00 [60.00-70.00] | 66.00 [61.00-71.00] | 0.001 |
| **BMI, kg/m$^2$** | 25.89 ± 3.43 | 26.06 ± 3.63 | 25.82 ± 3.35 | 0.091 |
| **SBP, mmHg** | 133.97 ± 16.86 | 132.48 ± 15.84 | 134.52 ± 17.19 | 0.002 |
| **DBP, mmHg** | 74.03 ± 10.05 | 74.40 ± 9.69 | 73.89 ± 10.18 | 0.205 |
| **Smoking** | 344 (5.4) | 74 (4.3) | 270 (5.7) | 0.027 |
| **Drinking** | 71 (1.1) | 15 (0.9) | 56 (1.2) | 0.343 |
| **Medical history, n (%)** | | | | |
| **HT** | 5,028 (78.3) | 1,322 (76.9) | 3,706 (78.8) | 0.096 |
| **Hyperlipidemia** | 3,807 (59.3) | 1,063 (61.8) | 2,744 (58.4) | 0.014 |
| **Family CVD** | 513 (8.0) | 116 (6.7) | 397 (8.4) | 0.030 |
| **Laboratory test** | | | | |
| **TC, mmol/L** | 4.04 [3.48-4.78] | 4.11 [3.55-4.85] | 4.02 [3.46-4.75] | 0.002 |
| **TG, mmol/L** | 1.40 [1.02-1.96] | 1.36 [1.01-1.88] | 1.42 [1.03-1.98] | 0.016 |
| **HDL-C, mmol/L** | 1.63 [0.98-1.31] | 1.16 [1.00-1.34] | 1.12 [0.97-1.29] | <0.001 |
| **LDL -C, mmol/L** | 2.39 [1.80-2.89] | 2.30 [1.85-2.98] | 2.27 [1.79-2.85] | 0.005 |
| **ALT, U/L** | 15.00 [10.00-21.00] | 15.00 [9.00-21.00] | 15.00 [10.00-22.00] | 0.001 |
| **AST, U/L** | 16.62 [12.00-22.00] | 17.00 [10.00-22.00] | 18.00 [13.00-22.00] | 0.001 |
| **TBIL, μmol/L** | 26.48 [11.20-42.70] | 36.50 [11.60-43.90] | 17.90 [11.10-42.30] | <0.001 |
| **DBIL, μmol/L** | 2.04 [1.60-2.73] | 2.03 [1.60-2.72] | 2.04 [1.60-2.73] | 0.729 |
| **γ-GGT, U/L** | 19.00 [11.00-27.00] | 18.00 [5.62-26.00] | 19.00 [12.00-27.00] | 0.003 |
| **ALB, g/L** | 40.70 [36.00-43.80] | 40.60 [33.65-43.70] | 40.70 [36.30-43.80] | 0.264 |
| **TBA, μmol/L** | 3.60 [1.60-11.00] | 3.90 [2.10-13.00] | 3.50 [1.90-10.50] | <0.001 |
| **hs-TNI, pg/mL** | 0.01 [0.00-7.00] | 0.01 [0.00-8.60] | 0.01 [0.00-5.90] | 0.222 |
| **BNP, pg/mL** | 55.45 [35.25-73.00] | 54.50 [33.00-69.00] | 55.80 [36.00-74.00] | 0.008 |
| **FBG, mmol/L** | 8.69 [6.58-12.90] | 8.36 [6.45-13.00] | 8.79 [6.62-12.87] | 0.150 |
| **HbA1c, %** | 7.40 [6.70-8.50] | 7.20 [6.50-8.20] | 7.50 [6.70-8.60] | <0.001 |
| **eGFR, mL/min/1.73m2** | 90.72 ± 11.58 | 91.22 ± 11.39 | 90.54 ± 11.65 | 0.152 |
| **Cr, μmol/L** | 45.84 ± 25.72 | 44.02 ± 26.23 | 46.51 ± 25.51 | 0.001 |
| **LVEF, %** | 64.58 ± 5.15 | 65.03 ± 4.90 | 64.43 ± 5.22 | 0.003 |
| **Medical treatment, n (%)** | | | | |
| **Aspirin** | 5,891 (91.7) | 1,344 (78.1) | 4,547 (96.7) | <0.001 |
| **P2Y12 inhibitors** | 4,763 (74.2) | 540 (31.4) | 4,223 (89.8) | <0.001 |
| **Statins** | 5,693 (88.7) | 1,386 (80.6) | 4,307 (91.6) | <0.001 |
| **Nitrate** | 3,009 (46.9) | 557 (32.4) | 2,452 (52.2) | <0.001 |
| **β blockers** | 3,936 (61.3) | 870 (50.6) | 3,066 (65.2) | <0.001 |
| **Insulin** | 1,496 (23.3) | 327 (19.0) | 1,169 (24.9) | <0.001 |
| **ARB/ACEI** | 1,439 (22.4) | 361 (21.0) | 1,078 (22.9) | 0.105 |

*T2DM, type 2 diabetes mellitus; CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate amino transferase; TBIL, total bilirubin; DBIL, direct bilirubin; γ-GGT, gamma-glutamyl transpeptidase; ALB, albumin; TBA, total bile acid; hs-TNI, hypersensitive troponin I; BNP, brain natriuretic peptide; HbA1c, glycosylated hemoglobin A1c; eGFR, estimate glomerular filtration rate; Cr, creatinine; LVEF, left ventricular ejection fraction; β-blockers, beta-blockers; ARB, angiotensin receptor blockers; ACEI, angiotensin converting enzyme inhibitors.*

independently associated with T2DM combined with CAD. In addition, in the CAD and MI subgroups, high levels of TBA may be protective against T2DM (**Table 5**).

# DISCUSSION

This study is the first to explore the relationship between TBA level and CAD, MI, and CAD or MI combined with T2DM in the population of menopausal women. First, this study revealed that both in the total sample and in the T2DM subgroup, TBA was significantly lower in patients with CAD. Under grouping according to the median TBA level, the percentages of CAD and MI showed a decreasing trend. Second, spline analysis indicated that with the increase of TBA level within the normal clinical range, the OR values of CAD, MI, and CAD or MI combined with T2DM showed a downward trend. Subsequently, in the GAM model and within the normal clinical range, the predicted probability for CAD, MI, and CAD or MI combined with T2DM showed a downward trend with the increase in TBA level. The logistic regression model demonstrated that low-level TBA was independently related to CAD and CAD combined with T2DM and that high-level TBA may be protective against MI and MI combined with T2DM.

## TBA and CVD

### BAs in Lipid and Glucose Metabolism

Since BAs are only synthetized by liver cells, they can be considered as the only quantitative markers reflecting the mechanisms of cholesterol decomposition and metabolism (1). Previous studies have indicated that BA functions go beyond the sole regulation of lipid digestion and cholesterol metabolism. BAs are considered signal molecules that interact with plasma membranes and nuclear receptors. These interactions regulate the synthesis of BAs and homeostasis of energy production, as well as other important physiological processes (15). Several studies have

**TABLE 3 |** Characteristics of patients in different TBA levels.

| Characteristics | <3.6 μmol/L (n=10,069) | 3.6~10 μmol/L (n=4731) | >10 μmol/L (n=5455) | P value |
|---|---|---|---|---|
| **Age, year** | 64.00 [59.00-69.00] | 65.00 [60.00-71.00] | 65.00 [60.00-70.00] | <0.001 |
| **BMI, kg/m$^2$** | 25.52 ± 3.43 | 25.25 ± 3.41 | 25.61 ± 6.01 | 0.031 |
| **SBP, mmHg** | 132.96 ± 17.38 | 131.92 ± 16.49 | 132.44 ± 16.88 | 0.094 |
| **DBP, mmHg** | 75.38 ± 10.43 | 73.89 ± 10.10 | 75.59 ± 10.42 | <0.001 |
| **Smoking** | 530 (5.3) | 273 (5.8) | 320 (5.9) | 0.217 |
| **Drinking** | 106 (1.1) | 57 (1.2) | 130 (2.4) | <0.001 |
| **Medical history, n (%)** | | | | |
| **HT** | 6,748 (67.0) | 3,165 (66.9) | 3,664 (67.2) | 0.959 |
| **Hyperlipidemia** | 4,977 (49.4) | 2,437 (51.5) | 4,025 (73.8) | <0.001 |
| **Family CVD** | 895 (8.9) | 353 (7.5) | 345 (6.3) | <0.001 |
| **T2DM** | 3,153 (31.3) | 1,588 (33.6) | 1,680 (30.8) | 0.006 |
| **Laboratory test** | | | | |
| **TC, mmol/L** | 4.27 [3.66-4.99] | 4.21 [3.61-4.95] | 4.19 [3.58-4.90] | <0.001 |
| **TG, mmol/L** | 1.34 [0.99-1.84] | 1.37 [1.00-1.88] | 1.36 [1.00-1.88] | 0.014 |
| **HDL-C, mmol/L** | 1.20 [1.04-1.40] | 1.17 [1.02-1.36] | 1.19 [1.03-1.39] | <0.001 |
| **LDL -C, mmol/L** | 2.42 [1.91-3.06] | 2.39 [1.89-3.04] | 2.36 [1.84-3.00] | <0.001 |
| **ALT, U/L** | 17.00 [13.00-23.00] | 16.00 [12.00-23.00] | 6.30 [5.80-7.70] | <0.001 |
| **AST, U/L** | 20.00 [17.00-24.00] | 20.00 [17.00-24.00] | 1.46 [0.62-4.80] | <0.001 |
| **TBIL, μmol/L** | 12.30 [10.10-15.40] | 12.20 [9.80-16.20] | 42.80 [40.10-45.60] | <0.001 |
| **DBIL, μmol/L** | 2.37 [1.85-3.03] | 2.25 [1.72-2.92] | 1.62 [1.44-1.83] | <0.001 |
| **γ-GGT, U/L** | 21.00 [16.00-28.00] | 21.00 [16.00-28.00] | 2.92 [2.13-4.12] | <0.001 |
| **ALB, g/L** | 42.50 [40.00-44.90] | 41.40 [39.20-43.80] | 22.00 [18.00-29.00] | <0.001 |
| **TBA, μmol/L** | 1.80 [1.20-2.60] | 5.20 [4.20-6.70] | 20.00 [15.00-28.00] | <0.001 |
| **hs-TNI, pg/mL** | 0.00 [0.00-0.01] | 0.00 [0.00-0.08] | 11.18 [8.44-14.40] | <0.001 |
| **BNP, pg/mL** | 42.00 [22.00-82.00] | 50.00 [26.00-88.00] | 58.80 [51.90-66.85] | <0.001 |
| **FBG, mmol/L** | 5.99 [5.24-8.40] | 6.51 [5.39-9.76] | 6.71 [4.10-14.93] | <0.001 |
| **HbA1c, %** | 6.10 [5.70-7.00] | 6.20 [5.80-7.10] | 6.30 [5.64-7.68] | <0.001 |
| **eGFR, mL/min/1.73m2** | 92.25 ± 11.05 | 91.26 ± 10.95 | 91.42 ± 11.28 | 0.001 |
| **Cr, μmol/L** | 58.18 ± 10.64 | 56.75 ± 15.34 | 9.40 ± 17.60 | <0.001 |
| **LVEF%** | 65.06 ± 5.16 | 64.77 ± 5.15 | 64.14 ± 4.80 | <0.001 |
| **Medical treatment, n (%)** | | | | |
| **Aspirin** | 8,793 (87.3) | 3,975 (84.0) | 4,576 (83.9) | <0.001 |
| **P2Y12 inhibitors** | 6,865 (68.2) | 3,115 (65.8) | 3,050 (55.9) | <0.001 |
| **Statins** | 8,462 (84.0) | 3,856 (81.5) | 4,545 (83.3) | 0.001 |
| **Nitrate** | 4119 (40.9) | 1864 (39.4) | 2095 (38.4) | 0.007 |
| **β-blockers** | 5,565 (55.3) | 2,533 (53.5) | 2,775 (50.9) | <0.001 |
| **Insulin** | 740 (7.3) | 368 (7.8) | 424 (7.8) | 0.518 |
| **ARB/ACEI** | 1,641 (16.3) | 811 (17.1) | 1,270 (23.3) | <0.001 |
| **Disease entity, n (%)** | | | | |
| **MI** | 991 (9.8) | 456 (9.6) | 507 (9.3) | 0.544 |
| **CAD** | 6,419 (63.8) | 2,931 (62.0) | 3,289 (60.3) | <0.001 |

*CAD, coronary artery disease; TBA, total bile acid; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate amino transferase; TBIL, total bilirubin; DBIL, direct bilirubin; γ-GGT, gamma-glutamyl transpeptidase; ALB, albumin; hs-TNI, hypersensitive troponin I; BNP, brain natriuretic peptide; HbA1c, glycosylated hemoglobin A1c; eGFR, estimate glomerular filtration rate; Cr, creatinine; LVEF, left ventricular ejection fraction; β-blockers, beta-blockers; ARB, angiotensin receptor blockers; ACEI, angiotensin converting enzyme inhibitors.*

established the important role of BAs in regulating cholesterol and TG metabolism. Moreover, the relationship between BAs and blood lipids may be bidirectional, that is, not only do BAs affect lipid and glucose metabolism or obesity, but TG metabolism also influences BA synthesis (16, 17). Interestingly, our study further supported this model for serum TBA in menopausal women. The relationship between different levels of TBA and different lipid types was variable. TC was negatively associated with low-level TBA in the CAD and CAD combined with T2DM populations, while high levels of TBA and LDL-C positively correlated in the CAD, MI, and CAD combined with T2DM populations. In addition, TG positively correlated with low- and high-level TBA in the CAD and MI populations, and high-level TBA positively correlated with TG in the CAD combined with T2DM subgroup. The relationship between other lipid components, including TC and HDL-C, and TBA may also depend on anabolic interactions between BAs and lipids to some extent.

## Relationship Between TBA and CAD Progression

The exact relationship between the circulating TBA levels and CAD is still unclear. Some previous reports have ruled out that any significant correlation between CAD and TBA level (17). However, in recent years, an increasing number of studies on the relationship between CAD and BAs have indicated that a connection may exist. In a study based on coronary computed tomography angiography (CTA), a higher circulating TBA level was found to be an independent predictor of coronary plaque instability. The authors proposed that high TBA levels may be

**TABLE 4** | Characteristics of patients with T2DM in different TBA levels.

| Characteristics | <3.8 μmol/L (n=3,282) | 3.8~10 μmol/L (n=1,459) | >10 μmol/L (n=1,680) | P value |
|---|---|---|---|---|
| **Age, year** | 65.00 [60.00-70.00] | 67.00 [62.00-72.00] | 66.00 [61.00-70.00] | <0.001 |
| **BMI, kg/m$^2$** | 25.77 ± 3.46 | 25.88 ± 3.34 | 25.97 ± 3.44 | 0.356 |
| **SBP, mmHg** | 134.70 ± 17.31 | 133.12 ± 16.25 | 133.83 ± 16.80 | 0.162 |
| **DBP, mmHg** | 74.32 ± 9.95 | 72.17 ± 9.49 | 74.57 ± 10.25 | <0.001 |
| **Smoking** | 166 (5.1) | 88 (6.0) | 90 (5.4) | 0.389 |
| **Drinking** | 32 (1.0) | 13 (0.9) | 26 (1.5) | 0.127 |
| **Medical history, n (%)** | | | | |
| **HT** | 2561 (78.0) | 1139 (78.1) | 1328 (79.0) | 0.691 |
| **Hyperlipidemia** | 1689 (51.5) | 820 (56.2) | 1298 (77.3) | <0.001 |
| **Family CVD** | 292 (8.9) | 123 (8.4) | 98 (5.8) | 0.001 |
| **Laboratory test** | | | | |
| **TC, mmol/L** | 4.07 [3.52-4.78] | 4.06 [3.50-4.84] | 3.98 [3.38-4.71] | 0.001 |
| **TG, mmol/L** | 1.37 [1.02-1.93] | 1.46 [1.03-1.99] | 1.43 [1.03-1.99] | 0.018 |
| **HDL-C, mmol/L** | 1.14 [0.99-1.33] | 1.11 [0.98-1.29] | 1.13 [0.98-1.31] | 0.016 |
| **LDL -C, mmol/L** | 2.29 [1.83-2.89] | 2.29 [1.81-2.95] | 2.23 [1.76-2.82] | 0.001 |
| **ALT, U/L** | 17.00 [13.00-23.00] | 17.00 [12.00-24.00] | 7.70 [6.80-9.20] | <0.001 |
| **AST, U/L** | 19.00 [16.00-23.00] | 19.00 [16.00-24.00] | 1.67 [0.71-5.48] | <0.001 |
| **TBIL, μmol/L** | 11.90 [9.80-14.70] | 11.54 [9.43-15.10] | 42.70 [39.90-45.60] | <0.001 |
| **DBIL, μmol/L** | 2.34 [1.84-2.98] | 2.23 [1.72-2.88] | 1.60 [1.43-1.81] | <0.001 |
| **γ-GGT, U/L** | 22.00 [17.00-29.00] | 22.00 [17.00-30.00] | 2.88 [2.12-4.11] | <0.001 |
| **ALB, g/L** | 42.20 [39.70-44.80] | 41.40 [39.10-43.80] | 21.00 [17.00-28.00] | <0.001 |
| **TBA, μmol/L** | 2.00 [1.30-2.70] | 5.20 [4.30-6.70] | 21.00 [15.28-28.00] | <0.001 |
| **hs-TNI, pg/mL** | 0.00 [0.00-0.02] | 0.00 [0.00-0.03] | 10.99 [8.27-14.04] | <0.001 |
| **BNP, pg/mL** | 44.00 [22.00-87.00] | 50.00 [28.00-93.00] | 59.30 [52.20-67.85] | <0.001 |
| **FBG, mmol/L** | 8.47 [6.74-11.84] | 9.27 [7.02-13.16] | 8.36 [4.70-18.00] | <0.001 |
| **HbA1c, %** | 7.40 [6.70-8.50] | 7.40 [6.70-8.40] | 7.60 [6.60-8.80] | 0.846 |
| **eGFR, mL/min/1.73m2** | 91.44 ± 11.75 | 90.25 ± 11.18 | 90.44 ± 11.61 | 0.017 |
| **Cr, μmol/L** | 58.70 ± 11.50 | 58.35 ± 14.40 | 9.92 ± 18.32 | <0.001 |
| **LVEF, %** | 64.84 ± 5.30 | 64.52 ± 5.17 | 64.05 ± 4.71 | <0.001 |
| **Medical treatment, n (%)** | | | | |
| **Aspirin** | 3030 (92.3) | 1334 (91.4) | 1527 (90.9) | 0.198 |
| **P2Y12 inhibitors** | 2556 (77.9) | 1109 (76.0) | 1098 (65.4) | <0.001 |
| **Statins** | 2913 (88.8) | 1287 (88.2) | 1493 (88.9) | 0.820 |
| **nitrate** | 1570 (47.8) | 675 (46.3) | 764 (45.5) | 0.252 |
| **β blockers** | 2050 (62.5) | 907 (62.2) | 979 (58.3) | 0.012 |
| **insulin** | 751 (22.9) | 328 (22.5) | 417 (24.8) | 0.218 |
| **ARB/ACEI** | 677 (20.6) | 291 (19.9) | 471 (28.0) | <0.001 |
| **Disease entity, n (%)** | | | | |
| **MI** | 419 (12.8) | 176 (12.1) | 187 (11.1) | 0.246 |
| **CAD** | 2456 (74.8) | 1059 (72.6) | 1186 (70.6) | 0.005 |

*CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; TBA, total bile acid; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate amino transferase; TBIL, total bilirubin; DBIL, direct bilirubin; γ-GGT, gamma-glutamyl transpeptidase; ALB, albumin; hs-TNI, hypersensitive troponin I; BNP, brain natriuretic peptide; HbA1c, glycosylated hemoglobin A1c; eGFR, estimate glomerular filtration rate; Cr, creatinine; LVEF, left ventricular ejection fraction; β-blockers, beta-blockers; ARB, angiotensin receptor blockers; ACEI, angiotensin converting enzyme inhibitors.*

associated with the severity of coronary artery stenosis and risk coronary artery plaque detected by CTA (18). In another study on the role of TBA in the progression of CAD in the overall population, also using spline analysis, the presence and severity of TBA and CAD showed an L-shaped relationship, and the breakpoint was close to the upper limit of the normal TBA level (10 μmol/L). That study showed that low TBA concentration is independently and significantly related to the presence and severity of CAD, especially in presence of MI (13). In our study, both in the general population of menopausal women and in the T2DM subgroup, the average level of TBA in the CAD patients was significantly lower compared with non-CAD group. In the spline analysis, there were also certain similarities. First, within the normal clinical range (0–10 μmol/L), in menopausal women, low-level TBA is related with CAD and MI alone, or combined with T2DM. The multivariate regression analysis confirmed that low-level TBA might indeed be independently related with CAD and CAD combined with T2DM.

## Relationship Between BA Excretion and Prognosis

As the main organic solute in bile, BAs are largely absorbed at the distal end of the small intestine, and then return to the liver where they are finally excreted, maintaining a continuous circulation of BAs between the liver and the intestine (19). A previous study found that BA excretion was significantly reduced in the CAD patients, which to a certain extent, supports the idea that CAD patients may have less BAs than non-CAD patients. Failure to effectively excrete BAs may be an independent risk factor for CAD (20). In addition, in a long-term follow-up study of BA excretion, stroke incidence, and mortality, the decrease in BAs and

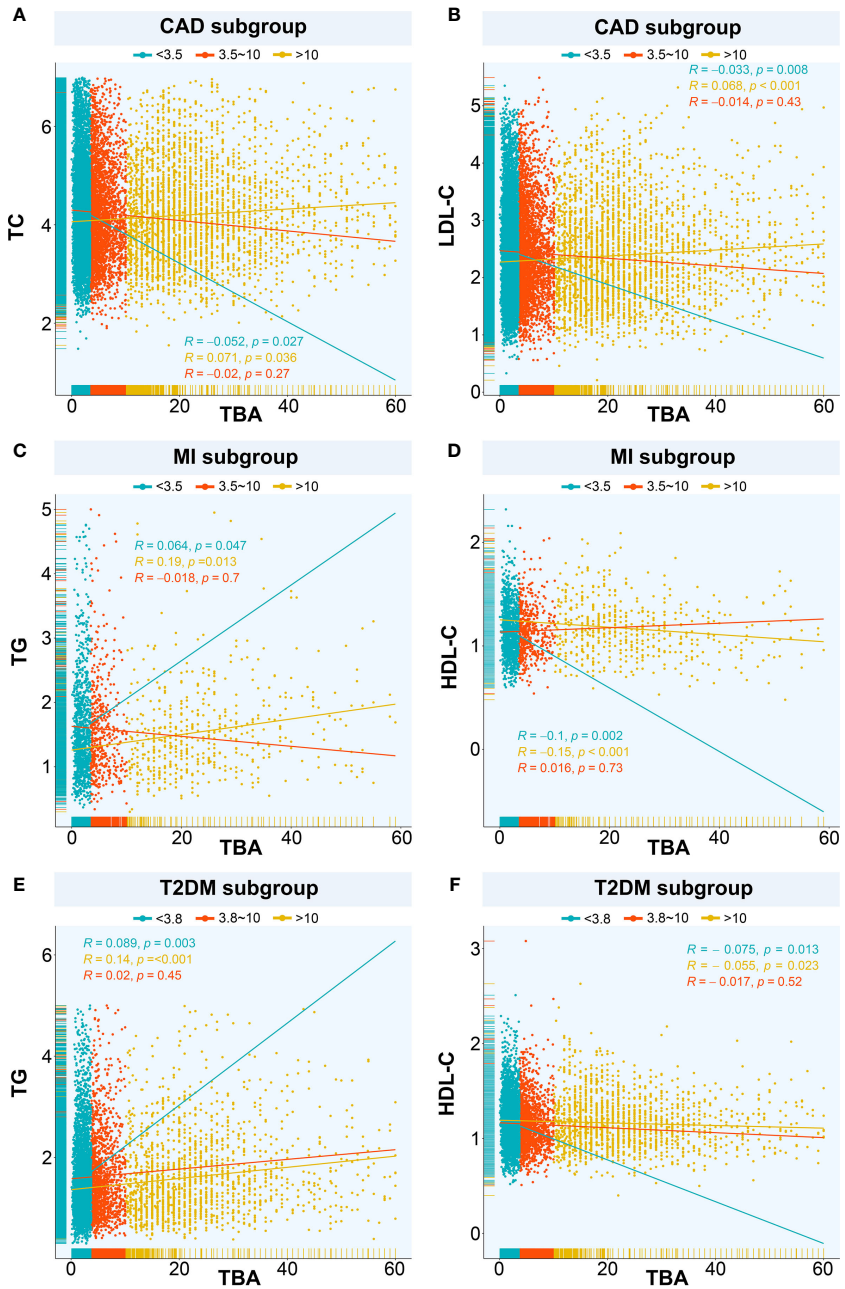


**FIGURE 2** | Relationships between TBA and lipids. The relationship between TBA and lipid composition in blood among the subgroups with different median TBA levels including **(A)** TBA and TC in CAD subgroup, **(B)** TBA and LDL-C in CAD subgroup, **(C)** TBA and TG in MI subgroup, **(D)** TBA and HDL-C in MI subgroup, **(E)** TBA and TG in T2DM subgroup, **(F)** TBA and HDL-C in T2DM subgroup. TBA, total bile acid; CAD, coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction; T2DM, type 2 diabetes mellitus.

secondary BA excretion was related to the risk of stroke, which pointed towards an association between atherosclerosis and BA excretion as an independent risk factor for cerebrovascular disease (21). The excretion of BAs as an indicator of CAD prognosis does not have quantitative standard references, and the prognostic value of TBA level for CAD has remained unclear. Therefore, we used abundant clinical data to establish a smooth spline based on GAM. For the first time in a population of menopausal women, we uncovered the predicted probabilities for different diseases based on the changes in serum TBA levels. Within the normal clinical range (0–10 μmol/L), as the level of TBA increased, the predicted probabilities of CAD, MI, and T2DM combined with these two diseases all showed a downward trend. This was largely consistent with the results obtained by the spline analysis, but at

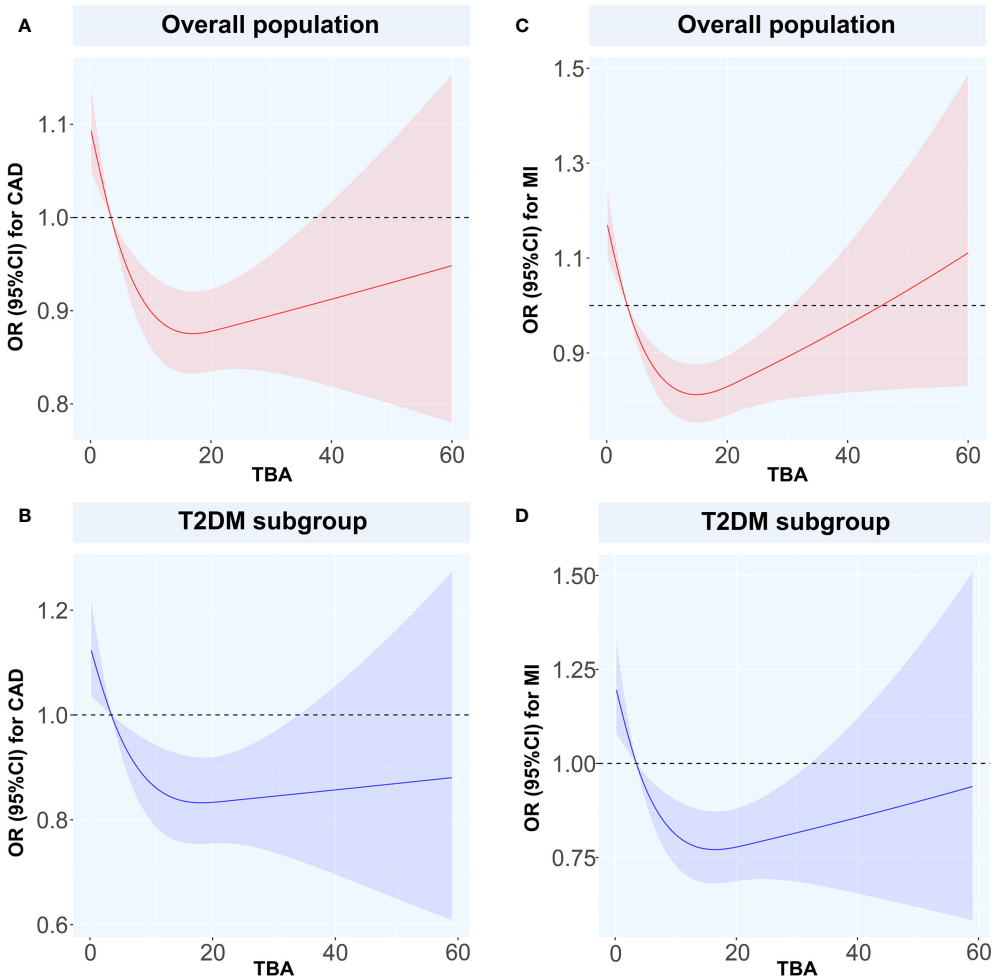


**FIGURE 3** | Restricted spline curves for the associations between TBA and MI or CAD Solid red and blue lines represent the odds ratio, and red and blue dashed area represent the 95% confidence intervals. **(A)** the relationship between TBA and CAD in overall population, **(B)** the relationship between TBA and CAD in T2DM subgroup, **(C)** the relationship between TBA and MI in overall population, **(D)** the relationship between TBA and MI in T2DM subgroup. TBA, total bile acid; CAD, coronary artery disease; MI, myocardial infarction; T2DM, type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval.

high-level TBA (> 10 μmol/L), as the level of TBA increased, the predicted probability of disease occurrence presented variable trends. This result underlines the need for further research in exploring the underlying mechanisms.

## Various Connections Between BAs and T2DM Combined or Not With CVD

T2DM and CVDs are common clinical comorbidities, and T2DM is a recognized risk factor for CAD. Although TBA is used as a CAD marker and closely relates to metabolism, its exact relationship with T2DM and whether it affects T2DM, and thereby further affects CAD, are not clear.

### Metabolic Characteristics of BAs Under T2DM Status

high-The abnormal composition of BAs in T2DM patients indicates that there might be an interaction between BA signal and insulin secretion capacity. In this regard, previous studies have found that fasting plasma TBA composition can be used as an indicator to predict and evaluate the progression and prognosis of T2DM (22). Moreover, changes in the circulating TBA levels are related to the pathogenesis of insulin resistance (IR) and T2DM and changing the composition of TBA may provide effective treatments for T2DM (23). A human study analyzing the changes in BA levels has revealed that compared with healthy controls, the concentration of one secondary BA, namely deoxycholic acid, was increased in T2DM patients, whereas the concentration of total BAs decreased (24). In another study, after adjusting for age and gender, the increase in the concentration of three 12α-hydroxylated secondary BA-cholic acids was associated with the occurrence of T2DM (25). Through Pearson's test, we found that both fasting insulin level and homeostasis model assessment of IR (HOMA-IR) significantly positively correlated with serum total bile acid ($P<0.05$) only in the CAD population (**Supplementary Table**). Previous studies have found that IR plays an important role in regulating BA metabolism,

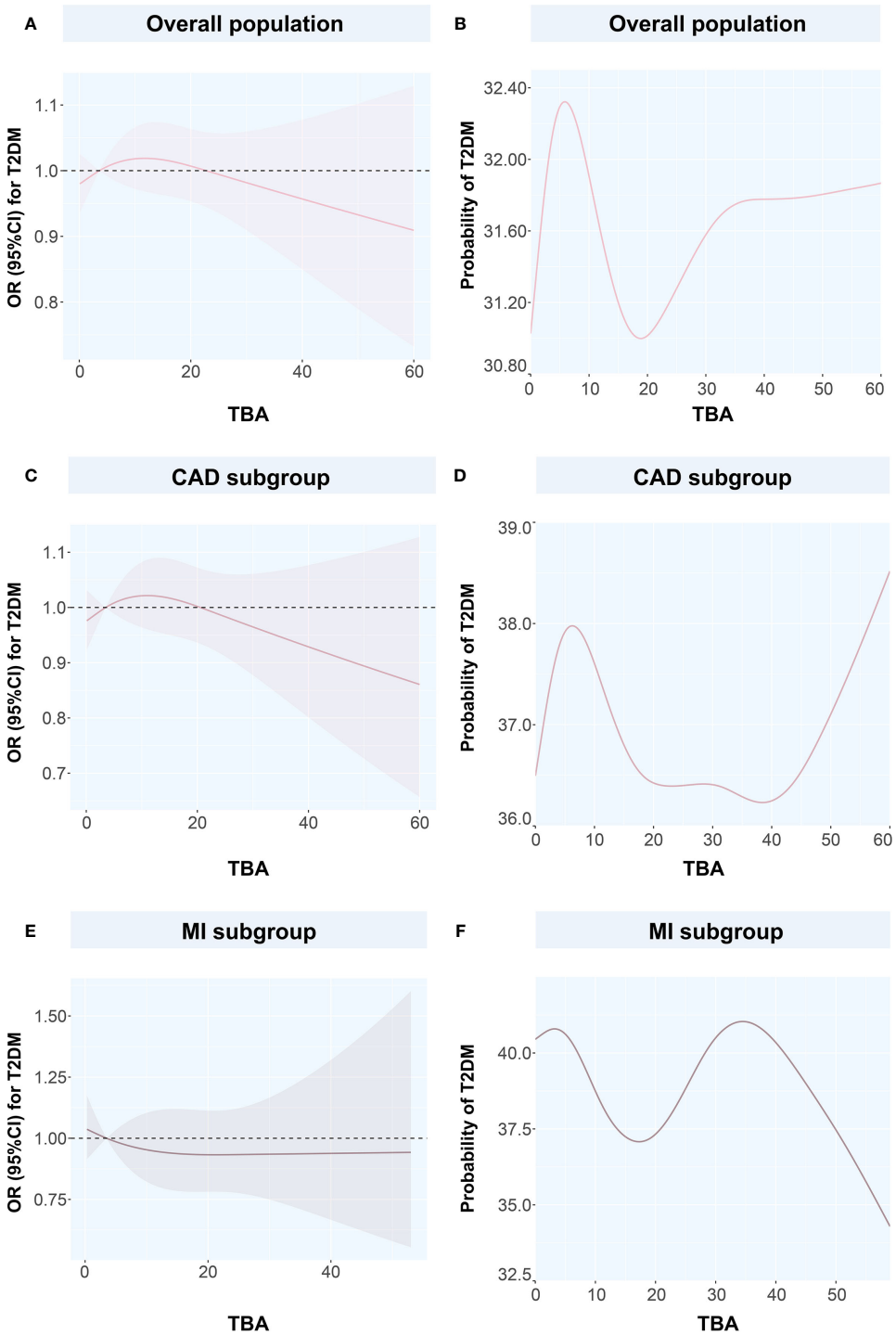


**FIGURE 4** | Restricted spline curves and generalized additive models for the associations between TBA and T2DM **(A)** the relationship between TBA and T2DM in overall population, **(B)** the relationship between TBA and probability of T2DM in overall population, **(C)** the relationship between TBA and T2DM in CAD subgroup, **(D)** the relationship between TBA and probability of T2DM in CAD subgroup, **(E)** the relationship between TBA and T2DM in MI subgroup, **(F)** the relationship between TBA and probability of T2DM in MI subgroup. Solid pink lines represent the odds ratio in **(A, C, E)**, while represent the probability of T2DM in **(B, D, F)**. Pink dashed area represents the 95% confidence intervals in **(A, C, E)**. TBA, total bile acid; T2DM, type 2 diabetes mellitus; CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

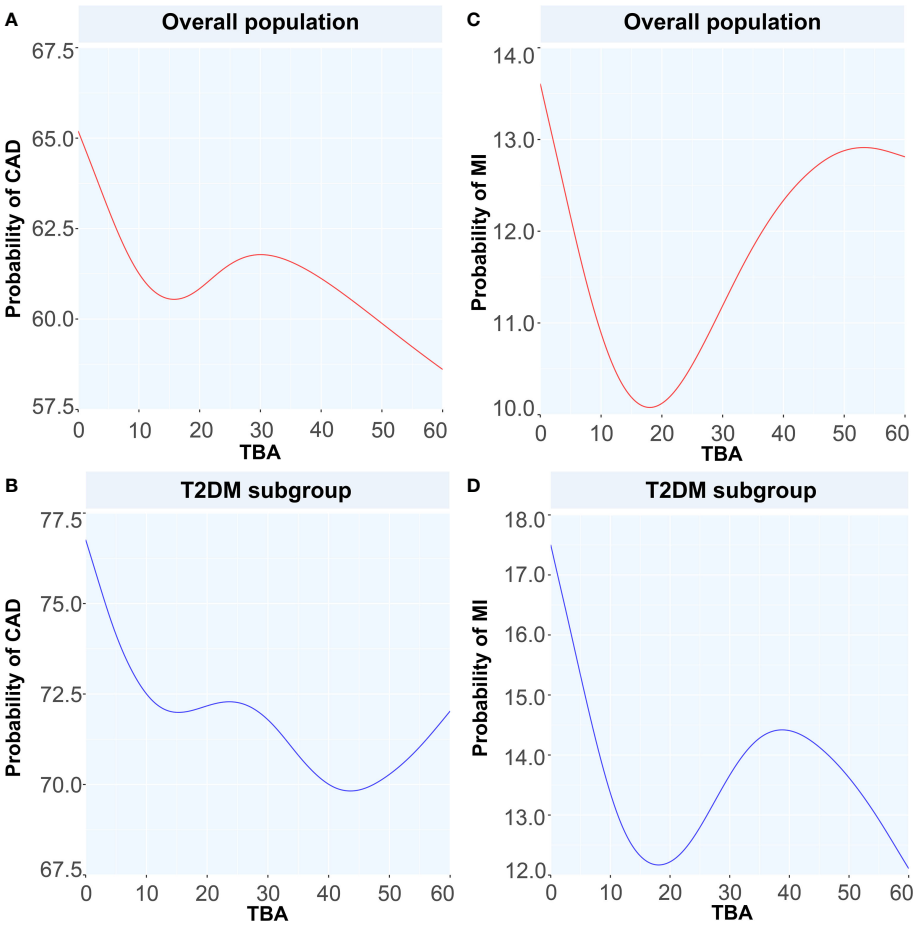


**FIGURE 5** | Generalized additive models for the associations between TBA and the probability of CAD and MI. Red lines represent the probability of diseases in overall population while blue lines represent the probability of diseases in T2DM subgroup. **(A)** the relationship between TBA and probability of CAD in overall population, **(B)** the relationship between TBA and probability of CAD in T2DM subgroup, **(C)** the relationship between TBA and probability of MI in overall population, **(D)** the relationship between TBA and probability of MI in T2DM subgroup. TBA, total bile acid; CAD, coronary artery disease; MI, myocardial infarction; T2DM, type 2 diabetes mellitus.

which was not related to diabetes status (26). In addition, it has been suggested that baseline TBA levels are positively associated with T2DM risk and longitudinal changes in glucose metabolism; meanwhile, IR may partially mediate the correlation between TBA and T2DM (27). Considering the influence of obesity, which is also a risk factor for CAD, Bishay et al. have suggested that IR is closely related to incommensurate alterations to the composition of BA pool, and they have emphasized that increased BAs in IR, rather than obesity possibly contribute to the defects in insulin signaling (28). In addition, although the spline analysis failed to describe a clear relationship between TBA variation and T2DM status in menopausal women, the smooth spline analysis based on GAM indicated the potential value of TBA level to predict the probability for T2DM in the CAD and MI populations. The predicted probability for T2DM showed similar profiles in the total and CAD populations, with an initial rising trend followed by a fall according to different TBA ranges.

In addition, compared with healthy individuals, the level and composition of TBA are variable in T2DM patients, further highlighting that BA metabolism may be involved in the pathogenesis of metabolic diseases (29). In our study, in both the total sample and the T2DM subgroup of menopausal women, within different ranges of TBA level, the predicted probability for CAD varied in trend and amplitude (about 3%) with similar patterns but became inconsistent at high levels of TBA. In contrast, in the MI subgroup, TBA varied within the different ranges, and the predicted probability was about 2% lower for MI than for MI combined with T2DM. Therefore, we speculate that when T2DM is combined with CAD and MI, the body may undergo additional metabolic changes, including those in TBA and other molecules, which may affect the pathogenesis of CAD and MI combined with T2DM.

## BA Receptors and Glucose Metabolism

Since the discovery of BA receptors, the importance of BAs as signaling molecules that regulate physiological functions has been attracting research attention. In the past 20 years, a large number of studies have revealed new functions of BAs as

**TABLE 5 |** Odds ratios of CAD, MI, T2DM and CAD/MI combined with T2DM in relation to the TBA levels.

| | TBA levels μmol/L | Unadjusted OR (95% CI) | P value | Adjusted OR (95% CI) | P value |
|---|---|---|---|---|---|
| **All population** | | | | | |
| **CAD** | <3.6 | 1.078 [1.002-1.161] | 0.045 | 1.352 [1.192-1.534] | <0.001 |
| | 3.6~10 | ref | | | |
| | >10 | 0.939 [0.833-1.058] | 0.302 | 0.932 [0.801-1.084] | 0.360 |
| **MI** | <3.6 | 1.066 [0.944-1.202] | 0.302 | 1.173 [0.945-1.455] | 0.148 |
| | 3.6~10 | ref | | ref | |
| | >10 | 0.794 [0.650-0.970] | 0.024 | 0.798 [0.614-1.038] | 0.093 |
| **T2DM** | <3.6 | 0.915 [0.848-0.988] | 0.023 | 0.998 [0.876-1.137] | 0.973 |
| | 3.6~10 | ref | | ref | |
| | >10 | 0.824 [0.727-0.935] | 0.003 | 0.849 [0.723-0.995] | 0.044 |
| **Patients with T2DM** | | | | | |
| **CAD** | <3.6 | 1.135 [0.986-1.307] | 0.077 | 1.451 [1.141-1.847] | 0.002 |
| | 3.6~10 | ref | | ref | |
| | >10 | 0.972 [0.771-1.226] | 0.811 | 1.005 [0.753-1.341] | 0.972 |
| **MI** | <3.6 | 1.128 [0.933-1.364] | 0.213 | 1.102 [0.773-1.570] | 0.591 |
| | 3.6~10 | ref | | ref | |
| | >10 | 0.679 [0.487-0.947] | 0.022 | 0.798 [0.517-1.232] | 0.309 |
| **Patients with CAD** | | | | | |
| **MI** | <3.5 | 1.027 [0.907-1.161] | 0.677 | 1.072 [0.81-1.336] | 0.534 |
| | 3.5~10 | ref | | ref | |
| | >10 | 0.796 [0.646-0.981] | 0.032 | 0.825 [0.630-1.082] | 0.825 |
| **T2DM** | <3.6 | 0.911 [0.830-0.999] | 0.047 | 0.991 [0.849-1.157] | 0.908 |
| | 3.6~10 | ref | | ref | |
| | >10 | 0.829 [0.709-0.969] | 0.019 | 0.883 [0.726-1.073] | 0.164 |
| **Patients with MI** | | | | | |
| **T2DM** | <3.5 | 0.983 [0.779-1.241] | 0.886 | 0.919 [0.599-1.410] | 0.699 |
| | 3.5~10 | ref | | ref | |
| | >10 | 0.644 [0.430-0.964] | 0.032 | 0.862 [0.508-1.463] | 0.862 |

*CAD, coronary artery disease; MI, myocardial infarction; T2DM, type 2 diabetes mellitus; TBA, total bile acid; OR, odds ratio; CI, confidence interval; ref, reference.*

signaling molecules and metabolic regulators. BAs regulate signals by activating nuclear receptors such as farnesoid X receptor (FXR), and G protein-coupled receptors such as Takeda G-protein receptor 5 (TGR5) (30, 31). FXR and TGR5 agonists may play an important role in the progression of atherosclerosis and vascular calcification (32). BAs exert additional control over cholesterol metabolism by regulating many FXR target genes. Interestingly, *in vitro*, FXR activation induces the expression of low-density lipoprotein (LDL) receptors and inhibits the proprotein convertase subtilisin/kexin type 9 (PCSK9) molecule (33). In addition, the activation of BA-mediated signaling pathways may be related to enhanced control of inflammation. Previous studies have found that the TGR5 signaling pathway inhibits the activation of inflammasomes (34). FXR is involved in glucose homeostasis and lipid metabolism. Furthermore, in the intestine, BAs activate the glycogen-like peptide 1 (GLP-1) pathway through the TGR5 receptor, release insulin, stimulate resting heat generation, and reduce inflammation. Outside the intestines, BAs send signals to FXR and TGR5 receptors in the adipose tissue, skeletal muscles, and pancreas through these two receptors to maintain glucose homeostasis (35–37). This shows that at the level of molecules and signaling pathways, the TBA in plasma might act on target receptors through blood circulation and participate in inflammatory responses and glucose metabolism by activating specific pathways, thereby further influencing CVDs.

## INSIGHT AND ENLIGHTENMENT

To summarize, circulating TBA levels may sometimes be a fluctuating parameter. Nevertheless, most evidence suggests that the level of circulating TBA is higher in metabolic diseases (38). In addition, previous studies have confirmed significant differences in BA levels between males and females (30). It has also been found that TBA characteristics may vary between different populations. Therefore, in this study, we focused on the specific population of menopausal women and explored the characteristics of serum TBA in CAD, MI, and CAD/MI in relation with T2DM status. This analysis provides a new reference basis to assess the relationship between clinically accessible TBA data and disease status.

## CONCLUSIONS

TBA is a common clinical biomarker that may often be ignored. As a serum marker potentially related to CAD and MI in menopausal women, TBA may be particularly valuable for patients with CAD, MI, and T2DM. As a predictor, TBA seems to present some degree of correlation with the predicted probability of disease.

## LIMITATIONS

First, our study was a single-center retrospective case-control study. Due to the large number of patients, follow-up data were unattainable. Further studies on the association between TBA and the disease are still needed in combination with the follow-up and prognosis information. Second, the TBA level in this study was based on the fasting measurement value of patients after admission. TBA data with repeated measurements were not obtained, so there may be some bias in the results.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this study will be available from the corresponding author on reasonable requests.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of Beijing Anzhen Hospital. The data retrospectively obtained from electronic medical records.

## AUTHOR CONTRIBUTIONS

XF and GZ made substantial contributions to manuscript writing. YZ, QG, and XF made substantial contributions to study design. XF, JY, and YL made contributions to data collection and analysis. YZ and QG revised this paper. All authors contributed to the article and approved the submitted version

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.754006/full#supplementary-material

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ERRATUM
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# Erratum: Myocardial Infarction and Coronary Artery Disease in Menopausal Women With Type 2 Diabetes Mellitus Negatively Correlate With Total Serum Bile Acids

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**Keywords: total serum bile acids, myocardial infarction, coronary artery disease, menopausal women, type 2 diabetes mellitus**

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Due to a production error, "high-" was inserted in the beginning of the first paragraph of **Discussion** and sub-section **TBA and CVD**, **BAs in Lipid and Glucose Metabolism**. The correction has been implemented and the "high-" was removed from both places.

The publisher apologizes for this mistake. The original version of this article has been updated.

frontiers in Endocrinology

ORIGINAL RESEARCH
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# U-Shaped Association Between Serum Uric Acid and Short-Term Mortality in Patients With Infective Endocarditis

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**Background:** Increased uric acid (UA) levels have been reported to be associated with poor clinical outcomes in several conditions. However, the prognostic value of UA in patients with infective endocarditis (IE) is yet unknown.

**Methods:** A total of 1,117 patients with IE were included and divided into two groups according to the current definition of hyperuricemia (UA>420 μmol/L in men and >360 μmol/L in women): hyperuricemia group (n=336) and normouricemia group (n=781). The association between the UA level and short-term outcomes were examined.

**Results:** The in-hospital mortality was 6.2% (69/1117). Patients with hyperuricemia carried a higher risk of in-hospital death (9.8% *vs*. 4.6%, p=0.001). Hyperuricemia was not an independent risk factor for in-hospital death (adjusted odds ratio [aOR]=1.92, 95% confidence interval [CI]: 0.92-4.02, p=0.084). A U-shaped relationship was found between the UA level and in-hospital death (p<0.001). The in-hospital mortality was lower in patients with UA in the range 250–400 μmol/L. The aOR of in-hospital death in patients with UA>400 and <250 μmol/L was 3.48 (95% CI: 1.38-8.80, p=0.008) and 3.28 (95%CI: 1.27-8.51, p=0.015), respectively. Furthermore, UA>400 μmol/L (adjusted hazard ratio [aHR]=3.54, 95%CI: 1.77-7.07, p<0.001) and <250 μmol/L (aHR=2.23, 95%CI: 1.03-4.80, p=0.041) were independent risk factors for the 6-month mortality.

**Conclusion:** The previous definition of hyperuricemia was not suitable for risk assessment in patients with IE because of the U-shaped relationship between UA levels and in-hospital death. Low and high levels of UA were predictive of increased short-term mortality in IE patients.

**Keywords: infective endocarditis, uric acid, prognosis, U-shaped, hyperuricemia**

## INTRODUCTION

Infective endocarditis (IE) is a rare but serious infectious disease that is defined by infection of the endocardial surface, native or prosthetic heart valves, or indwelling cardiac devices (1, 2). Although diagnostic technology and therapeutic strategies have improved significantly in recent decades, the prognosis of IE remains poor (3–5). Epidemiological data have indicated that the short-term mortality of IE is ~10% (6–8). Early identification of high-risk patients is essential for optimal therapeutic regimens.

Uric acid (UA) is the end product of purine nucleotides' degradation and is mainly eliminated by the kidney and the intestinal tract (9). It functions as a potent antioxidant extracellularly to scavenge free radicals; however, as an intracellular prooxidant, it can disturb the bioavailability of nitric oxide in the endothelium, activate the renin-angiotensin system, stimulate the proliferation of vascular smooth muscle cells, and promote inflammation (10). Therefore, accumulating evidence suggests a J- or U-shaped relationship between UA level and prognosis (11–13).

A similar phenomenon also occurs in an infectious state. Hypouricemia has been reported to be associated with disease severity and poor prognosis in patients with coronavirus disease 2019 (COVID-19) (14). By contrast, hyperuricemia can result in high mortality rate in patients with acute respiratory distress syndrome (15). IE is also an infectious disease that is frequently complicated with cardiac dysfunction (3). Increased UA is common in the setting of heart failure because of the increased production resulting from oxidative stress and decreased excretion due to renal insufficiency (16). However, few studies have explored the prognostic value of UA in patients with IE. Here, we investigated the nature of the link between UA and an adverse prognosis in patients with IE.

## METHODS

### Patient Enrolment

This was a retrospective study conducted in Guangdong Provincial People's Hospital. Consecutive patients between January 2009 and February 2020 were selected from the electronic medical records according to the *International Classification of Diseases 10* codes for endocarditis: I33.0 (acute and subacute infective endocarditis), I33.9 (acute endocarditis, unspecified), and T82.6 (infection and inflammatory reaction due to cardiac valve prosthesis). IE was diagnosed by pathologic or clinical criteria based on the modified Duke criteria (17). The exclusion criteria were as follows (i) age <18 years; (ii) noninfective vegetation; (iii) nosocomial IE; (iv) possible IE (17); and (v) missing UA data. For patients who were admitted with IE more than once, only the first episode of recorded IE was included for analysis. A total of 1,117 patients were included for the final evaluation (**Figure 1**).

### Ethical Approval of the Study Protocol

The study protocol was approved (GDREC2020098) by the ethics committee of Guangdong Provincial People's Hospital (Guangdong, China). Owing to the retrospective nature of the study, the ethics committee waived the need for written informed consent.

### Measurements and Data Collection

All participants underwent echocardiography within 24 h after hospital admission. Valve involvement and vegetations were evaluated. The left ventricular ejection fraction (LVEF) was obtained using the Simpson biplane method. Uric acid levels were measured on LX20, DXC800, or AU5800 systems (Beckman Coulter, Fullerton, CA, USA) based on a

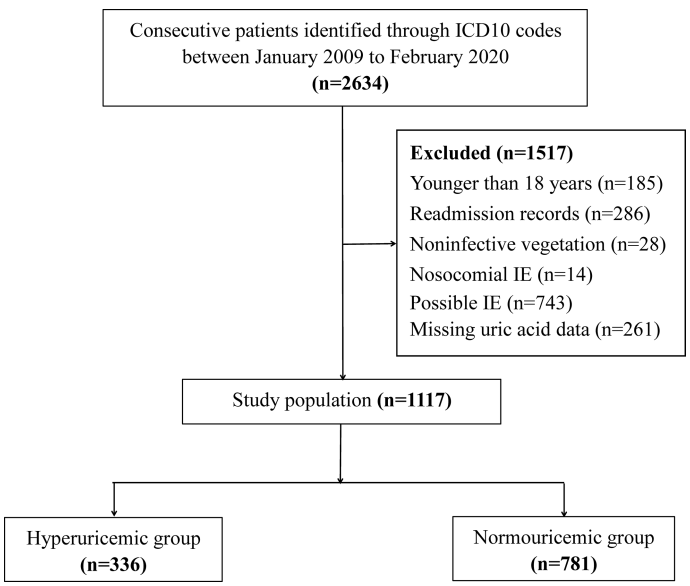


**FIGURE 1** | Flow chart of patient screening.

colorimetric method. A serum UA level of >420 μmol/L in men and >360 μmol/L in women was defined as hyperuricemia (10). The estimated glomerular filtration rate (eGFR) was calculated using the formula established by the Chronic Kidney Disease Epidemiology Collaboration (18). Demographics, medical history, results of laboratory tests and microbial culture, and treatment methods of the study population were collected from the electronic medical records by one researcher and checked randomly by another researcher. Clinical events were double-recorded. Inconsistent data were verified by a third researcher.

## Follow-up and Endpoints

Patients were followed-up *via* telephone for 6 months. In addition, the records for hospital readmission and outpatient-clinic interviews were reviewed for possible events. The primary endpoint was in-hospital mortality. The secondary endpoints were 6-month mortality (defined as any cause of death within 6 months after hospital admission), acute heart failure, and the need for renal replacement therapy (RRT) during hospitalization. The acute heart failure was defined as symptomatic heart failure at rest (New York Heart Association Class IV) and requiring inotropic support.

## Statistical Analyses

Normally distributed continuous data are presented as mean ± SD and were compared using the Student's *t*-test. Non-normally distributed continuous data with are presented as the median and interquartile range and were compared using the Mann–Whitney *U*-test. Categorical data are presented as percentages and were compared using the chi-square test or Fisher's exact test. Restricted cubic splines with three knots nested in the logistic regression analysis were used to flexibly model the association of UA with in-hospital mortality. Potential non-linearity was examined with a likelihood ratio test comparing the model with only a linear term against the model with linear and cubic spline terms. After careful visual inspection of the shape of UA's odds ratio (OR) curves for mortality, we identified the threshold of UA at the points, if any, where risk of mortality ceased to decline or started to rise steeply, as described in previous studies (19, 20). For convenient clinical application, the nearest integer was selected. The OR and 95% confidence interval (CI) were calculated. The association between variables and the 6-month mortality were assessed by Cox proportional hazard analyses. Significant variables in the univariate regression analysis were inputted into the multivariate regression analysis. A Kaplan–Meier curve was created to evaluate the cumulative 6-month mortality in patients with different levels of UA and compared using the log-rank test. Statistical analyses were undertaken using R-software (version 3.6.2; http://www.R-project.org) and SPSS 22.0 (IBM Corporation, Armonk, NY, USA). For all analyses, $p<0.05$ was considered to indicate statistical significance.

# RESULTS

## Patient Characteristics at Baseline

Among the 1,117 patients included in the present study, 336 (30.1%) had hyperuricemia. Patients with hyperuricemia were more likely to be men and have a history of hypertension, congenital heart disease, and hemodialysis than those with normouricemia. Patients with hyperuricemia also presented more usually with heart failure of New York Heart Association (NYHA) grade III/IV; had a higher body weight; higher levels of hemoglobin, fasting blood-glucose, and serum creatinine; but lower C-reactive protein (CRP) level, LVEF, and positive blood culture than those with normouricemia. The aortic valve was involved more often than the mitral valve in patients with hyperuricemia (**Table 1**).

## UA and In-Hospital Events

At the time of hospitalization, 69 (6.2%) patients died, 89 (8.0%) suffered acute heart failure, and 65 (5.8%) required RRT. The incidence of in-hospital death (9.8% *vs*. 4.6%, p=0.001; **Table 1**) and RRT (9.8% *vs*. 4.1%, p<0.001; **Table 1**) were significantly higher in patients with hyperuricemia than those with normouricemia. However, hyperuricemia was not an independent risk factor for in-hospital death after adjustment for age, hypertension, diabetes, prosthetic valve, NYHA Class III/IV, white blood cell (WBC), platelet <150×109/L, anemia, fasting blood-glucose, logCRP, eGFR<60 mL/min/1.73 $m^2$, LVEF, aortic valve vegetation, mitral valve vegetation, and surgical treatment (adjusted OR=1.92, 95%CI: 0.92-4.02, p=0.084; **Table 2**). A U-shaped trend was observed between the UA level and in-hospital death (p<0.001; **Figure 2A**). The in-hospital mortality was low in patients with UA in the range of 250–400 μmol/L (**Figure 2A**). Patients with UA>400 μmol/L suffered the highest risk of in-hospital death (10.1% *vs*. 2.7% *vs*. 7.3%, p<0.001; **Figure 3**) and RRT (10.1% *vs*. 4.1% *vs*. 3.3%, p<0.001; **Figure 3**).

Multivariate logistic regression analysis showed that compared with UA in the range of 250–400 μmol/L, UA>400 μmol/L (adjusted OR=3.48, 95%CI: 1.38-8.80, p=0.008; **Table 2**) and <250 μmol/L (adjusted OR=3.28, 95%CI: 1.27-8.51, p=0.015; **Table 2**) were significantly associated with in-hospital death after adjustment for confounding factors. In addition, The U-shape relationship was similar to the univariable model without adjustment (p<0.001; **Figure 2B**).

## UA and 6-Month Mortality

In total, 1,037 (92.8%) patients completed the 6-month follow-up, and the 6-month mortality rate was 9.5%. The Kaplan–Meier curve indicated that the cumulative 6-month mortality was significantly higher in patients with UA>400 μmol/L (log-rank test=22.4, p<0.001; **Figure 4**). In addition, UA>400 μmol/L (adjusted hazard ratio [HR]=3.54, 95%CI: 1.77-7.07, p<0.001; **Table 3**) and <250 μmol/L (adjusted HR=2.23, 95%CI: 1.03-4.80, p=0.041; **Table 3**) were independent risk factors for 6-month mortality in the multivariate Cox survival analysis.

# DISCUSSION

To our knowledge, this is the first study to explore the prognostic role of serum UA levels in patients with IE. We discovered a U-shaped relationship between the UA level and in-hospital

**TABLE 1** | Baseline characteristics.

| Clinical variables | Hyperuricemic group (n = 336) | Normouricemic group (n = 781) | P value |
|---|---|---|---|
| Age (year) | 46.5 ± 15.4 | 44.7 ± 15.7 | 0.070 |
| Female gender, n (%) | 81 (24.1) | 264 (33.8) | 0.001 |
| Body weight (kg) | 59.7 ± 12.7 | 55.5 ± 10.4 | <0.001 |
| Comorbidities, n (%) | | | |
| Hypertension | 71 (21.1) | 110 (14.1) | 0.003 |
| Diabetes | 33 (9.8) | 51 (6.5) | 0.056 |
| Rheumatic heart disease | 60 (17.9) | 118 (15.1) | 0.250 |
| Congenital heart disease | 120 (35.7) | 224 (28.7) | 0.020 |
| History of hemodialysis, n (%) | 6 (1.8) | 3 (0.4) | 0.042 |
| Prosthetic valve, n (%) | 24 (7.1) | 41 (5.2) | 0.215 |
| NYHA Class III/IV heart failure, n (%) | 143 (42.6) | 212 (27.1) | <0.001 |
| WBC ($\times10^9$/L) | 9.7 ± 4.3 | 9.9 ± 4.3 | 0.504 |
| Platelet ($\times10^9$/L) | 187.8 (124.6,269.0) | 207.0 (127.8,278.8) | 0.133 |
| Hemoglobin (g/L) | 108.3 ± 25.0 | 102.8 ± 20.6 | 0.001 |
| Fasting blood-glucose (mmol/L) | 5.4 ± 1.9 | 5.2 ± 1.4 | 0.150 |
| CRP (mg/L) | 16.8 (7.1,39.4) | 35.3 (14.0,67.2) | <0.001 |
| Serum creatinine (umol/L) | 92.0 (77.0,133.0) | 70.7 (57.8,85.9) | <0.001 |
| eGFR<60 ml/min/1.73m$^2$ | 109 (32.4) | 65 (8.3) | <0.001 |
| LVEF (%) | 63.8 ± 8.0 | 65.5 ± 6.8 | 0.001 |
| Vegetation, n (%) | 323 (96.1) | 757 (96.9) | 0.495 |
| Vegetation present, n (%) | | | |
| Aortic valve | 180 (53.6) | 277 (35.5) | <0.001 |
| Mitral valve | 163 (48.5) | 486 (62.2) | <0.001 |
| Aortic+Mitral valve | 47 (14.0) | 76 (9.7) | 0.037 |
| Other sites | 27 (8.0) | 70 (9.0) | 0.614 |
| Blood culture positive, n (%) | 176 (52.4) | 517 (66.2) | <0.001 |
| Surgical treatment, n (%) | 249 (74.1) | 556 (71.2) | 0.319 |
| Embolic events, n (%) | 57 (17.0) | 160 (20.5) | 0.172 |
| Length of hospital stay (days) | 32 (19,44) | 35 (20,46) | 0.072 |
| In-hospital events, n (%) | | | |
| Acute heart failure | 33 (9.8) | 56 (7.2) | 0.133 |
| Renal replacement treatment | 33 (9.8) | 32 (4.1) | <0.001 |
| Death | 33 (9.8) | 36 (4.6) | 0.001 |

*NYHA, New York Heart Association; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.*

mortality. The current definition of hyperuricemia was not an independent risk factor for in-hospital death in patients with IE. Both UA>400 μmol/L and <250 μmol/L were independently associated with in-hospital and 6-month mortality, which could be considered an optimal threshold for predicting poor prognosis in IE patients.

In our analysis, 30.1% IE patients had hyperuricemia, which could be attributed to a high incidence of renal and cardiac insufficiency in IE. In healthy individuals, two-thirds of daily UA is excreted by the kidneys and the remaining one-third is eliminated through the intestinal tract. In renal dysfunction, the renal excretion of UA reduced and the intestinal excretion is compromised by impairment of UA transporters, resulting in a high serum UA levels (21). With respect to heart failure, increased production of UA because of increased xanthine-oxidase activity as well as decreased renal excretion of UA because of renal hypoperfusion together contribute to an increase in the UA level (16). In addition to clinical manifestations in these two conditions, hyperuricemia could serve as an important indicator for unfavorable outcomes. Hyperuricemia plays a part in the progression of chronic kidney diseases (13, 22), as well as potentiating the risk of

**TABLE 2** | Univariate and multivariate logistic regression analysis for in-hospital mortality.

| | Hyperuricemia (UA>420 μmol/L in men and >360 μmol/L in women) | | UA>400 μmol/L *vs*. 250-400 μmol/L | | UA<250 μmol/L *vs*. 250-400 μmol/L | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | p | OR (95% CI) | P | OR (95% CI) | p |
| **In-hospital death** | | | | | | |
| Model 1: unadjusted | 2.25 (1.38-3.68) | 0.001 | 4.08 (2.13-7.82) | <0.001 | 2.87(1.40-5.86) | 0.004 |
| Model 2: multivariate adjusted* | 1.92 (0.92-4.02) | 0.084 | 3.48 (1.38-8.80) | 0.008 | 3.28(1.27-8.51) | 0.015 |

*UA, uric acid; OR, odds ratio; CI, confidence interval; NYHA, New York Heart Association; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.*
**Adjusted variables included age, hypertension, diabetes, prosthetic valve, NYHA Class III/IV, WBC, platelet <150×10$^9$/L, anemia, fasting blood-glucose, lgCRP, eGFR<60ml/min/1.73m2, LVEF, aortic valve vegetation, mitral valve vegetation and surgery treatment.*

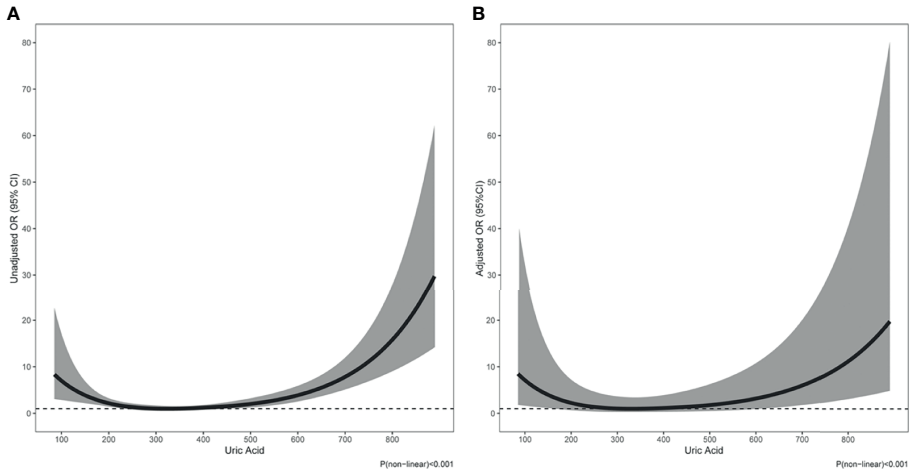


**FIGURE 2** | The association of uric acid with in-hospital death **(A)** Unadjusted; **(B)** Adjusted.

acute kidney injury during hospitalization (23, 24). In patients with acute/chronic heart failure, an increased UA level is believed to correlate independently with increased short-term and long-term mortality as well as re-hospitalization (25–27). However, the clinical importance of hyperuricemia in IE is still unclear.

We showed that hyperuricemia was a risk factor for in-hospital mortality, but its significance was lost after multivariate regression analysis. A U-shaped relationship between the UA level and in-hospital mortality for IE patients was discovered, which was consistent with previous studies (11–13). It was believed that the

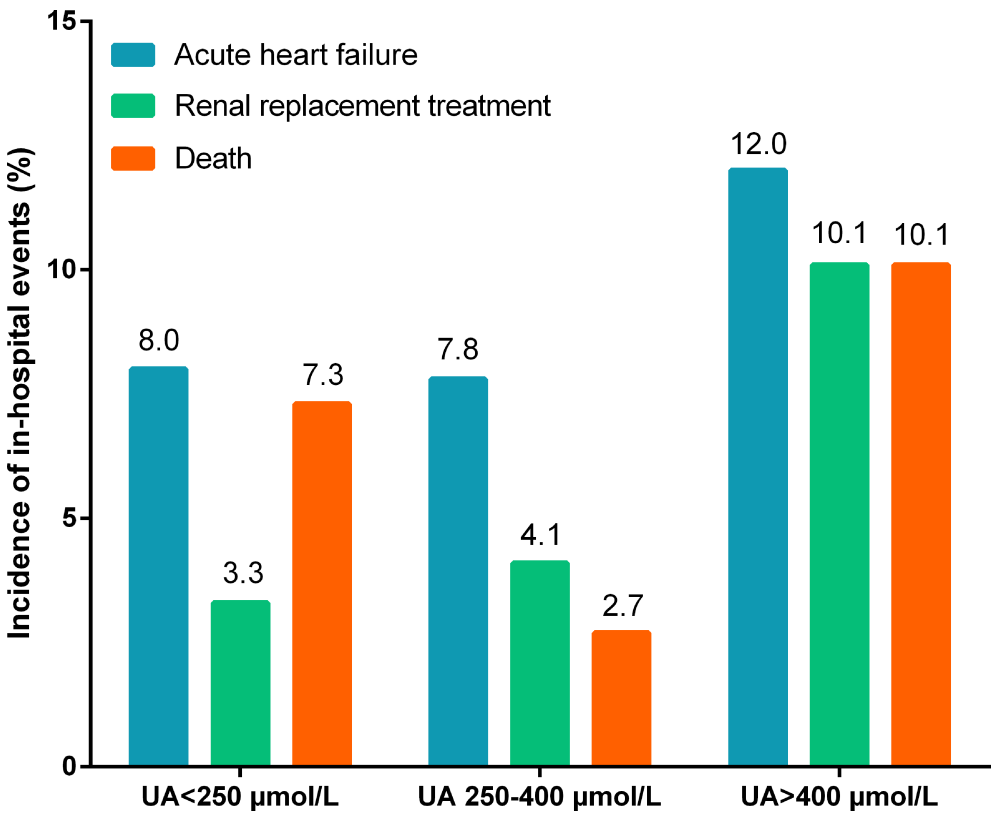


**FIGURE 3** | Incidence of in-hospital events according to different uric acid levels.

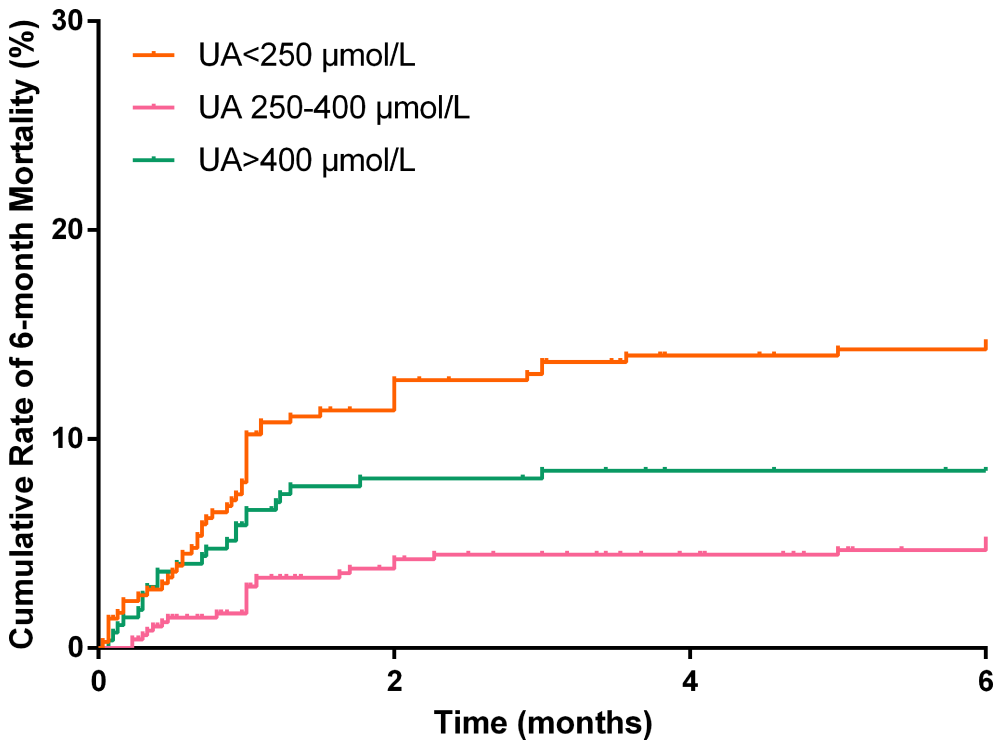


**FIGURE 4** | Cumulative rate of 6-month mortality according to different uric acid levels.

previous definition of hyperuricemia (UA>420 μmol/L in men and >360 μmol/L in women) was not sufficiently valid for predicting clinical outcomes in certain pathological conditions. The J- or U-shaped relationship was gradually accepted. A recent study conducted by Chen et al. included 1,854 patients with COVID-19 infection and showed a U-shaped association between UA and composite outcomes (28). Their cut-off values were ≥423 μmol/L and ≤278 μmol/L, which was close to our findings.

This U-shaped relationship might be explained by the dual antioxidant and prooxidant effect of UA in inflammatory conditions. In humans, >50% antioxidant capacity in blood plasma comes from UA (29). In addition, UA has been shown to be effective in preventing viral infection by enhancing T-cell responses and secretion of type-I interferons (30, 31). However, UA levels have been observed to decrease continuously in people with infectious diseases (32, 33). Consistently, the UA level was lower in patients with a positive blood culture in our study. Hypouricemia has a prognostic value to some extent. An extremely low level of UA diminishes the antioxidant capacity of plasma in severe sepsis and indicates a poor outcome (34).

Despite its protective effect, an extremely high UA level may signify more harm than benefit during severe inflammation. Our results suggested that an increased UA level was associated with a poor outcome in IE. The latter is a microbial infection on the endocardial surface, wherein the spread of pathogenic organisms into the bloodstream can trigger a systemic inflammatory response syndrome. The incidence of sepsis in 294 IE patients was nearly 30% in a study conducted by Krajinovic et al. (35). Chuang et al. showed that the UA level was positively correlated with the Acute Physiology and Chronic Health Evaluation (APACHE) score in patients with sepsis, which supported its role in reflecting illness severity (36). Septic patients with hyperuricemia tend to require greater vasopressor support (37). Lee et al. found that in patients with acute respiratory distress syndrome, the mortality was higher in cases with normal-to-high UA level than in patients with a low UA level (15). The possible underlying mechanism might be explained by the excessive prooxidant effect in a hyperuricemia environment that leads to endothelial dysfunction, increased activity of xanthine oxidase, increased oxidative stress, inappropriate activation of the renin–angiotensin–aldosterone system, impaired renal autoregulatory response, and release of proinflammatory chemokines (38).

Our study has some limitations. First, this was a single-center study with a small sample size (1,117 cases with 69 events). We used package "pwr2ppl" (version 0.2.0) in R software to calculate power for logistic model. Our study included 1117 cases with 69 events that had 82% to 99% power for detecting ORs of 1.50 to 1.90 at an alpha level of 0.05, with 6% in-hospital mortality and 0.20 correlation between UA and other covariates. Therefore, we think that the sample size is relatively powerful to detect the association between UA and mortality. Second, this study was retrospective in nature. Although we adjusted for most potential confounding factors in the multivariate analysis, residual factors might have affected the results. Third, the UA level is different in

**TABLE 3 |** Univariate and multivariable Cox regression analysis for 6-month mortality.

| Clinical variables | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | P | HR (95% CI) | P |
| Age | 1.04 (1.02-1.05) | <0.001 | 1.01 (0.99-1.03) | 0.603 |
| Female gender | 0.68 (0.43-1.09) | 0.109 | | |
| Weight | 1.00 (0.99-1.02) | 0.712 | | |
| Hypertension | 1.60 (1.00-2.55) | 0.049 | 0.86 (0.45-1.63) | 0.639 |
| Diabetes | 2.34 (1.35-4.06) | 0.002 | 1.41 (0.63-3.18) | 0.406 |
| Rheumatic heart disease | 2.01 (1.28-3.15) | 0.002 | 0.91 (0.46-1.81) | 0.790 |
| Congenital heart disease | 0.98 (0.64-1.50) | 0.916 | | |
| History of hemodialysis | 1.38 (0.19-9.93) | 0.747 | | |
| Prosthetic valve | 4.50 (2.73-7.43) | <0.001 | 2.87 (1.36-6.04) | 0.006 |
| NYHA class III or IV | 4.53 (2.98-6.88) | <0.001 | 3.33 (1.85-5.98) | <0.001 |
| WBC | 1.08 (1.04-1.11) | <0.001 | 1.02 (0.97-1.07) | 0.471 |
| Platelet $<150\times10^9$/L | 2.55 (1.72-3.80) | <0.001 | 1.83 (1.10-3.05) | 0.020 |
| Anemia | 2.90 (1.59-5.31) | 0.001 | 2.15 (0.96-4.79) | 0.061 |
| Fasting blood-glucose | 1.30 (1.22-1.40) | <0.001 | 1.12 (1.02-1.23) | 0.022 |
| lgCRP | 3.75 (2.21-6.36) | <0.001 | 1.64 (0.86-3.14) | 0.135 |
| eGFR<60ml/min/1.73m$^2$ | 3.90 (2.60-5.85) | <0.001 | 1.00 (0.97-1.03) | 0.841 |
| LVEF | 0.96 (0.94-0.98) | <0.001 | 2.41 (1.30-4.45) | 0.005 |
| Aortic valve vegetation | 2.80 (1.85-4.24) | <0.001 | 0.99 (0.56-1.74) | 0.968 |
| Mitral valve vegetation | 0.45 (0.30-0.67) | <0.001 | 0.21 (0.12-0.35) | <0.001 |
| Surgery treatment | 0.19 (0.13-0.29) | <0.001 | 2.87 (1.36-6.04) | 0.006 |
| UA, μmol/L | | | | |
| 250-400 | 1 [Reference] | - | 1 [Reference] | - |
| <250 | 1.72 (0.97-3.05) | 0.062 | 2.23 (1.03-4.80) | 0.041 |
| >400 | 3.01 (1.86-4.90) | <0.001 | 3.54 (1.77-7.07) | <0.001 |

*NYHA, New York Heart Association; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.*

male and female patients, but the newly identified cut-off value of UA was not distinguished based on because of the small study cohort. Last, telephone interviews, hospital-readmission records, and outpatient clinic interviews were employed during the follow-up period, but some patients showed poor compliance.

## CONCLUSIONS

Low and high levels of UA were independent risk factors for in-hospital and 1-year mortality in patients with IE. The previous definition of hyperuricemia (UA>420 μmol/L in men and >360 μmol/L in women) was not suitable for risk stratification because of the U-shaped trend between the UA level and adverse outcomes. UA>400 or <250 μmol/L might be more valuable predictors of outcome than the previous cut-offs, especially for infectious diseases.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The study protocol was approved (GDREC2020098) by the Ethics Committee of Guangdong Provincial People's Hospital (Guangdong, China). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

DY and JC contributed to the conception or design of the work. XW, BF, XC, and WC contributed to the acquisition or interpretation of data for the work. ZW and GJ contributed to statistical analysis. XW, BF, and XC drafted the manuscript. DY and JC critically revised the manuscript. Everyone gave final approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy. All authors contributed to the article and approved the submitted version.

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# Triglyceride and Glucose Index and Sex Differences in Relation to Major Adverse Cardiovascular Events in Hypertensive Patients Without Diabetes

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**Introduction:** Studies from recent decades have suggested that women have a lower risk of cardiovascular disease than men due to their characteristics, but hyperglycemia and hyperinsulinemia caused by IR (insulin resistance) might reverse this gender-protective effect. This study examined whether there were sex differences in the relationship between IR [evaluated by triglyceride and glucose index (TyG index)] and major adverse cardiovascular events (MACEs) in hypertensive patients without diabetes.

**Methods:** This was a *post-hoc* analysis of the Systolic Blood Pressure Intervention Trial (SPRINT). We explored the relationship between TyG index and MACEs by multivariate Cox proportional hazard regressions and two-piecewise linear regression models. The primary endpoint was MACEs, same as SPRINT, defined as a composite of myocardial infarction, stroke, heart failure, and/or death from cardiovascular causes. We used multiple adjustment models for all regressions.

**Results:** A total of 9,323 patients from the SPRINT were included in our analysis. TyG index was significantly related to the risk of MACEs in every adjusted model. Each 1 unit increase in TyG index increased the risk of MACEs in total participants (HR, 1.40; 95% CI, 1.20–1.64; P<0.01) and men (HR, 1.42; 95% CI, 1.18–1.71; P=0.02). However, TyG index was not associated with MACEs among female hypertensive patients (HR, 1.33; 95% CI, 0.97–1.82; P=0.0776). There was no interaction between the sex and TyG index (P for interaction= 0.73). We also used the two-stage linear regression model and did not find any threshold effect. There was no significant interaction in other confounders.

**Conclusion:** We found the TyG index was associated with MACEs in the hypertensive patients, and there was no gender difference between the TyG index and MACEs.

**Keywords: triglyceride and glucose index (TyG index), major adverse cardiovascular events (MACEs), sex differences, Systolic Blood Pressure Intervention Trial (SPRINT), hypertension**

## BACKGROUND

Insulin resistance (IR) is a key metabolic abnormality leading to the development of T2DM (1), and recent studies have also found that it could independently predict the development of cardiovascular disease (CVD) and chronic kidney disease (CKD) (2). Hyperinsulinemic-Euglycemic Clamp (HIEC), as the gold standard for the diagnosis of insulin resistance, is very difficult to be carried out in clinical work due to its difficulty of operation and equipment requirements (3). Triglyceride and glucose index (TyG index) was one of the alternative methods for evaluating insulin resistance (4) and was proved to be consistent with the HIEC in several studies (5–7). Therefore, as the accessibility of its measurement indicators (fasting plasma glucose and fasting triglyceride levels), TyG was used in numerous cardiovascular studies as an alternative to insulin resistance (8–12).

The lipotoxicity and glucotoxicity of the IR were the main factors in the development of cardiovascular diseases. Moreover, previous studies found an independent relevance between TyG and the major adverse cardiovascular events (MACEs), including acute coronary syndrome (8–10), stroke (11), and CVD death (12). However, this correlation might be different in both genders, due to their differences in physiology, cultural behavior, and environmental factors (13–15). In recent decades scientific literature has indicated that women have a lower risk of cardiovascular events due to their characteristics than men. However, the protection in female patients might be reverted with IR-related disorders due to the hyperglycemia and hyperinsulinemia caused by IR (16, 17).

The population included in the previous studies is the general population (12). Few studies included a large proportion of the elderly, and the elderly tended to have more cardiovascular risk factors. Moreover, previous studies did not distinguish between diabetic and non-diabetic patients. Previous studies from different regions also found that there might be gender differences in the correlation between the TyG index and MACEs (12, 18, 19). To better study these issues, we used the data from the Systolic Blood Pressure Intervention Trial (SPRINT) (20) to evaluate the relationship between TyG index and MACEs in a hypertension population and further explore the correlation between gender differences.

## METHOD

We performed a *post-hoc* analysis of the SPRINT. The limited dataset was obtained from the National Institutes of Health Biologic Specimen and Data Repository Information Coordinating Center (https://biolincc.nhlbi.nih.gov/studies/sprint/).

### Study Population

SPRINT was a randomized, controlled trial conducted at 102 clinical sites in the United States. The rationale, design, and main results of SPRINT have been previously published (20, 21). Briefly, SPRINT was designed to test whether the intensive management of systolic BP to <120 mmHg reduces cardiovascular disease events compared with standard BP management (<140 mmHg). The recruited participants were between the ages of 50 and 75 and had at least one of the following: presence of clinical or subclinical cardiovascular disease other than stroke; chronic kidney disease (defined as eGFR 20–59 ml/min/1.73 m2); Framingham risk score for 10-year CVD risk ≥15% based on laboratory work done in the last 12 months; or if patients were aged 75 years or older. Because the blood pressure trial of ACCORD study did not come to a good conclusion within the diabetic patients (22). Exclusion criteria were that patients had type 2 diabetes, prior stroke, and standing systolic BP <110 mmHg at the screening visit. The SPRINT showed that intensive blood pressure management significantly reduced cardiovascular mortality and all-cause mortality compared to standard management.

### Exposure Variables

TyG index was defined as TyG=Ln [fasting triglycerides (mg/dl) × fasting glucose (mg/dl)/2]. We used the baseline fasting triglycerides and fasting glucose to calculate the TyG index (7, 23). We divided the population into three groups according to the size of the TyG index. The first group was the reference.

The primary endpoint of our study was major cardiovascular adverse events (MACEs), defined as a composite of myocardial infarction, stroke, heart failure, and/or death from cardiovascular causes. The definitions of MI, stroke, heart failure, and outcomes were the same as SPRINT and presented elsewhere. The outcomes were adjudicated.

### Statistical Analysis

The baseline characteristics and outcomes of patients were expressed as frequencies and percentages for categorical variables. Means and standard deviations (SDs) or median and interquartile ranges were used for continuous variables, depending on whether datasets were normally distributed (assessed using normal Q–Q plots). We used chi-square analysis to compare categorical variables. We used analysis of variance or the Mann–Whitney U test to compare continuous variables in accordance with the distribution type.

The adjusted variables in this study were selected based on their clinical importance. Three multivariate Cox proportional hazard regressions were constructed to estimate the association of the baseline TyG index with the risk of MACEs by calculating the hazard ratio (HR) and 95% confidence interval (CI). The validity of the proportionality assumption was verified by scaled Schoenfeld residuals. Model 1 was an unadjusted model. Model 2 was adjusted for age, treatment arm, and ethnicity. Model 3 was further adjusted for age, treatment arm, ethnicity, baseline body mass index, smoking status, chronic kidney disease (CKD) subgroup, cardiovascular disease (CVD) subgroup, number of antihypertensive agents, aspirin used, and statin used at baseline.

To account for the TyG index as a continuous variable, we constructed a Cox proportional hazards regression model (Model 3). The TyG index was used to calculate the HR for outcomes. Then restricted cubic spline models with four knots at the fifth, 35th, 65th, and 95th percentiles were built to detect any non-linear relationship between TyG index and mortality. We

used two-piecewise linear regression models to elucidate how the associations differed by the threshold point. The threshold value was estimated by trying all possible values and choosing the threshold point with the highest likelihood. A logarithmic likelihood ratio test was employed to compare the differences in associations when using one-line linear regression models *vs.* two-piecewise linear regression models.

We performed the interaction and stratified analyses by sex, treatment arm, age (<75 years and ≥75 years), systolic blood pressure tertile (≤132 mmHg, 132–145 mmHg, and ≥145 mmHg), Framingham 10-y cardiovascular disease risk score (≤15%, >15%), smoking status, CVD subgroup, CKD subgroup, Black race, aspirin use, and statin use.

All analyses were performed using statistical software packages R (The R Foundation; http://www.R-project.org) and EmpowerStats (X&Y Solutions, Inc., Boston, MA, USA; http://www.empowerstats.com). P values <0.05 (two-sided) were considered statistically significant.

# RESULTS

## Baseline Characteristics of Included Hypertension Patients

Among the total 9,323 patients with hypertension from the SPRINT trial, there are 38 patients whose TyG index cannot be calculated. The median follow-up was 3.26 years. After follow-up, 155 (4.99%) MACEs occurred in the low TyG group, 196 (6.30%) in the middle group, and 210 (6.76%) in the high TyG group. Low TyG group patients had higher HDL-C levels and GFR. High TyG group patients had a higher Framingham 10-y cardiovascular disease risk score. **Table 1** provides the detailed baseline characteristics of the patients with hypertension included in the study population.

## Tertiles of TyG Index and MACEs

The association between the TyG index and MACEs in patients with hypertension is presented in **Table 2**. No matter in which model, the TyG index was significantly related to the risk of MACEs. In Model 3, the third tertile has the highest risk of MACEs (HR, 1.45; 95% CI, 1.17–1.81; P<0.01). When the TyG index grouping was regarded as a continuous variable, this trend did not change (HR, 1.20; 95% CI, 1.08–1.34; P<0.01). A similar trend was observed in male patients. Although there were similar trends among female patients, the statistical difference was not significant.

## TyG Index as a Continuous Variable and MACEs

As shown in **Table 3**, when we used the TyG index as a continuous covariate, each 1 unit increase in TyG index increased the risk of MACEs in total participants (HR, 1.40; 95% CI, 1.20–1.64; P<0.01) and men (HR, 1.42; 95% CI, 1.18–1.71; P<0.01). However, the TyG index was not associated with MACEs among female hypertensive patients (HR, 1.33; 95% CI, 0.97–1.82; P=0.08). Restricted cubic splines were used to flexibly model and visualize the relationship between the TyG index and MACEs. With the increase of the TyG index, the risk of MACEs increased. When the TyG index was close to nine, the trend of increasing the risk of MACEs slowed down (**Figure 1**). There was no interaction between the sex and TyG index (P for interaction= 0.73).

Next, we used the two-stage linear regression model to calculate the threshold effect. **Table 3** shows the results of the two-stage linear regression model. The inflection point was 8.71 in all participants; on the left inflection point, the effect size, 95% CI, and P value were 1.66, 1.21–2.27, and <0.01, respectively; on the right inflection point, HR, 1.20; 95% CI, 0.89–1.62; P=0.22. However, the log likelihood ratio test was 0.21. This means that the two-stage linear regression model was not better than the one-line linear regression models.

## Interaction and Sensitivity Analyses

The results of the interaction and stratified analyses are presented in **Figure 2**. Generally, the TyG index was significantly associated with the risk of MACEs across various subgroups. There was no significant interaction in the confounders.

# DISCUSSION

In this *post hoc* analysis, we observed that a high baseline TyG index was associated with the risk of MACEs in old hypertensive patients. And there was no significant interaction in sex and the TyG index.

TyG index, which is calculated from fasting triglyceride and blood glucose, is a reliable and surrogate biomarker to assess insulin resistance. The accuracy of TyG index in the diagnosis of insulin resistance was consistent with that of HIEC and HOMA-IR (4). A recent meta-analysis involving 5,731,294 participants reported that a higher TyG index might be independently associated with a higher incidence of atherosclerotic cardiovascular diseases (ASCVDs), CAD, and stroke in people without ASCVDs at baseline (24). To the best of our knowledge, this study is the first study to evaluate the relationship between the TyG index and adverse outcomes within the population of hypertensive patients without diabetes. These patients tended to have a higher risk of ASCVDs. A similar previous study focusing on the general population reached a similar conclusion to ours. The average age of the population we included in the study was much older than them, and the value of the inflection point we obtained was smaller than theirs. This explains to a certain extent that as patients age and cardiovascular risk factors increase, the risk of adverse events caused by an increase in the TyG index is higher (12). The association between the TyG index and the risk of MACEs should be interpreted as insulin resistance reflected by the TyG index. Insulin resistance is a condition where the body tissues became resistant to insulin, resulting in a disorder of lipid and glucose metabolism (25). The main characteristic of dyslipidemia caused by insulin resistance is the lipid triad, including hypertriglyceridemia, low HDL cholesterol, and small and dense LDL. In a normal physiological state, insulin could degrade apoB and reduce VLDL synthesis through activation of PI3K. However, in the insulin

**TABLE 1** | Baseline characteristics and crude end points of the study participants.

| | *TYG Tertile* | | | |
|---|---|---|---|---|
| | **Low** | **Middle** | **High** | ***P*-Value** |
| N | 3,104 | 3,111 | 3,108 | |
| TYG Scores | 8.02 ± 0.23 | 8.55 ± 0.13 | 9.19 ± 0.35 | <0.01 |
| Intensive treatment | 1,554 (50.06%) | 1,580 (50.79%) | 1,528 (49.16%) | 0.44 |
| BMI | 28.58 ± 5.84 | 30.01 ± 5.86 | 30.96 ± 5.34 | <0.01 |
| Age, y | | | | |
| Overall | 69.15 ± 9.65 | 68.26 ± 9.35 | 66.31 ± 9.03 | <0.01 |
| ≥75 y, n (%) | 1,037 (33.41%) | 929 (29.86%) | 659 (21.20%) | <0.01 |
| Race, n (%) | | | | <0.01 |
| Non-Hispanic Black | 1,228 (39.56%) | 911 (29.28%) | 646 (20.79%) | |
| Hispanic | 210 (6.77%) | 339 (10.90%) | 429 (13.80%) | |
| Other | 54 (1.74%) | 48 (1.54%) | 72 (2.32%) | |
| Non-Hispanic White | 1,612 (51.93%) | 1,813 (58.28%) | 1,961 (63.10%) | |
| Black Race, n (%) | 1,281 (41.27%) | 955 (30.70%) | 694 (22.33%) | <0.01 |
| Baseline blood pressure, mmHg | | | | |
| Systolic | 140.13 ± 15.63 | 139.60 ± 15.76 | 139.27 ± 15.34 | 0.90 |
| Diastolic | 77.47 ± 12.26 | 77.80 ± 11.62 | 79.11 ± 11.88 | <0.01 |
| Distribution of systolic blood pressure, n (%) | | | | 0.16 |
| ≤132 mmHg | 999 (32.18%) | 1,070 (34.39%) | 1,055 (33.94%) | |
| >132 to <145 mmHg | 1,002 (32.28%) | 994 (31.95%) | 1,030 (33.14%) | |
| ≥145 mmHg | 1,103 (35.53%) | 1,047 (33.65%) | 1,023 (32.92%) | |
| Serum creatinine, mg/dl | 1.06 ± 0.33 | 1.07 ± 0.34 | 1.09 ± 0.35 | 0.03 |
| Estimated GFR, ml* $min^{-1}$*1.73 $m^{-2}$ | 73.04 ± 20.79 | 71.32 ± 20.49 | 70.88 ± 20.44 | <0.01 |
| Fasting HDL cholesterol, mg/dl | 60.92 ± 15.64 | 52.19 ± 12.18 | 45.52 ± 10.75 | <0.01 |
| Fasting LDL cholesterol, mg/dl | 107.00 ± 31.53 | 113.91 ± 34.58 | 116.36 ± 38.31 | <0.01 |
| Fasting total cholesterol, mg/dl | 181.38 ± 37.67 | 187.61 ± 38.91 | 201.35 ± 44.08 | <0.01 |
| Fasting total triglycerides, mg/dl | 67.33 ± 14.77 | 107.56 ± 17.30 | 202.86 ± 119.89 | <0.01 |
| Fasting glucose, mg/dl | 93.36 ± 9.93 | 98.25 ± 11.15 | 104.83 ± 16.16 | <0.01 |
| Statin use, n (%) | 1,278 (41.52%) | 1,387 (44.87%) | 1,381 (44.65%) | 0.01 |
| Aspirin use, n (%) | 1,578 (50.97%) | 1,625 (52.32%) | 1,544 (49.77%) | 0.13 |
| Smoking status, n (%) | | | | 0.51 |
| Never smoked | 1,414 (45.55%) | 1,358 (43.65%) | 1,339 (43.08%) | |
| Former smoker | 1,286 (41.43%) | 1,339 (43.04%) | 1,338 (43.05%) | |
| Current smoker | 401 (12.92%) | 409 (13.15%) | 428 (13.77%) | |
| Framingham 10-y cardiovascular disease risk score, % | 17.73 ± 9.33 | 20.02 ± 10.79 | 22.52 ± 11.76 | <0.01 |
| No. of antihypertensive agents | 1.80 ± 1.04 | 1.83 ± 1.04 | 1.87 ± 1.04 | 0.06 |
| Not using antihypertensive agents, n (%) | 309 (9.95%) | 282 (9.06%) | 289 (9.30%) | 0.46 |
| MACEs | 155 (4.99%) | 196 (6.30%) | 210 (6.76%) | 0.01 |

*GFR, glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein.*

*Plus–minus values are means ± SD. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for glucose to millimoles per liter, multiply by 0.05551.*

*Race and ethnic group were self-reported.*

*Black race includes Hispanic black and black as part of a multiracial identification.*

*The body mass index is the weight in kilograms divided by the square of the height in meters.*

resistance state, this degradation is inhibited, resulting in increased VLDL synthesis (26). Hyperglycemia caused by insulin resistance could induce long-term epigenetic modifications of the NF-κB promoter, leading to mitochondrial dysfunction and endoplasmic reticulum stress (27). This could lead to an increase in reactive oxygen species and inflammatory factors, which could impair endogenous nitric oxide release and cause endothelial dysfunction (28). These insulin resistance states could promote the development of cardiovascular and cerebrovascular diseases (29, 30). Insulin resistance was also significantly associated with coronary artery plaque vulnerability, leading to the occurrence of acute coronary syndrome (31, 32). Therefore, these metabolic disorders promoted endothelial dysfunction, cardiovascular remodeling, oxidative stress, inflammatory factors release that exacerbated elevated blood pressure and artery stiffness, all of which were major risk factors for cardiovascular diseases (30, 33).

Previous studies have found gender differences in the relationship between the TyG index and the risk of major adverse cardiovascular events. The Kailuan cohort study found that the TyG index had an interaction with gender in predicting myocardial infarction, and the TyG index had a better predictive efficiency in women (34). Just like other traditional cardiovascular risk factors, there were gender differences in the relationship between the TyG index and cardiovascular diseases. The sex difference in the association between traditional cardiovascular risk factors could be explained by increased insulin resistance in menopausal women with reduced estrogen levels, which lead to a higher risk of cardiovascular diseases (35). However, our study found no interaction between the TyG index and gender in predicting MACEs. The reason for the inconsistency with the Kailuan cohort study may be the difference in study population size and the short follow-up time. The average age of the population

**TABLE 2 |** TyG index tertile and MACEs.

| TyG scores tertile | Hazard ratio (95% CI) P-value | | |
|---|---|---|---|
| | **Model 1** | **Model 2** | **Model 3** |
| Male | | | |
| 1 | Ref | Ref | Ref |
| 2 | 1.24 (0.96, 1.60) 0.10 | 1.32 (1.02, 1.71) 0.03 | 1.27 (0.98, 1.65) 0.07 |
| 3 | 1.34 (1.05, 1.72) 0.02 | 1.62 (1.26, 2.09) <0.01 | 1.46 (1.13, 1.90) <0.01 |
| TyG index tertiles as a continuous variable | 1.15 (1.02, 1.30) 0.02 | 1.27 (1.12, 1.44) <0.01 | 1.20 (1.06, 1.37) <0.01 |
| Female | | | |
| 1 | Ref | Ref | Ref |
| 2 | 1.27 (0.87, 1.84) 0.21 | 1.34 (0.92, 1.95) 0.12 | 1.32 (0.90, 1.92) 0.16 |
| 3 | 1.26 (0.86, 1.85) 0.24 | 1.45 (0.97, 2.15) 0.07 | 1.41 (0.94, 2.10) 0.10 |
| TyG index tertiles as a continuous variable | 1.12 (0.93, 1.35) 0.23 | 1.20 (0.99, 1.46) 0.06 | 1.18 (0.97, 1.44) 0.09 |
| All participants | | | |
| 1 | Ref | Ref | Ref |
| 2 | 1.25 (1.01, 1.54) 0.04 | 1.33 (1.07, 1.64) <0.01 | 1.29 (1.04, 1.60) 0.02 |
| 3 | 1.32 (1.07, 1.63) <0.01 | 1.56 (1.26, 1.94) <0.01 | 1.45 (1.17, 1.81) <0.01 |
| TyG index tertiles as a continuous variable | 1.14 (1.03, 1.27) <0.01 | 1.25 (1.12, 1.39) <0.01 | 1.20 (1.08, 1.34) <0.01 |

*Model 1, unadjusted; model 2, adjusted for age, treatment arm, and ethnicity; model 3, full adjusted model, adjusted for age, treatment arm, ethnicity, baseline body mass index, chronic kidney disease (CKD) subgroup, cardiovascular disease (CVD) subgroup, aspirin used, and statin used. Ref, reference.*

**TABLE 3 |** Results of two-piecewise linear-regression model.

| | **Male** | **Female** | **Total** |
|---|---|---|---|
| One linear-regression model | 1.42 (1.18, 1.71) P<0.01 | 1.33 (0.97, 1.82) P=0.08 | 1.40 (1.20, 1.64) P<0.01 |
| Inflection point (K) | 8.72 | 7.76 | 8.71 |
| <K Effect size β (95% CI) | 1.84 (1.26, 2.69) P<0.01 | 30.84 (0.04, 22031.46) 0.3065 P=0. 31 | 1.66 (1.21, 2.27) P<0.01 |
| >K Effect size β (95% CI) | 1.14 (0.81, 1.61) P=0.45 | 1.26 (0.90, 1.75) P=0.18 | 1.20 (0.89, 1.62) P=0. 22 |
| Log likelihood ratio test | 0.118 | 0.274 | 0.214 |

*Two-piecewise linear-regression model was used to calculate the threshold effect of the TyG index. If the log likelihood ratio test >0.05, it means the two-piecewise linear regression model is not superior to the single-line linear regression model.*

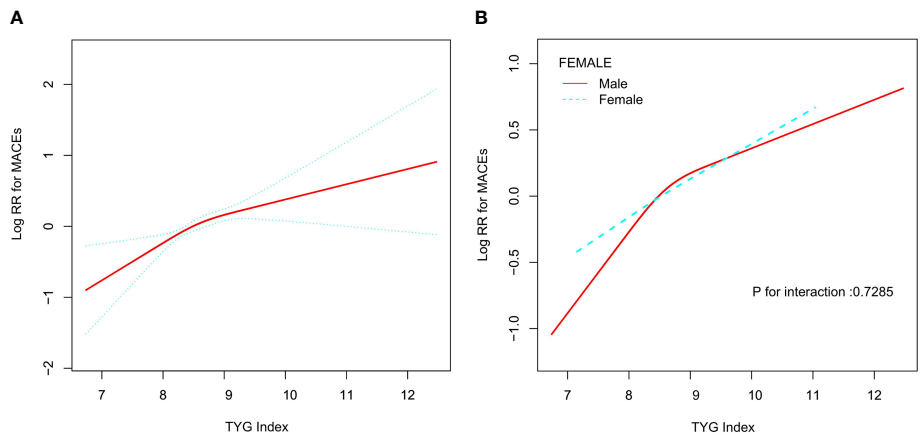


**FIGURE 1** | Relationship between Tyg index and MACEs. **(A)** Relationship between Tyg index and MACEs in all patients. The red line is the trend line and the blue line is the 95% confidence interval. **(B)** Relationship between Tyg index and MACEs grouped by sex. Male: red line; Female: blue line.

included in our study was significantly greater than in that study. This might weaken the gender differences caused by menopausal women.

Despite the above strengths and potential clinical implications, this research had some limitations that should be considered when interpreting the results. First, this was a *post-hoc* analysis, and the original study was not designed to examine the relationship between the TyG index and MACEs. Second, the study had a short follow-up time, and this limited the application of the results of this study.

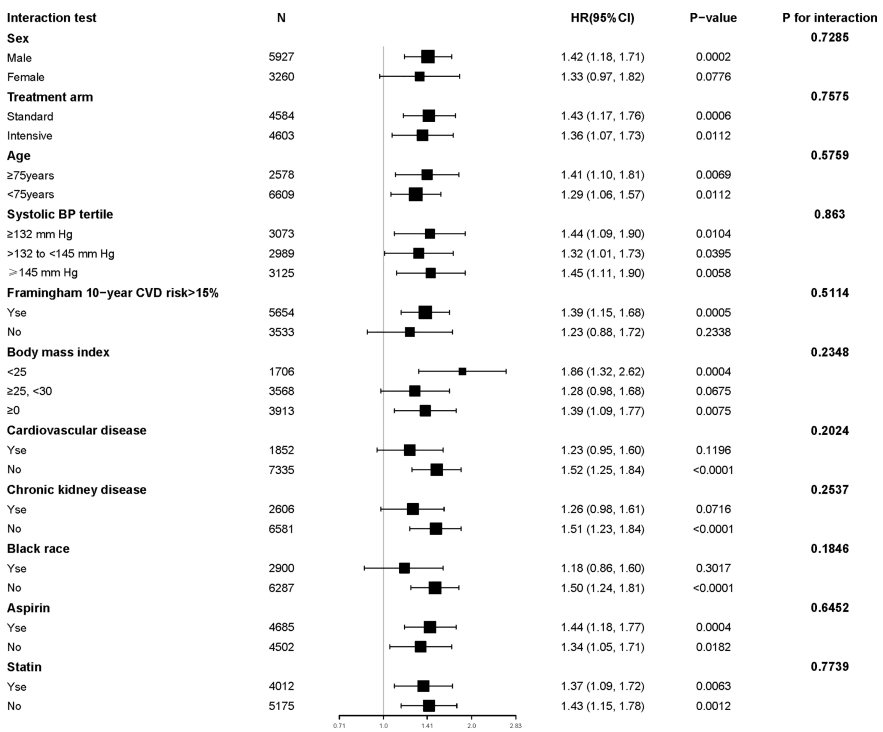


**FIGURE 2** | Subgroup analysis of the relationship between Tyg index and MACEs.

## CONCLUSION

In this *post-hoc* analysis using data from the SPRINT, we found that the TyG index was associated with MACEs in the hypertensive patients, and there was no gender difference between the TyG index and MACEs.

## AUTHOR CONTRIBUTIONS

KY wrote the paper. KY applied for the database and made statistical analysis. WL was responsible for the revision of the paper. Both authors contributed to the article and approved the submitted version.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://biolincc.nhlbi.nih.gov/studies/sprint/.

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REVIEW
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# Hyperuricemia and the Risk of Heart Failure: Pathophysiology and Therapeutic Implications

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The association between hyperuricemia and cardiovascular disease (CVD) has been reported and studied in the past two decades. Xanthine oxidase (XO) induced uric acid (UA) serves as a risk factor and has the independent prognostic and functional impact of heart failure (HF), but whether it plays a positive role in the pathogenesis of HF has remained unclear. Growing evidence suggest the up-regulated XO avtivity and increased production of free oxygen radical (ROS) correspondingly are the core pathogenesis of HF with hyperuricemia, which results in a whole cluster of pathophysiologic cardiovascular effects such as oxidative stress, endothelial dysfunction, vascular inflammation, left ventricular (LV) dysfunction as well as insulin resistance (IR). The use of XO inhibition represents a promising therapeutic choice in patients with HF due to its dual effect of lowering serum UA levels as well as reducing ROS production. This review will discuss the pathophysiologic mechanisms of hyperuricemia with HF, the targeted therapeutic interventions of UA lowering therapies (ULT) with XO inhibition and mechanism underlying beneficial effects of ULT. In addition, the review also summarizes current evidence on the role of ULT in HF and compares CV risk between allopurinol and febuxostat for practical and clinical purposes. Guidelines and implementation of CV risk management in daily practice will be discussed as well.

**Keywords: hyperuricemia, heart failure, cardiovascular disease, pathophysiology, treatment**

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## INTRODUCTION

Hyperuricemia is commonly defined as a serum UA concentration > 6.8 mg/dL, resulting predominantly from reduced renal excretion of uric acid (UA) (1). In recent years, the prevalence of hyperuricemia has been increasing worldwide and was seen in 14.6% of the US population (estimated 32.5 million individuals) in 2015. A number of epidemiological studies have shown that hyperuricemia is associated with the development of cardiovascular disease (CVD), chronic kidney disease (CKD), diabetes and metabolic diseases. Among them, the relationship between hyperuricaemia and heart failure (HF) has gained much attention for many years (2, 3).

As a systemic disease, HF represents hemodynamic failure and neuroendocrine activation of multiple other organs and systems. HF represents a growing public health burden with mortality rates and prevalence expecting to increase by 46% from 2012 to 2030, resulting in more than 8

million US adults with HF (4). In developed countries, it is reported that HF is the leading cause for non-elective hospitalizations for patients over 65 (5).

To date, a clear pathophysiological link between hyperuricemia and HF has yet to be confirmed. However, UA has been related to many of the established risk factors for HF, implying that hyperuricemia may play a vital role in HF. Findings from experimental studies indicated that the presence of hyperuricemia independently predicted the development of HF, including in individuals with normal cardic function. A systematic review and meta-analysis that reported HF morbidity and outcomes of adult patients found that hyperuricemia was associated with an increased risk of incident HF [hazard ratio (HR) 1.65, 95% confidence interval (CI) 1.41—1.94], and for every 1 mg/dL increased in UA, the odds of development of HF increased by 19% (6). Studies have demonstrated that hyperuricemia could be not only a risk factor but a strong and independent predictor of adverse outcomes of HF. Yuta et.al (7) had a finding regarding hyperuricemia and poor outcomes in 516 consecutive hospitalized HFpEF patients with decompensated HF, showing that hyperuricemia was significantly associated with increased incidence of all-cause death (p=0.016). Serum UA is a simple and inexpensive laboratory measurement with a wide range of clinical applications. Studies specifically found that UA could be a better predictor in HF of disease progression and impaired prognosis than BNP, but the threshold of predicting poor outcome has not been standardized yet. What's more, the debate is ongoing whether UA is merely a marker of poor prognosis or an active participant in pathogenesis of HF. Although strong evidence is emerging to prove a causal relationship between hyperuricemia and HF, most Mendelian randomization studies suggest serum urate is noncausal for comorbid traits (8). Additionally, asymptomatic hyperuricemia has been reported to increase a significant risk for cardiometabolic disorders (9). Serum UA levels can provide prognostic information alone or can be used in combination with other indicators of cardiac function, including left ventricular EF%. There are two major HF phenotypes, reduced versus preserved ejection fraction heart failure (HFrEF, HFpEF). Alberto et.alproved that in patients with HFrEF and HFpEF, hyperuricemia was related to the primary out of hospitalization or death, and the prevalence of hyperuricemia and the strength of its relationship with the primary outcome was greater in those with HFpEF. A study of elderly hypertensive outpatients found a strong inverse relationship between SUA and EF% for patients with mild to moderate HF. Moreover, elevated serum UA levels are independent predictor of mortality in patients with moderate-to-severe HF (10). The relationship between UA and EF% is independent of kidney function and diuretic usage, which excludes the possibility that the impaired renal excretion of UA is responsible for the association between hyperuricaemia and left ventricular dysfunction, even if this assumption requires further confirmation (e.g.by measures of 24-h uric acid excretion). Recent clinical evidence supported an expanded role for xanthine oxidase (XO) pathway in the pathogenesis of HF, as up-regulated activity of XO and increased reactive oxygen species (ROS) might lead to oxidative stress, endothelial dysfunction, vascular inflammation, etc, having detrimental effects on HF. In addition, as a UA lowering drug, allopurinol was reported to ameliorate outcomes in HF patients and become a marker of improved survival in the Seattle Heart Failure Model. These findings stimulated a growing research interest on the potential benefits of UA lowering therapies (ULT) and mechanisms underlying their effects on HF. In this review, we will discuss the underlying pathophysiology of hyperuricemia involved in the pathogenesis of HF. In particular, we will pay special attention to the potential effects and clinical implications of ULT on the progression of HF by reviewing most of available data on the medications related to hyperuricemia management. Finally, we will consider topics that need further research with the aim to decrease the HF burden of patients with hyperuricemia.

## HYPERURICEMIA: PATHOPHYSIOLOGY IN HF

Although the causal relationship between hyperuricemia and HF remains unknown, experimental and clinical studies have suggested hyperuricemia may be pathogenic and participates in the pathophysiology of HF by serving as a bridging mechanism mediating the deleterious effects on HF. **Figure 1** illustrates the possible pathophysiological mechanisms linking hyperuricemia and HF.

### Oxidative Stress

Up-regulated XO can contribute to the pathogenesis of HF through the oxidative stress induced by XO-derived UA and ROS. As an antioxidant, UA is capable of neutralizing dangerous pro-oxidants (11). However, evidence suggests that UA may function as a pro-oxidant in the hydrophobic intracellular environment, either by generating free radicals or by stimulating nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase. But more data must be collected to confirm this theory. Certainly, UA can reflect the potential activity of XO (12). Different from UA, XO-derived ROS plays a greatly clear role in promoting oxidative stress in HF. There are various sources of ROS within the cells, such as free radical superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and peroxynitrite ($ONOO^-$) (13). XO is the major CV sources of ROS in higher mammals that thought to have a prominent effect on cardiac function. The increased markers of oxidative stress have been observed in animal models of HF, which supports the theory that ROS may be the result of the progression of myocardial failure.

Increased ROS and UA production can lead to excess oxidative stress, protein and lipid peroxidation, DNA mutagenesis and eventually contribute to irreversible cardiomyocytes damage. Yu et.al (14) found a novel mechanism of UA-induced endothelial dysfunction-oxidative stress with an activation of the renin-angiontensin system (RAS) in human vascular endothelial cells. ROS interacts readily with endothelium-derived nitric oxide (NO) to produce $ONOO^-$ (15), which decreases NO bioavailability (16) and starts a cascade of detrimental oxygen radical effects on endothelial cell, causing vascular endothelial dysfunction in HF. In addition, excess cardiac fibrosis is considered as an important detrimental factor of chronic heart failure (CHF). ROS plays a role

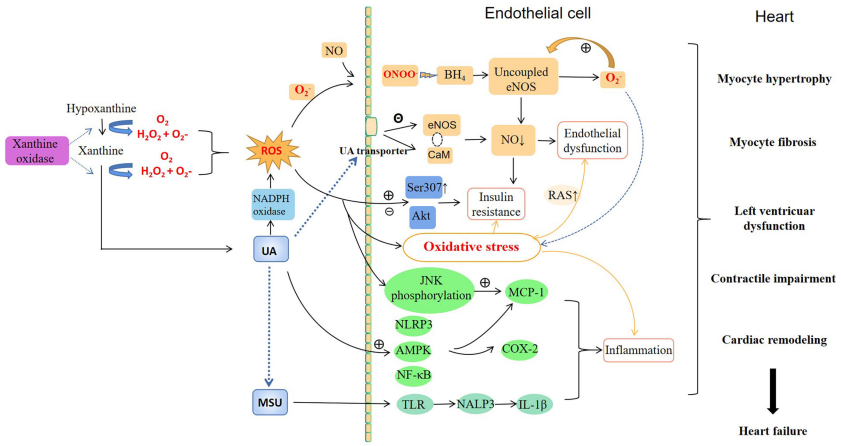


**FIGURE 1** | Potential mechanisms by which hyperuricemia might mediate HF.

in cardic fibrosis by inducing cardiac fibroblast proliferation and activating XO-mediated matrix metalloproteinases (MMP), leading to extracellular remodelling (17). James et.al (18) reported that ROS depressed $Ca^{2+}$ accumulation by sarcoplasmatic reticulum (SR) and $Ca^{2+}$ ATPase of SR, inducing a decrease in cardiac contractility. Allopurinol diminished the ROS effects on myofilament $Ca^{2+}$ sensitivity, contributing to the improvement of LV contractile function and efficiency. What's more, cardiomyocyte ROS and MMP activation may play a causative role in the myofibrillar degeneration, and are responsible for myosin and troponin degradation during ischemia/reperfusion injury of the heart, resulting in LV dysfunction (19). Accumulating data shows that this impaired oxidative metabolism is the core pathogenesis of HF with hyperuricemia, and it is implicated in the development of endothelial dysfunction, myocardial fibrosis, LV remodelling, and contractility impairment responsible for worse clinical status in patients with HF.

## Endothelial Dysfunction

Endothelial dysfunction can be defined as the condition that impairs the balance between endothelium-dependent vasodilation and constriction. NO is generated by endothelial NO synthase (eNOS), and reduced NO bioavailability contributes to endothelial dysfunction and oxidative stress, which is the key mechanism of CV risk and dysfunction. Experimental studies have indicated that UA absorbes into endothelial cells *via* UA transporters and induces oxidative stress, inflammation, or proliferation of vascular smooth muscle cells (VSMC), contributing to endothelial dysfunction through a reduction of endothelial NO bioavailability (20). Studies reported UA could attenuate eNOS activity and NO production or in human umbilical vein endothelial cells (HUVEC) by significantly decreasing the interaction between eNOS and calmodulin (CaM) or enhancing protein kinase C (PKC)-dependent eNOS phosphorylation (21, 22).

In environments of high ROS, $O_2^-$ reacts avidly with vascular NO to form $ONOO^-$. Considered as a highly active oxygen radical, $ONOO^-$ leads to lipid peroxidation and destroys endothelial membrane, accelerating the development of endothelial dysfunction (16). Moreover, $ONOO^-$ can oxidize tetrahydrobiopterin, the vital eNOS cofactor, so that eNOS failures to produce NO but $O_2^-$. The process is referred to as eNOS uncoupling (23). $O_2^-$ can also induce endothelium injury directly and further promote eNOS uncoupling through a vicious cycle, contributing to ventricular remodeling and HF ultimately. Ajit ed.al (24) found that ROS regulated canonical Wnt signaling, inducing vascular endothelial dysfunction *via* redox regulatory protein p66(Shc)-regulated ROS.

## Vascular Inflammation

It is well documented that hyperuricemia induces vascular inflammation *via* multiple mechanisms including oxidative stress, VSMC proliferation, and endothelial cells injury. A finding has been shown that soluble UA could release chemokines and adhesion molecules and interfere with the formation of endothelial cell tubes in HUVECs in a dose-dependent way (25). Hui et.al (26)revealed that UA induced inflammation *via* Nod-Like Receptor Protein 3 (NLRP3)-inflammasome-mediated VSMC proliferation. AMP-activated protein kinase (AMPK) and nuclear factor-κB (NF-κB) are major pathways in mediating inflammatory response and participating in the expression of inflammatory factors induced by UA (27, 28). The inflammatory response caused by the deposition of monosodium urate (MSU) in the synovium can be recognized by Toll-like receptor (TLR), and then activates NACHT-PYD-containing protein 3 (NALP3) inflammasome. This is the most important mechanism of IL-1βsecretion. MSU-triggered neutrophils adhere to the endothelium, traverse through the vessel wall and reach the site of inflammation, contributing to a proinflammatory response by producing immune mediators (29).

Although ROS is essential for vascular homoeostasis, excessive ROS may cause vascular damage. UA and MSU can mediate the generation of ROS, which induces inflammation and promotes the progression of HF. ROS production is observed in MSU treated macrophages and the use of antioxidants or

knockdown of NAPDH subunit expressions can inhibit MSU-induced inflammasome activation (30, 31). ROS induces the phosphorylation of c-Jun N-terminal kinases (JNK), contributing to the production of monocyte chemotactic protein-1 (MCP-1) in macrophages (32). Therefore, inflammation from soluble UA, MSU and ROS might together contribute to the progression of HF, but it is often difficult to distinguish which mechanism acts first in CVD.

### Left Ventricular Dysfunction

Decompensation of cardic function can be observed in patients with HF, leading to LV dilation, hypertrophy, and myocardial fibrosis, further results in remodeling of the ventricular structure. LV dysfunction contributes to reduced ventricular compliance and decreased systolic function, consequently causes HF. The mechanisms of hyperuricemia causing LV dysfunction have not been definitely investigated. Several studies show elevated UA levels might result in the echocardiographic abnormalities related to HF by affecting endothelial function and inflammation. UA has been reported to inhibit both NO production by vascular endothelial cells and their proliferation and migration, prompting LV development (33). Microvascular dysfunction caused by inflammation can result in deposition of collagen with subsequent reduced ability of the myocardium to contract and relax, developing into HF with preserved ejection fraction (34, 35). In addition, recent studies have shown that hyperuricemia significantly induces cardiomyocyte apoptosis, interstitial fibrosis, diastolic dysfunction and ventricular remodeling through activation of calpain-1 and endoplasmic reticulum (ER) stress (36) or a ROS-dependent endothelin-1 (ET-1) pathway (37), accelerating the occurrence and deterioration of CVD.

### Insulin Resistance

Studies have found myocardial insulin mediates energy uptake by increasing the absorption of glucose and plays an essential role in protection against post-ischemic HF, while IR promotes the progression of HF. The causal relationship between hyperuricemia and IR has not been clearly determined and is under investigation. It was reported that increased UA concentration could reduce NO levels and further reduce the insulin sensitivity. Other reports showed that UA induced IR by increasing tissue NADPH oxidase or hs-CRP level, the latter was found to be an independent predictor of homeostatic model assesssment-insulin resistance (38). Oxidative stress induced by ROS may play a causal role in IR-related CV complications (39). Li Zhi et.al (40) reported hyperuricemia could increase ROS production and inhibit insulin-induced glucose uptake in H9c2 and primary cardiomyocytes. N-acetyl-L-cysteine (ROS scavenger) pretreatment could reverse the inhibitory effect. The mechanism may be that hyperuricemia increases phospho-IR substrate 1 (Ser307) and inhibits phospho-protein kinase B (Akt) response to insulin in myocardial tissues. It is reported that ULT in patients with hyperuricemia can improve IR. A double-blind crossover trial that randomly assigned patients to benzbromarone or placebo indicated that patients with hyperuricemia and HF showed an improvement in IR index (placebo, 5.4+/-2.6; benzbromarone, 3.0+/-1.7; $P<0.05$) (41). However, whether hyperuricemia has a causal relationship with IR and diabetes remains controversial and the mechanisms of myocardial IR induced by UA have not been fully elucidated. Nonetheless, it still can be a novel potential mechanism of CVD related to hyperuricemic.

## THERAPEUTIC IMPLICATIONS

The important contribution of hyperuricemia to the pathophysiology of HF may indicate that therapeutic strategies aimed at ULT may beneficially influence the course of the disease. In the next paragraphs we will explore the potential mechanisms of ULT on HF, discuss CV effects of drugs for hyperuricemia treatment and further compare whether allopurinol or febuxostat is more effective in treating HF.

### Mechanism Underlying Beneficial Effects of ULT

Currently, two potent classes of ULT medications are commonly used in clinical practice: XOI (e.g., allopurinol, febuxostat) and increasing UA excretion drugs (e.g., benzbromarone, probenecid). Studies have shown that ULT is associated with reduced risk of HF in hyperuricemic patients but the potential mechanisms remain uncertain. Moreover, it has not yet been definitely proved whether the cardioprotective effects of ULT are due to XO inhibition or UA reduction.

Actually, XO is a critical source of ROS that accounts for a range of detrimental processes in the pathophysiology of HF. XO inhibition treatment from ULT shows a beneficial effect on the outcomes in HF patients. Allopurinol has been reported to improve myocardial oxidative stress and attenuate cardiac fibrosis in cardiac diastolic dysfunction (42). George et.al (43) showed a dose-response curve for allopurinol and its effect on endothelial function that allopurinol significantly increased forearm blood flow response to acetylcholine. Febuxostat has been shown to control the formation of ROS and act against vascular inflammation promoted by oxidative stress (43). However, benzbromarone was reported to have no influence on BNP levels, NYHA functional class, or LV ejection fraction (LVEF) (42). The results demonstrated that UA lowering without XO inhibition might not improve hemodynamic impairment in pathophysiology of HF. What's more, several studies have illustrated the effect of XO inhibition on improved LV ejection fraction, cardiac remodeling, and peripheral perfusion (44, 45). Recently, studies have demonstrated that SGLT2i can dramatically improve clinical outcomes in diabetes, especially HF and progression of kidney disease. Factors that may contribute to these findings include: (1) improved glycemic control, (2) reduced serum UA levels, (3) reduction in all-cause mortality, CV mortality and improving HF (46, 47). A meta-analysis of randomized controlled trials showed SGLT2i significantly reduced SUA levels compared to controls [total weighted average difference (WMD) -37.73μmol/L, 95% CI (-40.591, -34)] (46, 47). Serum UA decreased in SGLT2i users owing to the increased urinary excretion rate of UA, which is

due to the inhibition of UA reabsorption mediated by the effect of the drug on the GLUT9, located at the collecting duct of the renal tubule (48). Therefore, SGLT2i has great benefits in reducing the risk of CV events in T2DM patients with hyperuricemia.

Accumulating evidence has suggested that blocking ROS accumulation may become a promising new treatment option for hyperuricemia. However, current studies confirm that ULT benefits young hypertensive patients, but the effect on HF has shown contradictory clinical outcomes. A network meta-analysis suggested that allopurinol therapy did not have a significantly low risk of mortality in terms of HF but might offset the adverse effects associated with long-term hyperuricemia in patients with HF (49). Givertz et al. found that after treatment of HF patients with allopurinol and placebo, There was no significant difference in changes in clinical status, 6-minute walk distances, and LVEF between two groups at 24 weeks, which may result from the study duration being not long enough to observe the benefits of XO inhibition (50). There has been no high quality RCT comparing allopurinol with placebo on clinical CV events. Therefore, an appropriate methodological approach is needed to evaluate the efficacy of XO inhibition and give a better description of the characteristics of HF patients. A longitudinal cohort in Taiwan found that serum UA ≥8 or <4 mg/dL could independently predict the elderly > 65 years with higher all-cause and CVD-related mortality (51). And when serum UA <4 mg/dL, the risks of mortality increased as serum UA levels decreased (52). High doses of allopurinol may have association with loss of CV protection (53). These results question the hypothesis that "the lower is better" regarding serum UA levels. U or J shaped association between serum urate and CV adverse outcomes was reported by some observational studies (51, 52). This may be due to the fact that UA is an important antioxidant, and low serum UA level may represent a decrease in total antioxidant capacity, which becomes an incentive for increasing CV risk. As have discussed above, there is still a controversy about ULT on CV outcomes in hyperuricemic patients with HF. Conversely, With regard to the impact of HF on ULT, furosemide prescribed for patients with HF may eliminate the inhibitory effect of allopurinol on XO (54). Therefore, the minimal effective dose of diuretics should be kept in order to decrease the risk of CVD. Underlying mechanisms and beneficial effects of ULT are needed to be further explore and prove in future studies. Besides, CV safety trials are required before guidelines recommend reducing UA below a certain threshold.

## Comparative CV Risk Between Allopurinol and Febuxostat

Studies reporting on the relationship between ULT and CV risk have demonstrated conflicting results (**Table 1**). To date, most studies have been limited to allopurinol until the cardiovascular safety of febuxostat and allopurinol in patients with gout and cardiovascular morbidities (CARES) trial was initiated (58). Higher all-cause and CV mortality were found in patients with febuxostat gradually. Therefore, it is necessary to discuss and evaluate CV safety in allopurinol versus febuxostat.

As a XO enzyme inhibitor, allopurinol becomes currently the accepted first-line treatment as ULT for hyperuricemia. However, observational studies on whether the use of allopurinol may be associated with improved CV outcomes are inconclusive. Study showed allopurinol could significantly lower LVEF and improved CFR (61). In an RCT of 65 patients with coronary disease, allopurinol markly prolonged the time to the total exercise time (58s median increase, p=0.0003), the time to angina (38s median increase, p=0.001), and ST-segment depression (43s median increase, p=0.0002) (55). The study by de Abajo et al. found that allopurinol appeared to increase CV protection with greater duration of treatment and higher dose (62). The finding is consistent with the results from Wei et al. who observed a dose dependency with reduced CV events and mortality in HF in high dose compared with low-dose group (HR=0.63; 0.44 to 0.91). Recently, A study in Taiwan found that CVD could increase the risk of allopurinol hypersensitivity (63).

Compared with allopurinol, febuxostat provides highly selective and effective inhibition of XO and has higher UA lowing activity. Clinical trials found that febuxostat may improve oxidative stress status or ameliorate inflammation of hemodialysis patients with endothelial dysfunction (64, 65). In a phase II, multicenter, placebo-controlled study of 189 patients with gout, febuxostat was well tolerated in patients with gout and did not show an increased risk of CV complications (57). However, the safety of febuxostat shows conflicting results. FDA adverse event reporting system (AERS) in US reported febuxostat-related CV thromboembolic events from the database (66). The CARES trial conducted by the US Food and Drug Administration (FDA) has observed that the major CV events of febuxostat group were similar to those associated with allopurinol treatment. However, all-cause and CV mortality were higher in the febuxostat group comparable to those of allopurinol [HR for death from any cause, 1.22 (95% CI, 1.01 to 1.47); HR for CV death, 1.34 (95% CI, 1.03 to 1.73)] (58). Therefore, CARES results do not support the use of febuxostat as first-line treatment in ULT. However, there are some uncertainties from the results of CARES, such as high discontinuation rate and loss (67). With regard to the results in CARES, Montenegro et.al (68) proposed a possible explanation that febuxostat was more effective in blocking the reduction of XO-dependent nitrite levels, as compared with allopurinol, which might reduce beneficial effects of NO in CV homeostasis. Of note, there is no evidence to suggest that febuxostat is linked with greater CV risk than no XOI treatment. Nevertheless, the FDA has issued a black-box warning to restrict the use of febuxostat to gout patients who have failed or cannot tolerate maximum dose of allopurinol (69). However, subsequent studies still have shown inconsistent outcomes. A study showed that febuxostat might favorably affect CV mortality compared with allopurinol in elderly patients with mild-to-moderate HF (59). Recently, a randomized, blinded-endpoint trial in patients with gout in the UK, Denmark, and Sweden reported the long-term use of febuxostat didn't contribute to an increased risk of death or serious adverse outcomes compared with allopurinol (60). In HFrEF patients with elevated UA levels, XOI with allopurinol did not improve clinical status,

**TABLE 1** | Studies to assess or compare the effect of XO inhibitors in CVD.

| Study | Study design | Population | Mean follow-up | Treatment | Results | CV risk by treatment |
|---|---|---|---|---|---|---|
| **Comparison between a XO inhibitor versus placebo** | | | | | | |
| Awsan Noman et al (55), (UK) | Randomized, double-blind, placebo-controlled, crossover study | Chronic stable angina | 12-weeks | Allopurinol | Allopurinol prolonged the time to the total exercise time (58s median increase, p=0.0003), the time to angina (38s median increase, p=0.001), and ST-segment depression (43s median increase, p=0.0002) | Reduced |
| Li Wei et al (56), (Scotland) | Cohort study | Elderly (≥60 years old) | 5-years | Allopurinol | High-dose (≥300 mg) allopurinol had reduced risk of CV events (adjusted HR 0.69,95%CI 0.50–0.94) and mortality (adjusted HR 0.75,95% CI 0.59–0.94) | Reduced |
| Lhanoo Gunawardhana et al (57), (USA) | Phase II, multicenter, placebo-controlled, double-blind proof-of-concept study | Gout | 3-months | Febuxostat | Febuxostat lowered serum UA effectively and did not show an increased risk of CV complications | No difference |
| **Comparison between XO inhibitors** | | | | | | |
| William B. White et al (58), (USA) | Multicenter, double-blind, noninferiority trial | Gout with CVD | 32-months | Allopurinol *vs* Febuxostat | All-cause and CV mortality were higher in the febuxostat group than in the allopurinol group [HR for death from any cause, 1.22 (95% CI, 1.01 to 1.47); HR for CV death, 1.34 (95% CI, 1.03 to 1.73)]. | Higher risk in febuxostat |
| Arrigo Francesco Giuseppe Cicero et al (59), (Italy) | Cohort study (prospective) | Elderly with CHF | 5-years | Allopurinol *vs* Febuxostat | Febuxostat had a better CV outcome in patients treated with in comparison with allopurinol (The cumulative CV survival was 0.96 (95% CI 0.93–0.99) in febuxostat group and 0.89 (95% CI 0.84–0.93) in allopurinol group. | Lower risk in febuxostat |
| Isla S Mackenzie et al, (UK, Denmark (60), and Sweden) | Multicentre, prospective, open-label, non-inferiority trial | Elderly with Gout | 4-years | Allopurinol *vs* Febuxostat | Febuxostat is non-inferior to allopurinol therapy about the primary cardiovascular endpoint, and it is not associated with an increased risk of death or serious adverse events compared with allopurinol. | No difference |

*XO, xanthine oxidase; CVD, cardio vascular disease; UA, urate acid; CHF, Congestive heart failure.*

exercise capacity, quality of life, or LVEF at 24 weeks (50).To date, the exact effect on the CV risk between allopurinol and febuxostat has not been definitely proved and the mechanisms underlying these findings remain unanswered. More evidence is required to evaluate the CV risk of these drugs and guide clinical use in hyperuricemia.

## MANAGEMENT OF CV RISK

UA production and metabolism are complex processes and many enzymes are involved in the conversion of the adenine and guanine to UA. Initially, adenosine monophosphate (AMP) and guanine monophosphate (GMP) are converted into inosine and guanosine by deaminase and nucleotidase respectively, and are further converted into the purine bases hypoxanthine and guanine by purine nucleoside phosphorylase (PNP), which are finally oxidized by XO to form the final product UA (70). Therefore, XOI have thus been proposed as a strategy for reducing UA. In addition, for hyperuricemic patients combined with CV risk factors, not only medication but also life management should be implemented simultaneously. The European League Against Rheumatism (EULAR) guideline recommends that patients with gout should avoid excessive intake of meat and seafood, alcohol, and soft drinks that contain fructose. It has been reported that the metabolism of fructose stimulates the production of UA, because transient ATP consumption is usually accompanied by the production of AMP and stimulates the AMP deaminase (AMPD) to catalyze the degradation of AMP to inosine monophosphate and increase the degradation of purines (71). However, severe purine restriction can contribute to increased consumption of carbohydrates and saturated fats, which in turn lead to IR, triglycerides, and LDL cholesterol (72). Despite smoking has been found to be negatively correlated with gout recently (73), smoking cessation still be encouraged in the EULAR recommendations. It is reported that weight loss achieved by lifestyle changes reduced serum UA levels by 18% with a decrease in XOD activity (74). Furthermore, regular physical activity produces many cardioprotective effects including beneficial physiologic remodeling of the heart.

A retrospective matched cohort study found patients with gout had already an increased CV risk profile at the date of their incident diagnosis, and they were more likely to have prior CVD (75), which provides strong evidence for treatment of gout in primary care guidelines on CV risk management (75). International studies suggest that more than half of adverse outcomes can be prevented in patients with CVD risk by making sure everyone take aspirin, stay smoke free and control their blood pressure and lipid levels. These data indicate that all patients with gout are at high risk for CVD, and that screening and management of CVD risk may achieve a high therapeutic effect (76). Although there are guidelines for the administration of CV risk, management in clinical practice is often difficult due to a poor adherence to management in gout and therapies. Recently, among patients attending secondary care gout clinics in New Zealand, we found only 50% of eligible patients received aspirin treatment, 64% on β-blockersand and 53% on a statin (76). Nevertheless, a CVD care programme in New Zealand showed a successful implementation of CVD risk management. Patients with a 5-year CVD risk >10% were offered a single

**TABLE 2 |** Pathophysiologic mechanisms of hyperuricemia in HF and potential effects of ULT.

| Pathophysiological mechanisms | UA | ROS | Mechanism underlying beneficial effects of ULT |
|---|---|---|---|
| Oxidative stress | a. UA functions as a pro-oxidant in the hydrophobic intracellular environment (by generating ROS or stimulating NADPH oxidase)<br>b. UA induces endothelial dysfunction-oxidative stress with an activation of the RAS | a. ROS interacts with NO to produce $ONOO^-$ and starts detrimental oxygen radical effects on endothelial cell<br>b. ROS induces cardiac fibroblast proliferation and activates MMP and leads to cardic fibrosis and extracellular remodelling<br>c. ROS depresses $Ca^{2+}$ accumulation and $Ca^{2+}$ ATPase of SR, and decreases cardiac contractility | Allopurinol has been reported to improve myocardial oxidative stress and attenuate cardiac fibrosis in cardiac diastolic dysfunction (42) |
| Endothelial dysfunction | a. UA induces oxidative stress, inflammation, or proliferation of VSMC, and reduces endothelial NO bioavailability<br>b. UA attenuates eNOS activity and NO production or decreasing the interaction between eNOS and CaM or enhancing PKC-dependent eNOS phosphorylation | a. ROS-reduced $ONOO^-$ leads to lipid peroxidation and destroys endothelial membrane<br>b. $ONOO^-$ causes eNOS uncoupling<br>c. $O_2^-$ induces endothelium injury directly and further promotes eNOS uncoupling<br>d. ROS regulates canonical Wnt signaling and induces vascular endothelial dysfunction | Allopurinol had effects on endothelial function that significantly increased forearm blood flow response to acetylcholine (43) |
| Vascular inflammation | a. UA induces inflammation *via* NLRP3-inflammasome-mediated VSMC proliferation or AMPK and NF-κB signal pathways<br>b. MSU activates NALP3 inflammasome and secrets IL-1β | ROS induces the phosphorylation of JNK, and contributes to the production of MCP-1 in macrophages | Febuxostat has been shown to control the formation of ROS and act against vascular inflammation promoted by oxidative stress (43) |
| LV dysfunction | a. UA-induced inflammation can reduce ability of the myocardium to contract and relax<br>b. UA activates calpain-1 and ER stress and induces cardiomyocyte apoptosis, interstitial fibrosis and diastolic dysfunction | ROS leads to ventricular remodeling through a ET-1 pathway | Allopurinol diminished the ROS effects on myofilament $Ca^{2+}$ sensitivity, contributing to the improvement of LV contractile function and efficiency |
| IR | a. UA reduces NO bioavailability and generation of mitochondrial oxidative stress to result in IR<br>b. UA inhibits insulin-induced glucose uptake in H9c2 and primary cardiomyocytes | ROS plays a causal role in IR-related CV complications | Benzbromarone improved in IR index (41) |

*XO, xanthine oxidase; UA, urate acid; ROS, reactive oxygen species; ULT, uric acid lowering therapies; RAS, renin-angiotensin system; NO, nitric oxide; ONOO-, peroxynitrite; O2-, superoxide anion; MMP, matrix metalloproteinases; SR, sarcoplasmatic reticulum; VSMC, Vascular Smooth Muscle Cells; eNOS, endothelial nitric oxide synthase; CaM, calmodulin; PKC, protein kinase C; NLRP3, Nod-Like Receptor Protein 3; AMPK, AMP-activated protein kinase; NF-κB, nuclear factor-κB; MSU, monosodium urate; NALP3, NACHT-PYD-containing protein 3; IL-1β, Interleukin-1β; JNK, c-Jun N-terminal kinases; MCP-1, monocyte chemoattractant protein-1; LV, left ventricular; ER, endoplasmic reticulum; ET-1, endothelin-1; IR, insulin.*

intensive nurse-led intervention session and the great improvements were observed in blood pressure, prescriptions of aspirin and statins, and uptake of nicotine replacement products (77). Therefore, formal CVD risk assessments, more in-depth interventions and community long-term care support networks are necessary for hyperuricemic patients with CV risk. Moreover, we should not forget that patients often suffer from other comorbidities as well, and optimal preventive treatment requires to pay attention to these comorbidities. In the seventh Korean National Health and Nutrition Examination Survey from 2016 to 2017, there was an approximate U-shaped association between serum UA levels and 10-year CVD risk scores in males and the risk of CVD was the lowest when the serum UA level was 6.9 mg/dL. An approximately J-type association could be found in women (78). Therefore, it is necessary to appropriately manage UA levels in high-risk groups to reduce the risk of CVD. In a prospective cohort of 1193 patients with gout, serum UA ≥0.36 mmol/L was associated with increased overall mortality (HR=2.33, 95% CI 1.60 to 3.41) and CV-related mortality (HR= 2.05, 95% CI 1.21 to 3.45) (79). Maintaining serum UA <360μmol/L in a long term should be the main goal for these high-risk patients to reduce CV events and prolong patient survival. The EULAR guideline recommends that serum UA levels should be monitored at <360 mmol/L and a lower UA target (300mmol/L) is recommended for patients with severe gout (80). For asymptomatic hyperuricemia patients, Multidisciplinary consensus in Taiwan suggests they needn't immediate ULT, potential causes of hyperuricemia should be identified and appropriately dealt with, especially in diseases that may increase CV risks.

## CONCLUSIONS AND FUTURE RESEARCH

In the past two decades, a compelling body of evidence including both experimental and clinical has emerged, which directly links hyperuricemia with the development and progression of HF. The pathophysiologic mechanisms of hyperuricemia with HF is being

recently explored by the new data from experimental, epidemiological and clinical intervention trials (**Table 2**). This review suggests that the up-regulated XO avtivity and increased production of ROS correspondingly are the core pathogenesis of HF with hyperuricemia, which results in a whole cluster of pathophysiologic CV effects. Therefore, XO itself may serve as a novel and promising therapeutic target and XO inhibition may potentially lead to better clinical outcomes in HF. To date, there is no RCT that has compared XOIs with uricosurics on the clinical CV events so that large trials are warranted to demonstrate which medication is better. From a clinical perspective, clinicians need to identify the threshold of UA where ULT can effectively improve the adverse outcomes of HF without increasing the mortality. In the future, larger studies are conducted to determine whether ULT can further improve the clinical prognosis of the heterogeneous population of HF patients and analyze the sensitivity of different types of HF such as HFpEF and HFrEF to XO inhibition, thus carrying out targeted and individualized treatment. Although additional studies are needed to determine the threshold of UA for treatment initiation and to confirm optimal target levels, we believe that there is sufficient evidence to recommend routine screening for hyperuricemia in patients with HF as part of clinical practice and consider initiation of ULT among those who are hyperuricemic with evidence of deteriorating cardic function, unless there are specific contraindications.

## AUTHOR CONTRIBUTIONS

KS contributed to the conception and the writing of the article. CW and LX performed the framework. WL, BD and YZ contributed to the English grammar. ZW and YH gave the constructive discussions to the article. YW and YC revised important intellectual content critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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# Influence of the Triglyceride-Glucose Index on Adverse Cardiovascular and Cerebrovascular Events in Prediabetic Patients With Acute Coronary Syndrome

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**Background:** Cardiovascular disease and insulin resistance are closely related. The triglyceride-glucose (TyG) index is frequently used as an indicator of insulin resistance. However, there is scant information on the TyG index in the prediabetic population, nor is the prognostic significance of the index known for prediabetes and acute coronary syndrome (ACS) patients.

**Methods:** The clinical endpoint was a major adverse cardiovascular and cerebrovascular event (MACCEs), including cardiac-related death, non-fatal myocardial infarction, ischemia-driven revascularization, and stroke. The TyG index was calculated as = ln [(triglyceride level, mg/dL) × (glucose level, mg/dL)÷2] under fasting conditions.

**Results:** The study included 2,030 prediabetic patients with ACS. Patients were followed up for 2.5 years, during which the total incidence of MACCEs was 12%. After adjustment for covariates, the TyG index was found to be predictive of prediabetes with ACS (HR 4.942, 95%CI: 3.432-6.115, P<0.001). Using propensity score matching, 574 pairs were successfully matched, and the two groups were analyzed in terms of survival. This showed that there was a significantly greater incidence of MACCEs in patients with high TyG indices (HR 3.526, 95%CI: 2.618-4.749, P<0.001), mainly due to ischemia-driven revascularization and stroke.

**Conclusions:** The TyG index independently predicts future MACCEs and may be an important prognostic indicator for patients with prediabetes and ACS.

**Keywords: TyG index, prediabetes, ACS, prognosis, MACCEs**

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## BACKGROUND

The prevalence of diabetes has risen from 108 million to 422 million in the last thirty years, of which type 2 diabetes (T2DM) accounts for more than 90%, and studies estimate that it will increase to 642 million by 2040. Most patients go through a prediabetic stage before they develop diabetes (1, 2). The prevalence of prediabetes is also rising globally. Research shows that by 2030, more than 470 million people will suffer from prediabetes. Prediabetes is associated with co-existing insulin resistance (IR) and β-cell dysfunction, and these abnormalities begin before blood sugar changes are detected. Prediabetes is a complex, multi-factorial metabolic disorder, and its pathophysiology centers around IR, impaired incretin action, and high insulin secretion. Observational evidence has linked prediabetes with an elevated risk of nephropathy, diabetic retinopathy, and cardiovascular diseases (CVDs) (3–5).

IR is usually present in prediabetes, before development to T2DM (6). It has been found that the triglyceride-glucose (TyG) index is a practical method of evaluating IR, and the TyG index has been verified in multiple populations around the world, and its effectiveness and reliability was consistently showed in IR detecting (7, 8). Recent studies suggest that prediabetes is correlated with an increased risk of T2DM, CVD, dementia, and cancer; moreover, its incidence is increasing worldwide (9). In China, the prevalence of prediabetes rose from 15.5% in 2008 to 35.2% in 2017, with about 357 million people suffering from prediabetes (10, 11). Notably, the best control of glycemia and IR as in the case of metformin therapy could result in these patients in best clinical outcomes (12, 13). Moreover, asymptomatic diabetic patients with high TyG indices have a higher risk of coronary artery stenosis (14). Therefore, the TyG index may be a valuable biomarker for the development of diabetes (15, 16), allowing the effective screening and early detection of individuals at high risk for T2DM (17). In addition, it has been found that after adjustment for confounders, there was a close relationship between the TyG index and prediabetes (18).

In addition, the TyG index, which also measures IR, may be helpful for the early recognition of cardiovascular events, with higher TyG indices in high-risk groups related to an increased risk of CVD (19). Although there has been an increase in the number of studies on the TyG index and CVDs recently, there is still a lack of prognosis-related studies on acute coronary syndrome (ACS) in prediabetic patients. Identification of an effective means of evaluating the prognosis of prediabetic patients with ACS would assist the recognition of those at high risk of major adverse cardiovascular and cerebrovascular events (MACCEs) for closer monitoring or potential early intervention. Based on the results of follow-up, we aimed to explore the relationship between the prognosis and the TyG index in prediabetic patients with ACS.

**Abbreviations:** T2DM, type 2 diabetes; IR, insulin resistance; CVDs, cardiovascular diseases; TyG, triglyceride-glucose; ACS, acute coronary syndrome; MACCEs, major adverse cardiovascular and cerebrovascular events; HbA1c, glycosylated hemoglobin A1c; UAP, unstable angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; CABG, coronary artery bypass grafting; ROC, receiver operating characteristic; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; FBG, fasting blood glucose; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; Cr, creatinine; SUA, serum uric acid; CRP, C-reactive protein; hs-TNI, high sensitivity troponin I; MI, myocardial infarction; IQR, interquartile range; HRs, hazard ratios; RCS, restricted cubic spline; CI, confidence level; ACEI, angiotensin-converting enzyme inhibitor; AUC, area under curve; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HOMA-IR, Homeostasis model assessment of insulin resistance; CAC, coronary artery calcification; CAD, coronary artery disease; CTA, computed tomography angioplasty; ICU, intensive care unit; PCI, percutaneous coronary intervention; AMI, acute myocardial infarction.

## METHODS

### Study Design, Patient Population and Definitions

This single center, retrospective, observational study enrolled 2030 patients with prediabetes and ACS admitted to Anzhen Hospital for coronary angiography from August 2018 to September 2019. Using the definition of prediabetes, we discussed and reviewed previous studies and found that although the new glycosylated hemoglobin A1c (HbA1c) criteria identified fewer high-risk individuals than those with impaired fasting glucose, HbA1c (in the range of 5.7-6.4%) had a similar the predictive value to impaired fasting glucose alone (20). At the same time, considering that the fasting glucose of patients admitted to hospital may be affected by their diet, the final definition was based on the 2021 AHA Classification and Diagnosis of Diabetes, with an HbA1c range from 5.7% to 6.4% (21). ACS was defined based on appropriate guidelines, including unstable angina pectoris (UAP), ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) (22). The exclusion criteria were patients with (1) abnormal liver function: severe insufficiency with alanine transaminase (ALT) or aspartate transaminase (AST) over 5 upper limit of normal; abnormal kidney function: with estimated glomerular filtration rate (eGFR) < 30 mL/(min * 1.73 $m^2$); (2) incomplete baseline and follow-up clinical data; (3) a history of coronary artery bypass grafting (CABG); (4) any kind of cancer or other major diseases affecting long-term survival. The TyG index cut-off was calculated using the receiver operating characteristic (ROC) curve (TyG index = 8.83). The patients were assigned to two groups based on their TyG indices, the "high TyG index group" (TyG index ≥ 8.83) and the "low TyG index group" (TyG index < 8.83). Patients were matched through the 1:1 propensity score between the two groups, and 574 pairs were successfully identified for survival analysis. These details are shown in the flow chart (**Figures 1** and **2**).

### Data Collection and Laboratory Examination

We performed the data collection of clinical characteristics using case report form, including age, gender, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), previous medical history, laboratory examination and types of medication taken. The TyG index was defined as previously reported: TyG index = ln (fasting triglyceride (TG, mg/dL) ×

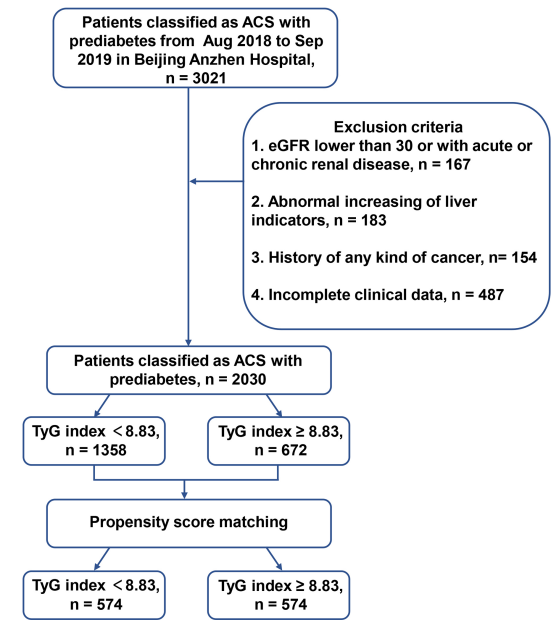


**FIGURE 1** | The flow chart of enrolled patients. ACS, acute coronary syndrome; HbA1c, glycosylated hemoglobin A1c; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; TyG, triglyceride-glucose.

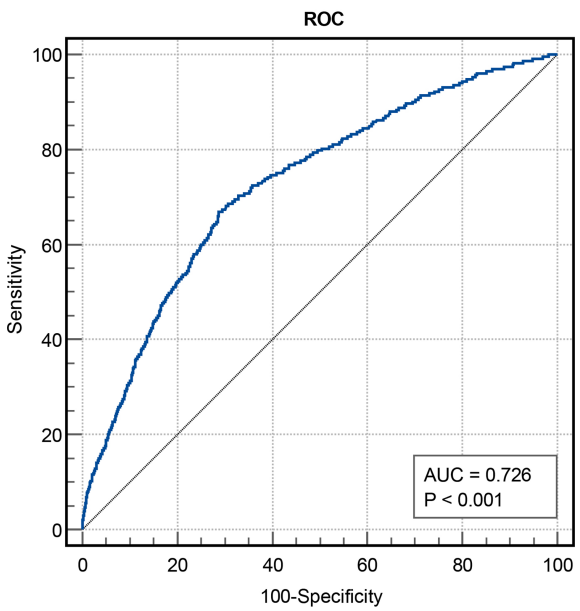


**FIGURE 2** | The predictive values of TyG index for the risk of MACCEs. TyG, triglyceride-glucose; MACCEs, major adverse cardiovascular and cerebrovascular events; AUC, area under curve.

fasting blood glucose level (FBG, mg/dL)/2) (23). The collection of peripheral venous blood samples was performed in the morning after an overnight fast and immediately sent for analysis to the central laboratory methods of Beijing Anzhen Hospital. The analysis included TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), creatinine (Cr), serum uric acid (SUA), FBG, HbA1c, C-reactive protein (CRP), high sensitivity troponin I (hs-TNI), amongst other hematological and biochemical parameters. All of the above biochemical variables were evaluated at baseline.

## Definition of Clinical Endpoints

All patients were routinely followed up at 3, 6 and 12 months, and then annually for 30 months. Information on adverse events was gained from patients or their families by telephone questionnaires. New-onset MACCEs was defined as the primary endpoint comprising cardiac-related death, non-fatal myocardial infarction (MI), ischemia-driven revascularization, and stroke. Secondary endpoints included the same events (24, 25), which were recorded for each patient during the 2.5-year follow-up after discharge. The first primary endpoint event that occurred during the follow-up was used for analysis. For multiple adverse outcomes during the follow-up, only the most severe event was used (cardiac death > MI/stroke > ischemia-driven revascularization).

## Statistical Analysis

Normally distributed continuous variables were represented as means ± standard deviation, while those with skewed distribution were shown as median (interquartile range [IQR]). Besides, the continuous variables between two groups were compared using one-way analysis of variance or the Kruskal-Wallis test, and the categorical variables were showed as frequencies and analyzed using test of chi-square. The Pearson and Spearman correlation analyses were applied to assess the relationship between two variables as appropriate. Time dependent Cox proportional hazards regression was performed to evaluate the hazard ratios (HRs) for MACCEs associated with the predictive values of TyG and variables. In multivariate analyses, in addition to age, gender (reference to male), BMI, SBP, DBP, smoking status, history of hypertension and hyperlipemia, levels of LDL-C, HDL-C, Cr, SUA, eGFR, brain natriuretic peptide (BNP), and CRP were adjusted. Restricted cubic spline (RCS) regression was used to show the graphical association between TyG index and MACCEs. ROC curves were used to assess the cut-off point of the TyG index for MACCEs. To match the patients between the high-TyG index and low-TyG index groups, a 1:1 propensity score-matched analysis was conducted (details in **Supplementary Material**). Kaplan–Meier analysis was performed to evaluate the time-related events and the evaluation of discrepancies was assessed by log-rank tests. Two-tailed P-values < 0.05 were regarded as statistically significant. All statistical analyses in this study used MedCalc version 20.0.3, SPSS version 22.0 and R version 4.0.0.

# RESULTS

## Baseline Characteristics

In total, 2030 patients were enrolled in the study. During 2.5 years of follow-up, 233 (11.5%) of the 2030 patients experienced

MACCEs, including 11 (0.5%) cardiac deaths, 29 (1.4%) non-fatal MIs, 180 (8.9%) ischemia-driven revascularizations and 41 (2.0%) strokes. Using stratification based on MACCEs occurrence, the baseline clinical characteristics of the overall population were grouped into MACCEs and non-MACCEs (**Table 1**). The TyG index levels in the MACCEs group (9.00 ± 0.51) were significantly elevated compared to those in the non-MACCEs group (8.59 ± 0.48) (P<0.001). The two groups also differed significantly in terms of TC, TG, HDL-C, Cr, SUA, FBG, HbA1c, hs-TNI, and use of angiotensin-converting enzyme inhibitor (ACEI) medication (P<0.05) but not in terms of other indicators. The area under the curve (AUC) of the TyG index for MACCEs was 0.726 (95% CI 0.691–0.761, P < 0.001, P<0.001). The TyG index of 8.83 was determined as the optimal cutoff point for predicting MACCEs with a sensitivity of 64.4% and a specificity of 71.7%. Patients with raised TyG indices tended seemed to be younger, with high BMI and DBP levels and a greater incidence of hypertension compared to the lower TyG index group. Besides these, the levels of LDL-C, TC, TG, Cr, SUA, FBG, and β-blocker use were higher in the group with high TyG indices (P<0.01), while the HDL-C levels were lower in the group with low TyG indices (P<0.001) (**Table 2**).

## The TyG Index and Indicators of Cardiovascular Risk

The correlations between traditional cardiovascular risk indicators or commonly-used risk indicators of CVD and the TyG index were examined. The TyG index was positively linked to DBP, history of hyperlipemia, and smoking status (P<0.05) while correlating negatively with gender, HDL-C and BNP (P<0.05) (**Figure 3**).

## The TyG Index and MACCEs

After the stratification of the MACCEs incidence, propensity score matching was performed (**Figure 4**). This showed

**TABLE 1** | Baseline clinical characteristics among the MACCEs and non-MACCEs group of overall population.

| Characteristics | Overall (n=2030) | Non-MACCEs (n=1797) | MACCEs (n=233) | P |
|---|---|---|---|---|
| **Demographic** | | | | |
| **Age, years** | 58.87 ± 10.27 | 59.02 ± 10.29 | 57.74 ± 10.06 | 0.074 |
| **Gender, (male%)** | 1505 (74.1) | 1327 (73.8) | 178 (76.4) | 0.449 |
| **BMI, kg/m$^2$** | 25.64 ± 3.31 | 25.59 ± 3.34 | 25.97 ± 3.08 | 0.107 |
| **SBP, mmHg** | 128.42 ± 16.83 | 128.46 ± 16.84 | 128.06 ± 16.77 | 0.735 |
| **DBP, mmHg** | 76.91 ± 10.86 | 76.86 ± 10.76 | 77.26 ± 11.63 | 0.603 |
| **Medical history, n (%)** | | | | |
| **Smoking** | 1026 (50.5) | 902 (50.2) | 124 (53.2) | 0.424 |
| **Hypertension** | 1214 (59.8) | 1074 (59.8) | 140 (60.1) | 0.982 |
| **Hyperlipemia** | 1412 (69.6) | 1252 (69.7) | 160 (68.7) | 0.813 |
| **Pre-PCI** | 479 (23.6) | 425 (23.7) | 54 (23.2) | 0.937 |
| **Laboratory results** | | | | |
| **LDL-C, mmol/L** | 2.41 ± 0.84 | 2.40 ± 0.85 | 2.46 ± 0.84 | 0.323 |
| **TC, mmol/L** | 4.04 ± 0.98 | 4.03 ± 0.98 | 4.16 ± 1.02 | 0.046 |
| **TG, mmol/L** | 1.38 ± 0.71 | 1.32 ± 0.64 | 1.86 ± 0.96 | <0.001 |
| **HDL-C, mmol/L** | 1.14 ± 0.26 | 1.15 ± 0.27 | 1.07 ± 0.22 | <0.001 |
| **TyG index** | 8.64 ± 0.50 | 8.59 ± 0.48 | 9.00 ± 0.51 | <0.001 |
| **Cr, μmol/L** | 70.86 ± 14.80 | 70.61 ± 14.54 | 72.82 ± 16.58 | 0.032 |
| **SUA, μmol/L** | 355.37 ± 88.15 | 352.97 ± 85.75 | 373.86 ± 103.20 | 0.001 |
| **eGFR, mL/(min* 1.73 m$^2$)** | 96.48 ± 13.09 | 96.50 ± 12.98 | 96.34 ± 13.94 | 0.858 |
| **BNP, pg/mL** | 47.33 ± 102.55 | 47.35 ± 101.94 | 47.16 ± 107.36 | 0.979 |
| **CRP, mg/L** | 3.17 ± 5.56 | 3.13 ± 5.55 | 3.48 ± 5.63 | 0.369 |
| **FBG, mmol/L** | 5.83 ± 1.26 | 5.77 ± 1.21 | 6.25 ± 1.56 | <0.001 |
| **HbA1c, %** | 6.00 ± 0.24 | 5.99 ± 0.24 | 6.05 ± 0.24 | 0.002 |
| **hs-TNI, pg/mL** | 0.36 ± 2.42 | 0.30 ± 2.12 | 0.80 ± 4.02 | 0.003 |
| **Clinical presentation, n (%)** | | | | |
| **STEMI** | 118 (5.8) | 98 (5.5) | 20 (8.6) | 0.076 |
| **NSTEMI** | 108 (5.3) | 94 (5.2) | 14 (6.0) | 0.732 |
| **UAP** | 1804 (88.9) | 1605 (89.3) | 199 (85.4) | 0.094 |
| **Medication, n (%)** | | | | |
| **Antiplatelet** | 2029 (100.0) | 1796 (99.9) | 233 (100.0) | 1.000 |
| **Statin** | 2026 (99.8) | 1793 (99.8) | 233 (100.0) | 1.000 |
| **ACEI** | 337 (16.6) | 284 (15.8) | 53 (22.7) | 0.010 |
| **ARB** | 1504 (74.1) | 1320 (73.5) | 184 (79.0) | 0.084 |
| **β-blocker** | 1608 (79.2) | 1422 (79.1) | 186 (79.8) | 0.872 |
| **Nitrate** | 1841 (90.7) | 1628 (90.6) | 213 (91.4) | 0.775 |

*BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PCI, percutaneous coronary intervention; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; TyG, triglyceride-glucose; Cr, creatinine; SUA, serum uric acid; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin A1c; hs-TNI, high sensitivity troponin I; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; UAP, unstable angina pectoris; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.*

**TABLE 2** | Baseline clinical characteristics of patients stratified by the optimal cutoff point of TyG index.

| Characteristics | Overall (n=2030) | Lower TyG index (<8.83, n=1358) | Higher TyG index (≥8.83, n=672) | P |
|---|---|---|---|---|
| **Demographic** | | | | |
| **Age, years** | 58.87 ± 10.27 | 59.70 ± 10.17 | 57.21 ± 10.28 | <0.001 |
| **Gender, (male%)** | 1505 (74.1) | 1021 (75.2) | 484 (72.0) | 0.140 |
| **BMI, kg/m$^2$** | 25.64 ± 3.31 | 25.39 ± 3.35 | 26.14 ± 3.18 | <0.001 |
| **SBP, mmHg** | 128.42 ± 16.83 | 128.45 ± 17.02 | 128.35 ± 16.45 | 0.907 |
| **DBP, mmHg** | 76.91 ± 10.86 | 76.52 ± 10.92 | 77.69 ± 10.72 | 0.022 |
| **Medical history, n (%)** | | | | |
| **Smoking** | 1026 (50.5) | 689 (50.7) | 337 (50.1) | 0.840 |
| **Hypertension** | 1214 (59.8) | 791 (58.2) | 423 (62.9) | 0.047 |
| **Hyperlipemia** | 1412 (69.6) | 939 (69.1) | 473 (70.4) | 0.603 |
| **Pre-PCI** | 479 (23.6) | 337 (24.8) | 142 (21.1) | 0.074 |
| **Laboratory results** | | | | |
| **LDL-C, mmol/L** | 2.41 ± 0.84 | 2.30 ± 0.82 | 2.63 ± 0.85 | <0.001 |
| **TC, mmol/L** | 4.04 ± 0.98 | 3.88 ± 0.93 | 4.37 ± 1.00 | <0.001 |
| **TG, mmol/L** | 1.38 ± 0.71 | 1.05 ± 0.32 | 2.05 ± 0.79 | <0.001 |
| **HDL-C, mmol/L** | 1.14 ± 0.26 | 1.18 ± 0.27 | 1.06 ± 0.23 | <0.001 |
| **TyG index** | 8.64 ± 0.50 | 8.37 ± 0.33 | 9.17 ± 0.31 | <0.001 |
| **Cr, μmol/L** | 70.86 ± 14.80 | 70.24 ± 14.10 | 72.12 ± 16.05 | 0.007 |
| **SUA, μmol/L** | 355.37 ± 88.15 | 345.40 ± 82.50 | 375.50 ± 95.54 | <0.001 |
| **eGFR, mL/(min* 1.73 m$^2$)** | 96.48 ± 13.09 | 96.55 ± 12.50 | 96.35 ± 14.21 | 0.742 |
| **BNP, pg/mL** | 47.33 ± 102.55 | 49.61 ± 104.01 | 42.72 ± 99.47 | 0.155 |
| **CRP, mg/L** | 3.17 ± 5.56 | 3.10 ± 5.66 | 3.31 ± 5.36 | 0.419 |
| **FBG, mmol/L** | 5.83 ± 1.26 | 5.54 ± 0.97 | 6.40 ± 1.56 | <0.001 |
| **HbA1c, %** | 6.00 ± 0.24 | 6.00 ± 0.24 | 6.00 ± 0.24 | 0.763 |
| **hs-TNI, pg/mL** | 0.36 ± 2.42 | 0.31 ± 2.40 | 0.45 ± 2.46 | 0.215 |
| **Clinical presentation, n (%)** | | | | |
| **STEMI** | 118 (5.8) | 79 (5.8) | 39 (5.8) | 0.999 |
| **NSTEMI** | 108 (5.3) | 68 (5.0) | 40 (6.0) | 0.431 |
| **UAP** | 1804 (88.9) | 1211 (89.2) | 593 (88.2) | 0.580 |
| **Medication, n (%)** | | | | |
| **Antiplatelet** | 2029 (100.0) | 1358 (100.0) | 671 (99.9) | 0.719 |
| **Statin** | 2026 (99.8) | 1354 (99.7) | 672 (100.0) | 0.381 |
| **ACEI** | 337 (16.6) | 212 (15.6) | 125 (18.6) | 0.101 |
| **ARB** | 1504 (74.1) | 1020 (75.1) | 484 (72.0) | 0.150 |
| **β-blocker** | 1608 (79.2) | 1049 (77.2) | 559 (83.2) | 0.002 |
| **Nitrate** | 1841 (90.7) | 1235 (90.9) | 606 (90.2) | 0.634 |

*BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PCI, percutaneous coronary intervention; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; TyG, triglyceride-glucose; Cr, creatinine; SUA, serum uric acid; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin A1c; hs-TNI, high sensitivity troponin I; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; UAP, unstable angina pectoris; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.*

significant differences between the low and high-TyG index groups in terms of gender, age, SBP, DBP, BMI, statin therapy, TC, SUA, LDL-C, Cr, BNP, CRP, eGFR, history of hyperlipemia, hypertension and smoking was not found.

### Prediction of MACCEs Using the TyG Index

Among the overall population, in the univariate Cox proportional hazard analysis, the TyG index, Cr, and SUA were found to be independently related with MACCEs (P<0.05). In the multivariate analysis, the variables gender, age, BMI, LDL-C, HDL-C, SBP, DBP, TyG index, Cr, SUA, eGFR, BNP, CRP, history of smoking, hypertension, and hyperlipemia were adjusted. It was found that after adjustment, only the TyG index was independently related to MACCEs (**Table 3**). Using RCS, we evaluated the shape of the association using penalized splines to examine the relationship between MACCEs risk and the TyG index. In populations of pre/post propensity score matching, with increased TyG index, the RCS curves showed the same trend of monotonic increase in the risk of MACCEs (**Figure 5**).

### The TyG Index and Clinical Outcomes

The occurrence of MACCEs, ischemia-driven revascularization, and stroke differed significantly between the groups (P<0.01), shown by pre/post propensity score matching and Cox proportional hazard analyses (**Table 4**). Kaplan–Meier survival analysis confirmed a greater incidence of MACCEs (P < 0.001), ischemia-driven revascularization (P < 0.001), and stroke (P = 0.002) in the higher TyG index group (**Figure 6**).

## DISCUSSION

This appears to be the first investigation of the relationship between prognosis and the TyG index in prediabetic patients with ACS. Firstly, the study included 2030 patients with

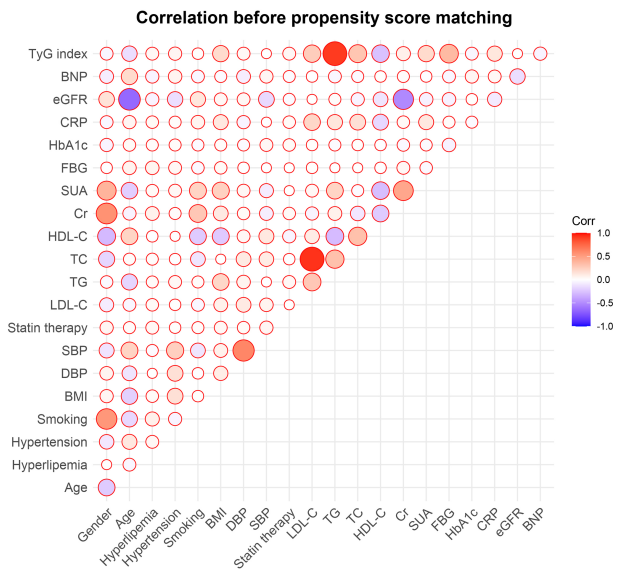


**FIGURE 3** | Correlations between the TyG index and traditional cardiovascular risk factors. TyG, triglyceride-glucose; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; HbA1c, glycosylated hemoglobin A1c; FBG, fasting blood glucose; SUA, serum uric acid; Cr, creatinine; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

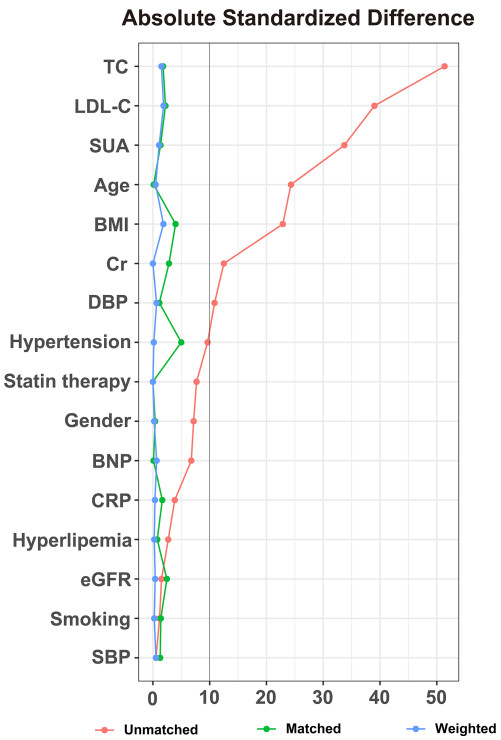


**FIGURE 4** | Absolute standardized differences in unweighted and propensity score-weighted data sensitivity analyses. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; SUA, serum uric acid; BMI, body mass index; Cr, creatinine; DBP, diastolic blood pressure; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

prediabetes and ACS who were followed up for two-and-a-half years during which the total incidence of MACCEs was 11.5%. After 1:1 propensity score matching, MACCEs incidence to be significantly greater in patients with high TyG indices, with ischemia-driven revascularization and stroke dominating. Secondly, multivariate Cox regression analysis of the overall population showed a significant link between the TyG index and MACCEs, and the RCS curve also indicated a consistent increase between the index and the HR both before and after matching.

## The TyG Index and Atherosclerosis

The TyG index has long been regarded as an IR indicator. IR in the liver is frequently estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). Kim et al. concluded that TyG index was more effective HOMA-IR in predicting coronary artery calcification (CAC) (26), and Park et al. have also pointed out that the TyG index predicts CAC progression, especially in adults with non-severe CAC (27). IR has also been found to influence plaque development by promoting apoptosis in vascular smooth muscle cells, macrophages and endothelial cells (28). In addition, studies have shown that prediabetic patients have more severe coronary atherosclerosis and plaque vulnerability than non-diabetic patients (29). Considering the substantial impact of diabetes on CAC, these results may be related to the increased prevalence of diabetes and the increase in the TyG index (30), where the critical mechanisms may include the activation of endothelial dysfunction, vascular inflammation, and oxidative stress by hyperglycemic injury (31). An observational study reported that in asymptomatic diabetic patients, the CAC score can be used effectively to assess obstructive coronary artery disease (CAD) by coronary computed tomography angioplasty (CTA) (32). Subsequently, observing coronary plaques through CTA, Won et al. also found a correlation between the TyG index and severity of CAD, suggesting that IR accompanied by CAC affects the progression of coronary plaques (33).

## Prediction of MACCEs by the TyG Index in Prediabetic Patients

### The TyG Index and Stroke

An association between the TyG index and increased probability of IR recurrence, neurological decline, and all-cause mortality has been found in patients with ischemic stroke (34). A higher TyG index can also predict a poor functional prognosis of acute ischemic stroke (35). In addition, the TyG index is able to forecast both the hospitalization and intensive care unit mortality after stroke, especially with ischemic stroke (36). Shi et al. considered that TyG could assist in the assessment of the probability of ischemic stroke (37). In addition, an increased

**TABLE 3** | Independent predictors of MACCEs in overall patients.

| Variables | Univariate analysis HR (95% CI) | P | Multivariate analysis HR (95% CI) | P |
|---|---|---|---|---|
| **Age** | 0.989 (0.977-1.001) | 0.073 | 1.009 (0.982-1.037) | 0.498 |
| **Male** | 1.122 (0.829-1.519) | 0.454 | 0.760 (0.412-1.401) | 0.379 |
| **BMI** | 1.033 (0.995-1.072) | 0.087 | 0.986 (0.944-1.030) | 0.524 |
| **SBP** | 0.999 (0.991-1.007) | 0.823 | 1.001 (0.990-1.013) | 0.802 |
| **DBP** | 1.003 (0.991-1.015) | 0.592 | 1.000 (0.983-1.017) | 0.984 |
| **Smoking** | 1.115 (0.862-1.442) | 0.409 | 1.092 (0.801-1.488) | 0.579 |
| **Hypertension** | 1.014 (0.780-1.317) | 0.920 | 0.984 (0.745-1.301) | 0.903 |
| **Hyperlipemia** | 0.969 (0.734-1.278) | 0.822 | 0.925 (0.698-1.225) | 0.579 |
| **LDL-C** | 1.083 (0.933-1.256) | 0.295 | 1.039 (0.532-2.031) | 0.672 |
| **HDL-C** | 0.298 (0.171-0.519) | <0.001 | 0.873 (0.338-2.251) | 0.778 |
| **TyG index** | 4.453 (3.507-5.653) | <0.001 | 4.942 (3.432-6.115) | <0.001 |
| **Cr** | 1.009 (1.001-1.018) | 0.025 | 1.014 (0.986-1.042) | 0.328 |
| **SUA** | 1.002 (1.001-1.004) | 0.001 | 1.000 (0.999-1.002) | 0.819 |
| **eGFR** | 0.999 (0.989-1.009) | 0.829 | 1.015 (0.981-1.051) | 0.378 |
| **BNP** | 1.000 (0.999-1.001) | 0.906 | 1.000 (0.999-1.001) | 0.955 |
| **CRP** | 1.010 (0.988-1.031) | 0.378 | 1.009 (0.986-1.032) | 0.458 |

*HR, hazard ratio; CI, confidence level; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TyG, triglyceride-glucose; Cr, creatinine; SUA, serum uric acid; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein.*

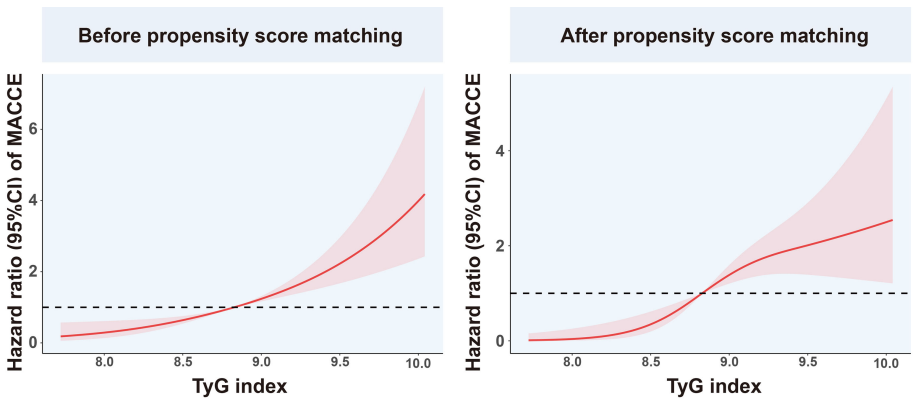


**FIGURE 5** | Restricted cubic spline analysis of the association between TyG index and MACCEs. TyG, triglyceride-glucose; MACCEs, major adverse cardiovascular and cerebrovascular events.

TyG index independently predicts the risk of ischemic stroke in the general population, and IR may be positively correlated with future stroke risk (38). Although studies have found that the correlation between BMI and stroke prognosis is not affected by the TyG index (39), it is interesting that Du et al. reported an association between the TyG-BMI index derived from the TyG index and ischemic stroke (40).

A recent registration study observed a relationship between elevated higher TyG indices and increased MACCEs risk with STEMI, and concluded that the TyG index can effectively predict outcomes in STEMI cases after percutaneous coronary intervention (PCI) (41). Subsequently, Zhang et al., in examining the relationship between the TyG index and MACCEs, concluded that the former was valuable in assessing both prognosis and risk stratification in T2DM patients after suffering acute myocardial infarction (AMI) (42). In our research, we found a 2.0% incidence of stroke in all patients during the 2.5-year follow-up. After propensity score matching, survival analysis showed that the risk of stroke and the TyG index were significantly different between higher and lower TyG groups, which is consistent with previous studies. In addition, the higher TyG index group had a poorer prognosis (HR 2.902, 95% CI: 1.431-5.885, P<0.001), and it was shown that the TyG index may be closely linked to the occurrence of stroke in prediabetic patients with ACS.

## The TyG Index and Cardiovascular Events

Evidence shows that the TyG index is predictive of cardiovascular events. Perusal of the recent literature shows that there is similar evidence in different populations of CAD. Firstly, in SAP patients, the TyG index correlated positively with cardiovascular events, indicating that TyG is a valuable indicator in predicting the clinical outcome of CAD patients (43). Concurrent studies confirmed that in SAP patients with T2DM, compared with the hemoglobin

**TABLE 4** | Estimated Kaplan–Meier events rates of 2.5 years follow-up.

| Adverse events | Overall population | | | | Propensity score-matched population | | | |
|---|---|---|---|---|---|---|---|---|
| | Lower TyG index (< 8.83, n = 1358) | Higher TyG index (≥8.83, n=672) | Adjusted HR (95% CI) | P | Lower TyG index (< 8.83, n = 574) | Higher TyG index (≥8.83, n=574) | HR (95% CI) | P |
| **MACCEs, n (%)** | 77 (5.7) | 156 (23.2) | 4.942 (3.432-6.115) | <0.001 | 42 (7.3) | 136 (23.7) | 3.526 (2.618-4.749) | <0.001 |
| **Cardiac death, n (%)** | 8 (0.6) | 3 (0.4) | 0.652 (0142-2.997) | 0.583 | 5 (0.9) | 2 (0.3) | 0.461 (0.099-2.138) | 0.322 |
| **MI, n (%)** | 10 (0.7) | 19 (2.8) | 4.844 (2.060-6.388) | <0.001 | 7 (1.2) | 15 (2.6) | 2.278 (0.968-5.360) | 0.059 |
| **Cardiac death/MI, n (%)** | 15 (1.1) | 22 (3.3) | 3.139 (1.470-6.703) | 0.003 | 10 (1.7) | 17 (3.0) | 1.684 (0.773-3.673) | 0.190 |
| **Ischemia-driven revascularization, n (%)** | 60 (4.4) | 120 (17.9) | 4.116 (3.612-6.246) | <0.001 | 34 (5.9) | 104 (18.1) | 3.455 (2.459-4.856) | <0.001 |
| **Stroke, n (%)** | 13 (1.0) | 28 (4.2) | 2.405 (1.647-3.307) | 0.001 | 7 (1.2) | 24 (4.2) | 2.902 (1.431-5.885) | 0.003 |

*HR, hazard ratio; CI, confidence level; TyG, triglyceride-glucose; MACCEs, major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction.*

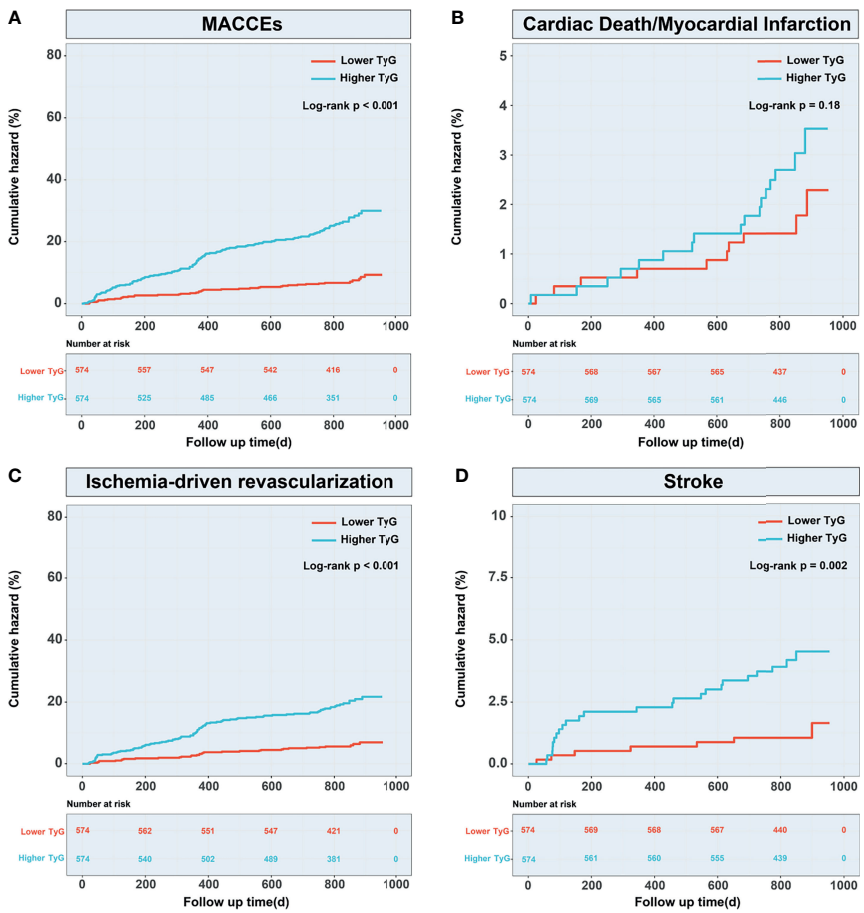


**FIGURE 6** | Kaplan–Meier curves for MACCEs and endpoint events according to the propensity score-matched population. **(A)** Kaplan–Meier curves for MACCEs; **(B)** Kaplan–Meier curves for cardiac death or non-fatal myocardial infarction; **(C)** Kaplan–Meier curves for ischemia-driven revascularization; **(D)** Kaplan-Meier curves for stroke. TyG, triglyceride-glucose; MACCEs, major adverse cardiovascular and cerebrovascular events.

glycation index, the TyG index is superior in terms of prognostic value (44).

In the ACS population, it has been found that high TyG indices may be related to a greater risk of major adverse cardiovascular events (MACE) in AMI patients (45). A recent study has shown that in NSTEMI patients, MACE occurred more frequently in patients with high TyG indices (46). In addition, the TyG index was also found to predict future MACE in patients with ACS (47).

Previous studies have shown that compared with HbA1c and TG, the TyG index is a valuable forecaster of future cardiovascular events, and may provide additional prognostic benefits for T2DM (48). For ACS patients treated with PCI, compared with FPG or HbA1c, the TyG index may be superior in predicting cardiovascular events (49). In non-diabetic patients, a higher TyG index predicts future ischemic heart disease, indicating that it may be a valuable indicator for assessing the risk of cardiovascular events in non-diabetic adults (50). Similarly, in non-diabetic ACS patients, higher TyG indices are associated with a greater incidence of revascularization or AMI and larger infarct size (51), suggesting that high TyG indices can effectively predict revascularization. However, few studies have addressed the prognosis of prediabetic patients with ACS. The existing studies only report a causal relationship between prediabetes combined with all-cause mortality and CVD (29), with the TyG index recognized as potentially useful in the early detection of patients at risk of developing CVDs and adverse outcomes (52). In addition, prediabetes as a metabolic disorder might affect the MACCEs *via* over-inflammation and oxidative stress through inflammatory/oxidative stress pathways at the levels of cardiac (fat) tissue and the atherosclerotic plaque (12, 13, 53). The same pathways could be more evidenced in over-weight subjects and influence the epigenetic, the MACEs, and be influenced (positively) by metformin therapy (54). Moreover, prediabetic patients have alteration of inflammatory markers and of the value of endothelial function that consequently could cause higher rate of MACEs also in absence of significant coronary stenosis (55). In summary, our study indicates a close link between the TyG index and MACCEs in patients with prediabetes and ACS, with higher TyG indices related to poor prognosis. As the levels of the TyG index are rising, this may be a prognostic indicator for prediabetic patients with ACS.

## CONCLUSIONS

The TyG index is an important simple composite index of IR in prediabetic patients, and high TyG indices may be significant prognostic indicators in prediabetic patients with ACS.

## LIMITATIONS

(1) This is a single center study with a small sample size, and more multi-center studies need to be performed to confirm the present results. In addition, the retrospective nature of our study potentially biases the result of analysis. However, the prevalence of clinical risk factors in our study population was similar to some contemporary trials and real-world registries (56, 57). This might be a relevant finding and potentially supports the generalizability of our results. (2) TyG indices were only calculated at admission for only once and might change in the 2.5-year follow-up. However, we were not able to measure changes in TyG indices over time. (3) Due to lack of fasting insulin data, the calculation of HOMA-IR was not performed in the present study. (4) Since our study mainly focused on the presence of MACCEs in patients during the follow-up, we did not carry out subsequent hematological examination on these patients, and it was not possible to determine whether the patients progressed from prediabetes to diabetes or non-diabetes. (5) Outcome events in this study were not adjudicated by a clinical events committee. This represents a possible limitation of results that should be acknowledged (58).

## DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this study will be available from the corresponding authors on reasonable requests.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of Beijing Anzhen Hospital. The data retrospectively obtained from electronic medical records.

## AUTHOR CONTRIBUTIONS

QYG, XXF, BZ, and GYZ made contributions to the acquisition of data, analysis and drafting of the manuscript. QYG, XXF, YL, and JQY made contributions to the acquisition and interpretation of data. QYG, YJZ and DMS made substantial contributions to conception and design. YYL and YJZ made substantial contributions to critical revision of the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

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# Association Between METS-IR and Prehypertension or Hypertension Among Normoglycemia Subjects in Japan: A Retrospective Study

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**Aim:** Our study aimed to investigate the association between the novel non–insulin-based metabolic score for insulin resistance (METS-IR) index and pre-hypertension (HTN) or HTN in normoglycemia Japanese participants.

**Methods:** The NAGALA medical examination program at Murakami Memorial Hospital in Gifu, Japan was found in 1994. 15,453 participants enrolled in this program from 2004 to 2015 was included in this retrospective study to explore the association between the METS-IR index and pre-HTN or HTN. Covariates included serum biomarkers and clinicodemographic characteristics. Logistic regression was applied to explore the association between METS-IR level and pre-HTN or HTN.

**Results:** This study includes a total of 15453 participants. The prevalence rates of pre-HTN and HTN were 28.55% (4412/15453) and 6.23% (962/15453), respectively. Adjusted for confounding factors in the multivariable logistic regression analysis models, when METS-IR was used as a categorical variable, high METS-IR was significantly associated with both pre-HTN (adjusted odds ratio (OR) = 1.95, 95% confidence interval (CI): 1.61–2.36) and HTN (adjusted OR = 2.12, 95% CI: 1.44–3.11). When METS-IR was used as a continuous variable, each 1 unit increase in METS-IR was associated with a 7% increase in the prevalence of pre-HTN (adjusted OR = 1.07, 95% CI: 1.06–1.08) and with a 13% increase in the prevalence of HTN (adjusted OR = 1.13, 95% CI: 1.10–1.16). Stratified analyses indicated a positive correlation between METS-IR and pre-HTN or HTN in normoglycemia subjects with different characteristics.

**Conclusions:** METS-IR levels are significantly associated with pre-HTN or HTN in normoglycemia individuals in Gifu, Japan. METS-IR may be used as a monitoring indicator for the development of HTN primary prevention and management strategies in the future, but it still needs more research to confirm.

**Keywords: metabolic score for insulin resistance (METS-IR), insulin resistance, prehypertension, hypertension, normoglycemia**

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# INTRODUCTION

Hypertension (HTN) and cardiovascular diseases (CVDs) caused by high blood pressure blood pressure have drawn great attentions in public health (1, 2). More recently, the prevalence of HTN has surged, resulting in the increase of blood pressure-related morbidity and death. Furthermore, the common concomitant status in prehypertensive and hypertensive individuals, such as abnormalities in glucose and lipid homeostasis, leads to a poorer long-term prognosis (3). In fact, dyslipidemia and impaired fasting glucose (IFG) were shown to be present in 41.9 percent and 40.7 percent of hypertensive individuals, respectively (4, 5). Aberrant glycolipid metabolism in hypertensive individuals considerably increases the risk of CVD in these patients. As a result, seeking for a better understanding of the glycolipid metabolism components in patients with HTN may help to alleviate the massive load of global disease.

Insulin resistance (IR), defined as the attenuation of insulin responsiveness in tissues, is a crucial mechanism in glycolipid metabolism (6). IR may be a substantially primary cause for CVD, according to current epidemiological and pathophysiology researches (6–8). The impact of IR on the pathophysiology of HTN has also been extensively studied (9). Up to now, the available the reference standard for evaluating the significance of IR is the hyperinsulinemic euglycemic clamp (HEC) (10). However, this approach of analyzing IR, is time-consuming, costly, and sophisticated, and it necessitates a large workforce. Consequently, it is unsuitable for routine clinical use. The development of non-insulin-based IR indicators has provided an easier and less expensive method to detect IR, especially in primary healthcare settings.

The metabolic score for insulin resistance (METS-IR) index is a recently developed index aimed to be a practical and efficient alternative biomarker of IR (11). The METS-IR index has a stronger correlation with the HEC than other non–insulin-based IR indexes (11). However, there are only few studies on the association between the METS-IR index and blood pressure, with studies limited only to China and Mexico (12–15). Further, the enrolled populations in these studies were mainly those with a history of HTN and, therefore, were taking antihypertensive drugs. As such, the conclusions of these studies are easily affected by the use of medicine. Moreover, the association of METS-IR index with pre-HTN and HTN in different ethnic is unknown. Thus, we aim to study the association between METS-IR index and pre-HTN and HTN in Japanese normoglycemia individuals, using a relevant database (16).

# METHODS

## Data Source

Our data source was the DATADRYAD database (http://www.Datadryad.org/). The information was obtained from the Dryad data package (Okamura, Takuro, et al., 2019), which can be downloaded for free for all researchers. Detailed citations were used to gain information on the study of Okamura, Takuro, et al. (dataset: 10.5061/dryad.8q0p192) (16). The following variables of enrolled participants in the database included: age, sex, waist circumference (WC), weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), alanine aminotransferase (ALT), fasting plasma glucose (FPG), total cholesterol (TC), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), high-density lipoprotein cholesterol (HDL-C), smoking status, exercise, fatty liver, alcohol consumption, triglycerides (TG), hemoglobin A1c (HbA1c), obesity phenotype, obesity, visceral fat obesity, ethanol consumption, diabetes mellitus (DM), and follow-up duration.

## Study Population

A healthcare program aimed to identify chronic cardiovascular disorders and improve public health conditions was carried out at the hospital in Gifu, Japan. Data collected from the program were used to create the NAfld in the Gifu Area, Longitudinal Analysis (NAGALA) database (16). From 2004 to 2015, participants were given a test, with 60% of them receiving one or two exams per year. Based on exclusion criteria of (1) incomplete relevant records; (2) steatohepatitis or hepatitis B or C; (3) alcoholism (alcohol consumption over 60 g/day for men and 40 g/day for women); (4) oral intake of medicine; and (5) blood glucose level >6.1, a total of 15453 participants (8441 men and 7034 women) were finally enrolled in the program. The ethical approval was provided by Murakami Memorial Hospital's ethical committee, and a written informed consent was obligately required for all participants.

## Data Collection and Measurements

The NAGALA database contained the medical history and lifestyle information of individuals based on a standardized questionnaire. Alcohol intake was measured by subdivision of alcohol and average weekly alcohol consumption over the previous month. Thus, there were four categories as followed: no or minimal drinker (40 g/week), light drinker (40–140 g/week), moderate drinker (140–280 g/week), and heavy drinker (>280 g/week) (17). Simultaneously, according to the habits of smoking, the participants can be allocated to three different groups: none, ex-smoker, or present smoker. The regular exercisers referred to those who participate in any type of sports more than one time per week regularly (18). Steatohepatitis should correspond to the definition of which diagnosed by abdominal ultrasonography (19). The definition of obesity was a body mass index (BMI) no less than 25 kg/m2 or higher (20–22). Visceral fat obesity refers to a waist circumference ≥90 cm in men or ≥80 cm in women (23). The METS-IR index was calculated as follows: Ln[(2 × fasting glucose (mg/dL))+fasting TG (mg/dL)] × BMI (kg/m$^2$))/(Ln[high-density lipoprotein cholesterol (mg/dL)]) (11). Pre-HTN and HTN were defined according to the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2019) (24). The JSH 2019 cut-offs of office blood pressure for defining HTN are SBP ≥140 and/or DBP ≥90 mmHg; elevated blood pressure, SBP 130–139 and/or DBP 80–89

mmHg; and high normal blood pressure, SBP 120–129 mmHg and DBP <80 mmHg). Participants with an elevated blood pressure and with high normal blood pressure were collectively considered as having pre-HTN in the current study.

## Statistical Analysis

Data were categorized into continuous and categorical variables. Continuous variables were further divided into two types based on the normality of their distribution. Normally distributed continuous variables were presented as the mean ± standard deviation and compared between groups using the Student's t-test. Meanwhile, non-normally distributed variables were presented as the median ± interquartile range (IQR) and compared between two groups using the Wilcoxon rank-sum test. Categorical variables were presented as percentages and compared using the chi-square test. The Kruskal-Wallis test or one-way analysis of variance were applied to assess the significance of differences in groups stratified by METS-IR index quartiles. The association between METS-IR index and pre-HTN or HTN was investigated using univariate and multivariate logistic regression analyses models. Univariate and multivariable logistic regression analyses were used to study the association between METS-IR index and pre-HTN or HTN. Three models were used: model 1, adjusted for sex and age; model 2, adjusted for age, sex, smoking status, alcohol consumption and WC; and model 3, adjusted for age, sex, smoking status, alcohol consumption, WC, ALT, AST, GGT, and TC levels.

In the models, the median value of the METS-IR index in each quadrant was utilized to perform linear trend tests. In addition, we used curve-fitting to assess the linear relationship between METS-IR and pre-HTN or HTN. To identify modifications and interactions, we used a stratified linear regression model and likelihood ratio test in subgroups of age (<65 or ≥65 years), sex (female or male), BMI (<25 kg/cm$^2$or ≥25 kg/cm$^2$), and WC (<90 cm in men, <80 cm in women vs. ≥90 cm in men and ≥80 cm in women). The software packages R (http://www.R-project.org, The R Foundation) and Free Statistics software versions 1.3 were used to perform all statistical analyses. Statistical differences were considered significant at P<0.05.

# RESULTS

## Population

A total of 20,944 individuals were included in the NAGALA cohort. 4350 individuals were excluded owing to missing data (n=874), known liver disease (n=416), heavy drinking habits (n=739), and baseline medication consumption (n=2,321). At the baseline examination, 323 and 808 patients with T2DM and fasting blood glucose > 6.1 mmol/L, respectively, were further excluded. Consequently, 15,453 individuals were included in this study. The patient selection flowchart is shown in **Figure 1**.

## Baseline Characteristics

The mean participant age was 43.7 ± 8.9 years, and 7034 (45.5%) were men. The mean baseline METS-IR was 31.2 ± 6.5. The detailed characteristics of the population by METS-IR index quartiles are available in **Table 1**. METS-IR level was positively associated with several variables as follows: age, BMI, waist circumference, total cholesterol, TG, fasting blood glucose, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, SBP, and DBP and inversely associated with HDL-C. Interestingly, the higher the METS-IR, the higher

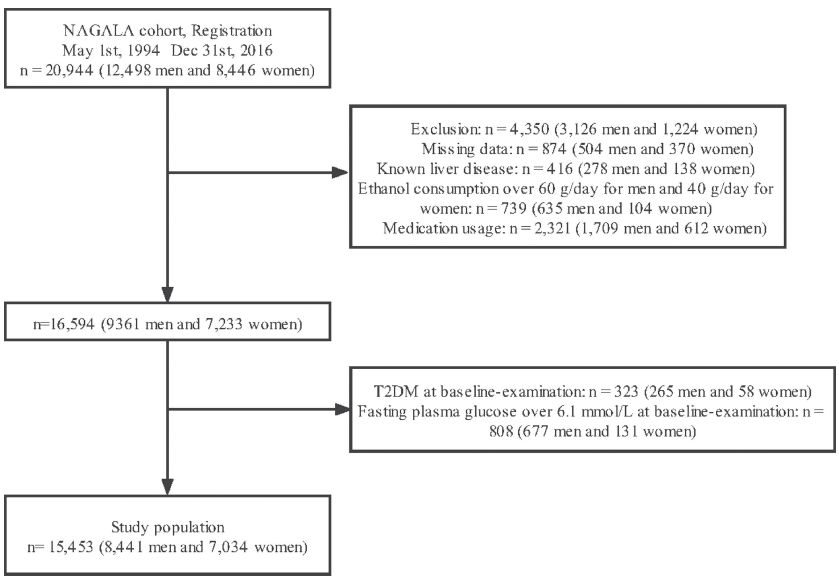



**FIGURE 1** | Flowchart of participant selection.

was the probability of high alcohol consumption and smoking. Meanwhile, women and those who exercised regularly had significantly lower METS-IR.

## Univariate and Multivariate Analyses of Prehypertension and Hypertension

Age, sex, BMI, WC, smoking status, alcohol intake, GGT, ALT, AST, HDL-C, TC, TG, HbA1c, fasting plasma glucose, and METS-IR were significantly all associated with pre-HTN and HTN (**Table 2**). METS-IR as continuous variables was calculated in **Table 2**. There was a linear relationship between METS-IR index and pre-HTN or HTN (**Figure 2**). After adjusting for different confounders, METS-IR was inversely associated with pre-HTN or HTN in all three models (**Table 3**). The odds ratios (ORs) of METS-IR were consistently significant in all three models irrespective of whether METS-IR was analyzed as a continuous variable or quartile (OR range 1.07–1.95, $p<0.05$ for pre-HTN; OR range 1.07–2.12, $p<0.05$, except quartile 2(Q2) =0.692 for HTN).

When METS-IR was evaluated as a continuous variable, in the full variables adjusted model (model 3), the adjusted OR was 1.07 (95% CI: 1.06–1.08) for pre-HTN and was 1.13 (95% CI: 1.1–1.16) for HTN. When METS-IR was analyzed as quartiles, also in model 3, the adjusted OR for pre-HTN in Q2, Q3, and Q4 were 1.18 (95% CI: 1.04–1.35), 1.40 (95% CI: 1.21–1.63), and 1.95 (95% CI: 1.61–2.36), respectively, with quartile 1 as reference. In the same model and analysis, the adjusted ORs for HTN in Q2, 3, and 4 were 1.07 (95% CI: 0.77–1.48), 1.41 (95% CI: 1.02–1.97), 2.12 (95% CI: 1.44–3.11), and 1.33 (95% CI: 1.18–1.5), respectively, with quartile 1 as reference. Moreover, it was statistically significant in all models (**Table 3**, p for trend <0.001), indicating that METS-IR was inversely associated with pre-HTN and HTN.

## Subgroup Analyses by Adjusted Potential Effect Confounders

Subgroup analyses were performed to assess the impact of METS-IR (per 1 unit increment) on pre-HTN and HTN in distinct subgroups (**Figure 3**). The association between METS-IR and pre-HTN or HTN was coordinated in the subgroups as follows: in pre-HTN, age (<65 years vs. ≥65 years; P-interaction = 0.037), sex (female vs. male; P-interaction = 0.001), BMI (<24 kg/m$^2$ vs. ≥24 kg/m$^2$; P-interaction = 0.068), and WC (<90 cm in men, <80 cm in women vs. ≥90 cm in men and ≥80 cm in women; P-interaction = 0.701); in HTN, age (<65 years vs. ≥65 years; P-interaction = 0.232), sex (female vs. male; P-interaction = 0.399), BMI (<24 kg/m$^2$ vs. ≥24 kg/m$^2$; P-interaction = 0.966), and WC (<90 cm in men,<80 cm in women vs. ≥90 cm in men and ≥80 cm in women; P-interaction = 0.079).

**TABLE 1** | Clinical characteristics of the study population according to METS-IR.

| Variables | Total (n = 15453) | Q1 (n = 3863) | Q2 (n = 3863) | Q3 (n = 3863) | Q4 (n = 3864) | p value |
|---|---|---|---|---|---|---|
| Age, (years) | 43.7 ± 8.9 | 41.6 ± 8.7 | 43.6 ± 8.9 | 45.0 ± 8.9 | 44.7 ± 8.6 | <0.001 |
| Male, n (%) | 7034 (45.5) | 809 (20.9) | 1756 (45.5) | 2679 (69.4) | 3175 (82.2) | |
| BMI, (kg/m2) | 22.1 ± 3.1 | 18.8 ± 1.3 | 21.0 ± 1.2 | 22.8 ± 1.4 | 25.9 ± 2.6 | <0.001 |
| WC, (cm) | 76.5 ± 9.1 | 67.1 ± 5.0 | 73.2 ± 5.1 | 78.9 ± 5.1 | 86.6 ± 6.8 | <0.001 |
| WHtR | 0.5 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | <0.001 |
| Alcohol consumption, n (%) | | | | | | <0.001 |
| None | 11802 (76.4) | 3290 (85.2) | 2970 (76.9) | 2764 (71.6) | 2778 (71.9) | |
| Light | 1754 (11.4) | 304 (7.9) | 462 (12) | 491 (12.7) | 497 (12.9) | |
| Moderate | 1357 (8.8) | 215 (5.6) | 314 (8.1) | 435 (11.3) | 393 (10.2) | |
| Heavy | 540 (3.5) | 54 (1.4) | 117 (3) | 173 (4.5) | 196 (5.1) | |
| Smoking status, n (%) | | | | | | <0.001 |
| Never | 9027 (58.4) | 3006 (77.8) | 2482 (64.3) | 1946 (50.4) | 1593 (41.2) | |
| Past | 2949 (19.1) | 412 (10.7) | 657 (17) | 923 (23.9) | 957 (24.8) | |
| Current | 3477 (22.5) | 445 (11.5) | 724 (18.7) | 994 (25.7) | 1314 (34) | |
| Habit.of.exercise, n (%) | | | | | | <0.001 |
| No | 12747 (82.5) | 3212 (83.1) | 3130 (81) | 3124 (80.9) | 3281 (84.9) | |
| Yes | 2706 (17.5) | 651 (16.9) | 733 (19) | 739 (19.1) | 583 (15.1) | |
| HDL-c, (mg/dL) | 56.5 ± 15.6 | 71.2 ± 14.6 | 60.3 ± 11.7 | 51.9 ± 9.9 | 42.7 ± 8.9 | <0.001 |
| TC, (mg/dL) | 198.2 ± 33.4 | 192.0 ± 31.8 | 194.6 ± 32.8 | 199.5 ± 33.2 | 206.8 ± 33.9 | <0.001 |
| TG, (mg/dL) | 65.0 (44.0, 99.0) | 42.0 (31.0, 57.0) | 55.0 (40.0, 74.0) | 74.0 (54.0, 100.0) | 117.0 (83.0, 164.0) | <0.001 |
| HbA1, (%) | 5.2 ± 0.3 | 5.1 ± 0.3 | 5.1 ± 0.3 | 5.2 ± 0.3 | 5.2 ± 0.3 | <0.001 |
| FPG (mg/dL) | 93.0 ± 7.4 | 88.7 ± 6.9 | 91.8 ± 6.9 | 94.3 ± 6.7 | 97.1 ± 6.5 | <0.001 |
| ALT, (IU/L) | 17.0 (13.0, 23.0) | 14.0 (11.0, 17.0) | 15.0 (12.0, 19.0) | 18.0 (14.0, 23.0) | 24.0 (18.0, 34.0) | <0.001 |
| AST, (IU/L) | 17.0 (14.0, 21.0) | 16.0 (13.0, 19.0) | 16.0 (13.0, 20.0) | 17.0 (14.0, 21.0) | 20.0 (16.0, 24.0) | <0.001 |
| GGT, (IU/L) | 15.0 (11.0, 22.0) | 12.0 (10.0, 15.0) | 13.0 (10.0, 17.0) | 17.0 (12.0, 24.0) | 22.0 (16.0, 33.0) | <0.001 |
| SBP, (mmHg) | 114.5 ± 15.0 | 106.4 ± 12.8 | 111.4 ± 13.2 | 116.9 ± 13.6 | 123.4 ± 14.6 | <0.001 |
| DBP, (mmHg) | 71.6 ± 10.5 | 65.9 ± 8.9 | 69.3 ± 9.4 | 73.3 ± 9.6 | 77.9 ± 10.0 | <0.001 |
| METS-IR | 31.2 ± 6.5 | 23.9 ± 1.7 | 28.2 ± 1.1 | 32.4 ± 1.4 | 40.1 ± 4.4 | <0.001 |

*Data were mean ± SD or median (IQR) for skewed variables or numbers (proportions) for categorical variables.*

*BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; HDL-c, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; ASL, aspartate aminotransferase; GGT, gamma glutamyl transferase; METS-IR, metabolic score for insulin resistance; Q1, Q2, Q3, and Q4 are quartiles of the metabolic score for insulin resistance(METS-IR).*

**TABLE 2** | Results of univariate analysis of prehypertension and hypertension.

| Variable | Prehypertension | | Hypertension | |
|---|---|---|---|---|
| | OR (95%CI) | p value | OR (95%CI) | p value |
| Age, (years) | 1.03 (1.03-1.04) | <0.001 | 1.07 (1.06-1.08) | <0.001 |
| Sex, n (%) | 2.84 (2.63-3.06) | <0.001 | 3.86 (3.31-4.50) | <0.001 |
| BMI, (kg/m2) | 1.3 (1.28-1.32) | | 1.44 (1.41~1.47) | <0.001 |
| WC, (cm) | 1.1 (1.09~1.10) | <0.001 | 1.14 (1.13~1.15) | <0.001 |
| Smoking status,n (%) | | | | |
| Never | ref | | ref | |
| Past | 1.94 (1.77~2.12) | <0.001 | 2.07 (1.76~2.44) | <0.001 |
| Current | 1.33 (1.22~1.45) | <0.001 | 1.31 (1.11~1.55) | 0.001 |
| Alcohol consumption | | | | |
| None | ref | | ref | |
| Light | 1.54 (1.38~1.71) | <0.001 | 1.76 (1.44~2.15) | <0.001 |
| Moderate | 1.95 (1.73~2.20) | <0.001 | 2.91 (2.39~3.54) | <0.001 |
| Heavy | 2.56 (2.12~3.09) | <0.001 | 4.72 (3.61~6.15) | <0.001 |
| ALT, (IU/L) | 1.04 (1.03~1.04) | <0.001 | 1.04 (1.04~1.04) | <0.001 |
| AST, (IU/L) | 1.05 (1.05~1.06) | <0.001 | 1.05 (1.04~1.06) | <0.001 |
| GGT, (IU/L) | 1.03 (1.03~1.03) | <0.001 | 1.03 (1.03~1.03) | <0.001 |
| HDL-c, (mg/dL) | 0.98 (0.98~0.98) | <0.001 | 0.97 (0.96~0.97) | <0.001 |
| TC, (mg/dL) | 1.01 (1.01~1.01) | <0.001 | 1.01 (1.01~1.02) | <0.001 |
| TG, (mg/dl) | 1.01 (1.01~1.01) | <0.001 | 1.01 (1.01~1.01) | <0.001 |
| HbA1c% | 1.71 (1.53~1.92) | <0.001 | 2.25 (1.83~2.77) | <0.001 |
| FPG, (mg/dl) | 1.08 (1.08~1.09) | <0.001 | 1.12 (1.11~1.13) | <0.001 |
| METS-IR | 1.13 (1.12~1.13) | <0.001 | 1.18 (1.17~1.19) | <0.001 |

*BMI, body mass index; WC, waist circumference; HDL-c, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; ALT, alanine aminotransferase; ASL, aspartate aminotransferase; GGT, gamma glutamyl transferase; METS-IR, metabolic score for insulin resistance.*

## DISCUSSION

After controlling for the variables in our population-based cross-sectional analysis, the results showed that the METS-IR, whether as a continuous or categorical variable, was positively and linearly associated with pre-HTN and HTN in normoglycemia subjects in Japan. The results were consistent in subgroups defined by age, sex, WC, and BMI (**Additional File 1: Tables S1**, **S2**).

Existing CVD risk assessment methods are unable to effectively assess the 10-year CVD risk in the prehypertensive populations (25); therefore, their CVD risk is unclear, highlighting an urgent need to develop more accurate and easy monitoring tools, particularly in primary care settings. Although HEC is the current gold standard for analyzing IR (26), it is mostly utilized only in research owing to its complexity. The most frequently used IR indicator in clinical and epidemiological studies is the homeostatic model assessment for insulin resistance (HOMA-IR), which is based on an insulin assay. However, its practical applicability is limited by its cost, particularly in less-developed areas (27). The METS-IR is a new substitute for IR that integrates traditional indicators (FPG, BMI, TG, and HDL) and shows good agreement with EHC and frequently sampled intravenous glucose tolerance (28).

The incidence of cardiovascular illnesses is higher in those with a BP of 120–129/80–84 mmHg and 130–139/85–89 mmHg than in those with a BP of 120/80 mmHg in Europe and the United States (29) and research in Japan (30, 31).Furthermore, the risk of HTN is also higher in those with a BP of 120–139/80–89 mmHg than those with a BP of 120/80 mmHg (32). Thus, we collectively defined those with an elevated blood pressure (SBP mmHg 130-139 and/or DBP 80–89 mmHg) and with high normal blood pressure (SBP 120–129 mmHg and DBP < 80 mmHg) based on JSH 2019 as having prehypertension in our study. Therefore, early detection and intervention for pre-HTN are important to avoid HTN. IR is a key aspect of pre-HTN and a necessary precursor to the early stages of the disease (3). However, up to now, there have been few studies on the relationship between BP and METS-IR. Fan et al. (14) compared the association of three alternative non-insulin-based IR surrogates with pre-HTN (defined SBP 120–139 and/or DBP 80–89 mmHg) in normoglycemia Chinese participants and found that only METS-IR, but not TG and triglycerides and high-density lipoprotein cholesterol (TG/HDL-c), was associated with pre-HTN, irrespective of the categorization of WC. Interestingly, we found that METS-IR was also associated with pre-HTN in normoglycemia Japanese subjects and may be of value in the management of pre-HTN and the prevention of prediabetes in different ethnic groups.

Our findings in the HTN group were also similar to those by Fan et al. (covariates included age, sex, and smoking), with more robust results by including more covariates (age, sex, smoking status, WC, alcohol consumption, ALT, AST, GGT, and TC). Omar et al. (12) report that the METS-IR was strongly associated with arterial stiffness and could predict the development of arterial HTN when combined with the Framingham Hypertension Risk Prediction Model. In addition, the METS-IR index was superior to other previously validated non–insulin-based IR measures, such as the TG index, TG/HDL-C index, and the HOMA-IR index in their findings. As a new IR index, METS-

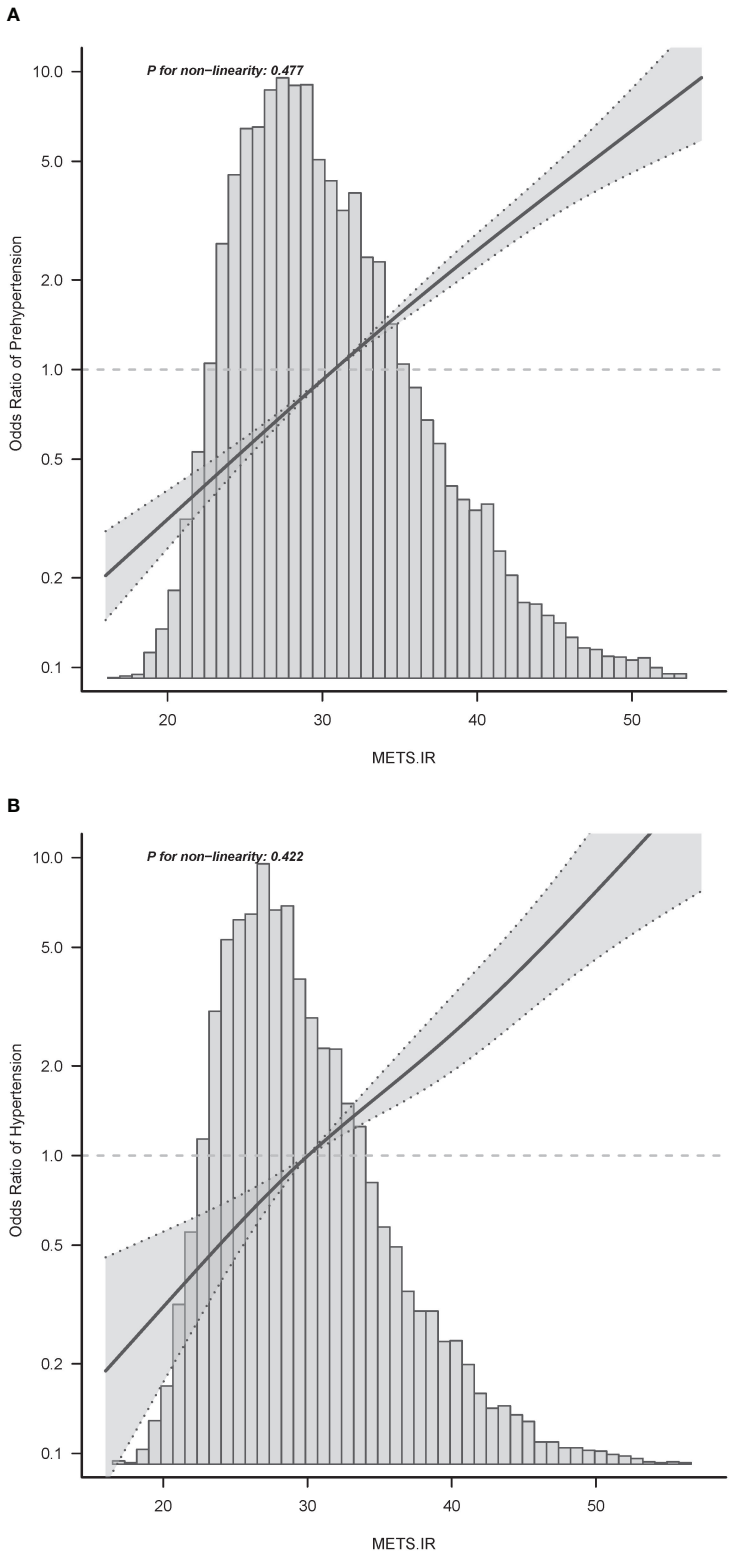



**FIGURE 2** | Associations between METS-IR index with prehypertension **(A)** or hypertension **(B)**. Odd ratios (ORs) were adjusted for age (continuous), sex (male or female), waist circumference (<90 or ≥90 in Men and <80(cm) ≥80 in Women), smoking status (never, past and current), alcohol consumption (none, light, moderate and heavy), total cholesterol (continuous) and triglyceride (continuous). Both P linearity, 0.001.

**TABLE 3 |** Multivariable-adjust ORs and 95%CI of the METS-IR index quartiles associated with prehypertension and hypertension.

| Variable | Unadjusted | | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|---|
| | OR (95%CI) | p value | OR (95%CI) | p value | OR (95%CI) | p value | OR (95%CI) | p value |
| Prehypertension | | | | | | | | |
| METS-IR | 1.13 (1.12~1.13) | <0.001 | 1.11 (1.10~1.11) | <0.001 | 1.06 (1.05~1.07) | <0.001 | 1.07 (1.06~1.08) | <0.001 |
| 1st Quartile (≤26.29) | Ref | | Ref | | Ref | | Ref | |
| 2st Quartile (26.29-30.14) | 1.86 (1.65~2.09) | <0.001 | 1.55 (1.37~1.75) | <0.001 | 1.16 (1.02~1.32) | 0.025 | 1.18 (1.04~1.35) | 0.012 |
| 3st Quartile (30.14-34.99) | 3.35 (2.98~3.75) | <0.001 | 2.39 (2.12~2.70) | <0.001 | 1.38 (1.20~1.60) | <0.001 | 1.40 (1.21~1.63) | <0.001 |
| 4st Quartile (≥34.99) | 7.04 (6.28~7.89) | <0.001 | 4.81 (4.24~5.44) | <0.001 | 2.02 (1.69~2.41) | <0.001 | 1.95 (1.61~2.36) | <0.001 |
| p for trend | 1.91 (1.85~1.98) | <0.001 | 1.69 (1.63~1.76) | <0.001 | 1.26 (1.19~1.34) | <0.001 | 1.24 (1.17~1.32) | <0.001 |
| Hypertension | | | | | | | | |
| METS-IR | 1.18 (1.17~1.19) | <0.001 | 1.17 (1.15~1.18) | <0.001 | 1.13 (1.11~1.15) | <0.001 | 1.13 (1.10~1.16) | <0.001 |
| 1st Quartile (≤26.29) | Ref | | Ref | | Ref | | Ref | |
| 2st Quartile (26.29-30.14) | 2.27 (1.67~3.10) | <0.001 | 1.77 (1.29~2.43) | <0.001 | 1.09 (0.78~1.50) | 0.622 | 1.07 (0.77~1.48) | 0.692 |
| 3st Quartile (30.14-34.99) | 5.78 (4.35~7.69) | <0.001 | 3.72 (2.77~5.01) | <0.001 | 1.55 (1.12~2.14) | 0.008 | 1.41 (1.02~1.97) | 0.04 |
| 4st Quartile (≥34.99) | 18.01 (13.72~23.64) | <0.001 | 10.92 (8.18~14.58) | <0.001 | 2.78 (1.94~3.99) | <0.001 | 2.12 (1.44~3.11) | <0.001 |
| p for trend | 2.73 (2.53~2.94) | <0.001 | 2.38 (2.19~2.58) | <0.001 | 1.5 (1.34~1.67) | <0.001 | 1.33 (1.18~1.50) | <0.001 |

*Model 1 adjust for age and sex.*

*Model 2 adjust for Model 1+WC, Smoking status, Alcohol consumption.*

*Model 3 adjust for Model 1+Model 2+ALT, AST, GGT, TC, TG.*

*Ref, reference; METS-IR, metabolic score for insulin resistance; WC, waist circumference; ALT, alanine aminotransferase; ASL, aspartate aminotransferase; GGT, gamma glutamyl transferase.*

IR was expected to become a predictor of incident HTN, and was a supplement to previously proven HTN risk prediction models.

Further, Liu et al. (13) indicated that, in normal-weight (BMI=18.5–23.9 kg/m2) Chinese adults but not in those with elevated BMI (≥24.0 kg/m2), METS-IR was strongly associated with HTN. The formula for METS-IR includes the BMI. BMI is a well-established predictor of HTN, and it influences blood pressure through various processes, including IR. And obesity is accompanied by a rise in the prevalence of HTN. Conversely, actively losing weight can dramatically alleviate high blood pressure (33, 34). Consequently, according to BMI, using the METS-IR index might have a potential impact on monitoring and managing HTN. Our study found that the METS-IR index is associated with both pre-HTN and HTN in different BMI categories (<25.0 kg/m$^2$ and ≥25.0 kg/m$^2$) in the Japanese, which may be due to racial differences in BMI. Moreover, in our study, the mean BMI of HTN group was the highest, then that of pre-HTN, and the lowest was that of the normotensive group (**Additional File 1: Table S3**). Therefore, weight control is an essential preventive and therapeutic strategy for IR. Surprisingly, in subgroup analysis based on WC, the close relationship of METS-IR with pre-HTN and HTN remained significant, thus highlighting the need for more research into the mechanism of METS-IR independent of WC.

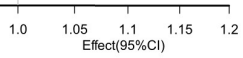
Pathophysiological data support an association among METSIR, arterial stiffness, and incident HTN. Hyperinsulinemia, hyperglycemia, dyslipidemia, HTN, and a pro-inflammatory state are all associated with IR, as are the consequences of disrupted insulin signaling at the endothelial cell level (endothelial cells and vascular smooth muscle cells). All of these factors lead to arterial stiffness and elevated arterial pressure (7, 35–38). As is known, the more activated the sympathetic nervous system is, as well as the more increased peripheral vascular resistance, and cardiac output, the higher the systemic blood pressure is, which are the most widely recognized theories linking IR and arterial HTN (39). Similarly, it is vital that the renin-angiotensin-aldosterone system would be fully activated by reduced insulin action, glucotoxicity, and MS, followed by risen tubular Na+ reabsorption and BP alterations (40). Endothelial dysfunction and decreased nitric oxide synthase activity are also caused by impaired insulin signaling, resulting in systemic vasoconstriction (41). We also found sexual dimorphism in METS-IR in both pre-HTN and HTN participants. Current evidence suggests that sex differences in IR may be related to the estrogen's potential protective effect in the development of IR (42). Garbis et al. (43) provided insights into insulin dysregulation in young females with polycystic ovary syndrome, using serum proteomics. Some proteins associated with β-estradiol, lipid metabolism, inflammation, and vitamin D, which are biological traits of cardiovascular physiology, may partly explain the mechanism of sexual dimorphism (44). However, the exact underlying mechanism requires further investigation. This emphasizes the need for including gender as a biological variable in preclinical investigations to better understand the pathophysiology of cardiometabolic disorders.

There were also several limitations in this study. First, the cross-sectional study design restricted the capability to determine causation. Second, the HOMA-IR of IR was not determined, because insulin levels were seldom identified in large epidemiological studies. Third, because the study data were obtained from Japanese subjects, the generalizability of the findings to other ethnic groups is unknown. Fourth, because this research is based on a secondary analysis of previously published research data, the procedures performed during the medical consultation, such as taking blood pressure measurements, are not entirely clear. Finally, all variables showed striking significance, but this could be because the sample size was not adjusted. However, the data analysis was based on a large sample, thus making the findings relatively reliable.

In conclusion, the METS-IR level is associated with pre-HTN or HTN in normoglycemia individuals in Japan. METS-IR may

**A**

| Subgroup | Total | Event (%) | OR (95%CI) | P for interaction |
|---|---|---|---|---|
| **Age,(y)** | | | | |
| <65 | 14299 | 4315 (30.2) | 1.13 (1.12~1.14) | 0.037 |
| >=65 | 192 | 97 (50.5) | 1.10 (1.02~1.18) | |
| **Sex** | | | | |
| Women | 6806 | 1310 (19.2) | 1.13 (1.12~1.15) | 0.001 |
| Men | 7685 | 3102 (40.4) | 1.11 (1.10~1.12) | |
| **BMI,(kg/m2)** | | | | |
| <25 | 12379 | 3159 (25.5) | 1.12 (1.11~1.13) | 0.055 |
| >=25 | 2112 | 1253 (59.3) | 1.09 (1.06~1.12) | |
| **WC,(cm)** | | | | |
| <90 in Men,<80 in Women | 12787 | 3538 (27.7) | 1.13 (1.12~1.14) | 0.701 |
| >=90 in Men,>=80 in Women | 1704 | 874 (51.3) | 1.13 (1.10~1.16) | |
| **Alcohol consumption** | | | | |
| none | 11203 | 3077 (27.5) | 1.13 (1.12~1.14) | 0.095 |
| light | 1621 | 596 (36.8) | 1.13 (1.10~1.16) | |
| moderate | 1208 | 513 (42.5) | 1.12 (1.09~1.15) | |
| heavy | 459 | 226 (49.2) | 1.10 (1.04~1.15) | |
| **Smoking status** | | | | |
| neve | 8542 | 2255 (26.4) | 1.14 (1.13~1.16) | <0.001 |
| past | 2695 | 1106 (41) | 1.12 (1.10~1.14) | |
| current | 3254 | 1051 (32.3) | 1.10 (1.08~1.12) | |




**B**

| Subgroup | Total | Event (%) | OR (95%CI) | P for interaction |
|---|---|---|---|---|
| **Age,(y)** | | | | |
| <65 | 10921 | 937 (8.6) | 1.19 (1.18~1.21) | 0.232 |
| ≥65 | 120 | 25 (20.8) | 1.20 (1.04~1.38) | |
| **Sex** | | | | |
| Women | 5724 | 228 (4) | 1.18 (1.15~1.21) | 0.399 |
| Men | 5317 | 734 (13.8) | 1.18 (1.16~1.20) | |
| **BMI,(kg/m2)** | | | | |
| <25 | 9773 | 553 (5.7) | 1.16 (1.13~1.19) | 0.761 |
| ≥25 | 1268 | 409 (32.3) | 1.17 (1.13~1.21) | |
| **WC,(cm)** | | | | |
| <90 in Men,<80 in Women | 9903 | 654 (6.6) | 1.17 (1.15~1.19) | 0.079 |
| ≥90 in Men,≥80 in Women | 1138 | 308 (27.1) | 1.20 (1.16~1.24) | |
| **Alcohol consumption** | | | | |
| none | 8725 | 599 (6.9) | 1.19 (1.17~1.22) | 0.081 |
| light | 1158 | 133 (11.5) | 1.23 (1.18~1.29) | |
| moderate | 844 | 149 (17.7) | 1.18 (1.13~1.23) | |
| heavy | 314 | 81 (25.8) | 1.16 (1.09~1.24) | |
| **Smoking status** | | | | |
| neve | 6772 | 485 (7.2) | 1.20 (1.18~1.23) | 0.004 |
| past | 1843 | 254 (13.8) | 1.19 (1.15~1.23) | |
| current | 2426 | 223 (9.2) | 1.17 (1.14~1.21) | |

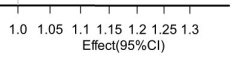



**FIGURE 3** | Subgroup analyses of the METS-IR and prehypertension **(A)** and hypertension **(B)**.

be used as a monitoring indicator for the development of HTN primary prevention and management strategies in the future, but it still needs more research to confirm.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Murakami Memorial Hospital's ethical committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

K-YH, JG, and YX designed the study. K-YH, JG, and ZW collected the data. K-YH, JG, SZ, and CXY analyzed the data. K-YH, JG, ZW, JL, SZ, CXY, and DL interpreted the result. K-YH wrote the first draft of the manuscript. YX contributed to the refinement of the manuscript. The final manuscript has been read and approved by the authors.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.851338/full#supplementary-material

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# Causal Association of Type 2 Diabetes Mellitus and Glycemic Traits With Cardiovascular Diseases and Lipid Traits: A Mendelian Randomization Study

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**Objective:** We aimed to evaluate the causal effect of type 2 diabetes mellitus (T2DM) and glycemic traits on the risk of a wide range of cardiovascular diseases (CVDs) and lipid traits using Mendelian randomization (MR).

**Methods:** Genetic variants associated with T2DM, fasting glucose, fasting insulin, and hemoglobin A1c were selected as instrumental variables to perform both univariable and multivariable MR analyses.

**Results:** In univariable MR, genetically predicted T2DM was associated with higher odds of peripheral artery disease (pooled odds ratio (OR) =1.207, 95% CI: 1.162-1.254), myocardial infarction (OR =1.132, 95% CI: 1.104-1.160), ischemic heart disease (OR =1.129, 95% CI: 1.105-1.154), heart failure (OR =1.050, 95% CI: 1.029-1.072), stroke (OR =1.087, 95% CI: 1.068-1.107), ischemic stroke (OR =1.080, 95% CI: 1.059-1.102), essential hypertension (OR =1.013, 95% CI: 1.010-1.015), coronary atherosclerosis (OR =1.005, 95% CI: 1.004-1.007), and major coronary heart disease event (OR =1.003, 95% CI: 1.002-1.004). Additionally, T2DM was causally related to lower levels of high-density lipoprotein cholesterol (OR =0.965, 95% CI: 0.958-0.973) and apolipoprotein A (OR =0.982, 95% CI: 0.977-0.987) but a higher level of triglycerides (OR =1.060, 95% CI: 1.036-1.084). Moreover, causal effect of glycemic traits on CVDs and lipid traits were also observed. Finally, most results of univariable MR were supported by multivariable MR.

**Conclusion:** We provided evidence for the causal effects of T2DM and glycemic traits on the risk of CVDs and dyslipidemia. Further investigations to elucidate the underlying mechanisms are warranted.

**Keywords: Mendelian randomization, diabetes, glycemic traits, cardiovascular disease, lipid**

## INTRODUCTION

Evidence from mounting prospective cohort studies has shown that type 2 diabetes mellitus (T2DM) is an independent risk factor of various cardiovascular diseases (CVDs) including coronary heart disease, heart failure (HF), stroke, peripheral artery disease (PAD) and so on (1–3). However, the causal effect of T2DM on CVDs could be confused by body mass index, age, sex, ethnicity, etc. in observational studies. Abnormal glycemic traits in the non-diabetic range, including fasting glucose (FG), fasting insulin (FI), and hemoglobin A1c ($HbA_{1c}$), were reported to be associated with CVDs (2–5). However, there are still conflict findings (6–11). Thus, the association remains uncertain. Patients with T2DM or abnormal glycemic traits were observed to predispose to the development of dyslipidemia such as increased low-density lipoprotein cholesterol (LDL-C), increased triglyceride, and decreased high-density lipoprotein cholesterol (HDL-C) (12). However, whether T2DM or abnormal glycemic trait is a cause or consequence of dyslipidemia is uncertain.

Mendelian randomization (MR) is an approach that relies on genetic variants that are considered to be allocated randomly at birth and is less subject to many confounders than observational studies (13). A previous MR study has investigated the relationship between T2DM and CVDs in a single cohort and revealed causal effects of T2DM on a range of CVDs (14). In our MR study, we pooled the estimates from two independent cohorts to ensure the robustness of the causal effects of T2DM on CVDs. Besides, we took three glycemic traits (FG, FI and $HbA_{1c}$) closely related to T2DM into consideration and conducted multivariable analyses to avoid bias of confounders brought by these traits. We further explored whether the causal effect of T2DM on CVDs was mediated by dyslipidemia using mediation analysis. Additionally, we evaluated whether genetically predicted T2DM or abnormal glycemic traits are causally associated with lipid traits.

## MATERIALS AND METHODS

### MR and Genome-Wide Association Studies (GWAS) Summary Data

MR is a genetic instrumental variable (IV)-based approach that utilizes single nucleotide polymorphisms (SNPs) as IVs to clarify the causal association between exposure and outcome. In this study, two-sample MR was used. Our MR analysis was based on three basic assumptions: (1) the IVs were robustly associated with the exposures (T2DM and glycemic traits); (2) the IVs affected the outcomes (CVDs and lipid traits) merely by their effect on exposures without any other causal pathways, which is also called no pleiotropic effect from the exposures; and (3) the IVs were not associated with any confounders which are present in the relation between the exposures and outcomes. To assure the reliability of the causal link between the exposures and outcomes obtained by MR, none of these assumptions should be violated (**Figure 1**).

The summary-level data were obtained from the OpenGWAS database developed by the MRC Integrative Epidemiology Unit (IEU) (https://gwasmrcieu.ac.uk/). Most of the datasets were publicly available and could be obtained by accessing application programming interfaces through convenient packages in R and Python (15, 16). Details on the phenotypes and consortiums are available in **Supplementary Table 1**.

### IVs for Exposures

We obtained the genetic instruments for T2DM from a meta-analysis of GWASs that included 74124 cases and 824006 controls from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium, which was derived from 32 GWASs conducted in populations of European ancestry (17). For the glycemic traits, the IVs for FG and FI were constructed from a meta-analysis of GWASs, which included 52 studies comprising up to approximately 133010 nondiabetic individuals from MAGIC (Meta-Analysis of Glucose and Insulin related traits Consortium) (18). The IVs for $HbA_{1c}$ were obtained from a meta-analysis of 82 cohorts that included up to 88355 European participants (19). All SNPs with a $p$ value $< 5 \times 10^{-8}$ were considered significant variants associated with phenotypes and included. We excluded SNPs with $r^2 < 0.001$ using linkage disequilibrium analysis. To avoid "weak instrument" bias, the F-statistic was calculated according to the formula $F = \frac{R^2(n-k-1)}{k(1-R^2)}$, where n, k, and $R^2$ represent the sample size, the number of SNPs, and the proportion of variance explained by the instrumental variants, respectively (20, 21). An F-statistic value > 10 was regarded as strong enough to avoid weak instrument bias (22). Finally, 286, 35, 18, and 38 SNPs served as IVs for T2DM, FG, FI, and $HbA_{1c}$, respectively (**Supplementary Table 2**).

### GWAS Summary Data for CVDs

A broad spectrum of CVDs were included in our study. Summary statistics were extracted from the Coronary Artery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM) plus the Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) for myocardial infarction (MI) and ischemic heart disease (IHD) (23); from the UKB for coronary atherosclerosis (CA), major coronary heart disease event (MCHDE), essential hypertension (HT), intracerebral hemorrhage and cardiovascular mortality (CM) (24); from the MEGASTROKE Consortium for stroke and ischemic stroke (IS) (25); from the Heart failure Events reduction with Remote Monitoring and eHealth Support (HERMeS) for HF (26); from the BioBank Japan (BBJ) for PAD (27); and from a meta-analysis including 6 studies (The Nord-Trøndelag Health Study (HUNT),

**Abbreviations:** T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; FG, fasting glucose; FI, fasting insulin; $HbA_{1c}$, hemoglobin A1c; MR, Mendelian randomization; GWAS, genome-wide association study; IV, instrumental variable; SNP, single nucleotide polymorphism; MI, myocardial infarction; IHD, ischemic heart disease; CA, coronary atherosclerosis; MCHDE, major coronary heart disease event; HT, essential hypertension; CM, cardiovascular mortality; IS, ischemic stroke; HF, heart failure; PAD, peripheral artery disease; AF, atrial fibrillation and fluttering; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA, apolipoprotein A; ApoB, apolipoprotein B; Lp(a), lipoprotein(a); IVW, inverse variance-weighted; MVMR, multivariable MR.

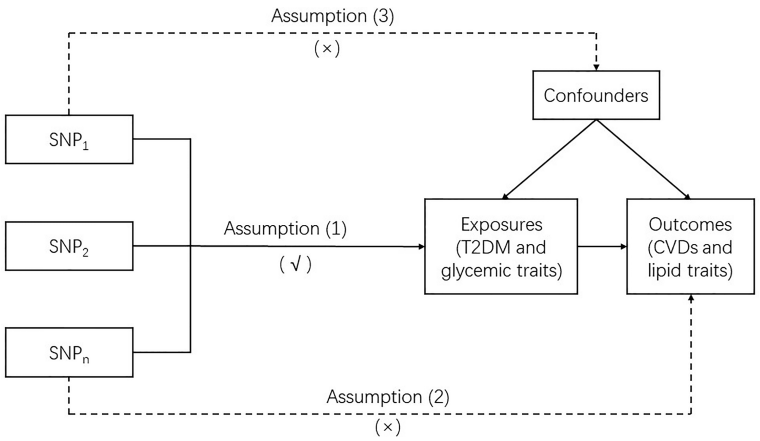


**FIGURE 1** | Mendelian randomization model.

deCODE, the Michigan Genomics Initiative (MGI), DiscovEHR, UKB, and the AFGen Consortium) for atrial fibrillation and fluttering (AF) (28).

To ensure the homogeneity of the study population and the reliabilities of the results, each CVD was derived from two independent large-scale cohorts. Therefore, summary-level data of each CVD were also extracted from the FinnGen consortium (study page: https://www.finngen.fi/en/; release 5: https://r5.finngen.fi/). According to the first occurrence, all CVDs were defined by the International Classification of Diseases (ICD)-10. The definition of each CVD is shown in **Supplementary Table 3**.

## GWAS Summary Data for Lipid Traits

We explored the following lipid traits measured in the UKB (20): HDL-C, LDL-C, triglycerides, apolipoprotein A (apoA), apolipoprotein B (apoB), and lipoprotein(a) [Lp(a)]. In addition, HDL-C, LDL-C, and triglycerides were explored again, utilizing the data from the Global Lipids Genetics Consortium (GLGC) to strengthen the credibility of the causal effects (29). We failed to reconduct analyses for the remaining three lipid traits due to the lack of data.

## Statistical Analyses

For the primary analyses, the univariable inverse variance-weighted (IVW) method was used to investigate the effects of different exposures on outcomes (30). Using the Wald ratio estimates of each SNP, the IVW method combines them into one cumulative causal estimate. Since the results of the IVW method could be affected by undetectable invalid IV bias or potentially unbalanced pleiotropy, different sensitivity analyses were performed to detect the robustness and validity of the MR results. First, the MR–Egger method was used to confirm the consistency of MR results and explore the horizontal pleiotropy effect through the intercept (31). Second, the heterogeneity of IVW and MR–Egger was calculated (32). A fixed-effects model was adopted to assess the IVW estimates when there was no significant heterogeneity; otherwise, a random-effects model was used. Third, we applied the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) method to recognize outlying SNPs, which might cause horizontal pleiotropy effects, and examine whether the causal effect would change after removing these outliers (33). Fourth, the weighted median, simple mode, and weighted mode were also employed to test the potential horizontal pleiotropy (34). Except for the analyses of apoA, apoB, and Lp(a), estimates of the causal effect from two independent cohorts were pooled using fixed-effects meta-analysis. It is also important to further evaluate whether the risk of CVDs in T2DM was mediated by dyslipidemia. Therefore, two-step MR was conducted to calculate the mediation effects of lipid traits in the relationship between T2DM and risk of CVDs (35).

For the complementary analyses, multivariable MR (MVMR) analysis was conducted using the IVW method, which incorporates different phenotypes as a single exposure into the MR analysis. In this study, since the relationship between T2DM and three glycemic traits was considered, we fitted a model with T2DM, FG, FI, and $HbA_{1c}$ to detect which phenotypes appeared to be significantly associated with the risk of CVDs or abnormal lipid traits.

All MR analyses were performed using R (version 4.1.1). In the univariable MR step, estimates were obtained with the "TwoSampleMR" package, recognizing outliers with the "MR-PRESSO" package. The MVMR was conducted with the "MendelianRandomization" package. MR results were reported as odd ratios (ORs) with 95% confidence intervals (CIs) per standard deviation or odds of objectively measured continuous or dichotomous variables. For the primary analyses, since we included analyses of 18 outcomes, a Bonferroni-corrected $p$ value less than 0.05 divided by 18 (that is, 0.0028) was regarded as a significant causal association to adjust for multiple testing. A $p$ value between 0.05 and 0.0028 was considered suggestive of a potential association.

# RESULTS

## Primary Analyses

Univariable MR was conducted and 286, 35, 18, and 38 SNPs associated with T2DM, FG, FI, and $HbA_{1c}$, respectively, were selected as IVs. A flow chart of the study was presented in **Supplementary Figure 1**. An overview of the main results of the primary analyses was shown in **Figure 2**.

### Causal Association of T2DM With CVDs and Lipid Traits

Genetically predicted T2DM was significantly associated with (ordered from largest estimate decreasing): PAD (OR = 1.207, 95% CI: 1.162-1.254, $p = 4.01 \times 10^{-22}$), MI (OR=1.132, 95% CI: 1.104-1.160, $p = 3.87 \times 10^{-22}$), IHD (OR = 1.129, 95% CI: 1.105-1.154, $p = 1.51 \times 10^{-28}$), stroke (OR = 1.087, 95% CI: 1.068-1.107, $p = 1.27 \times 10^{-19}$), IS (OR = 1.080, 95% CI: 1.059-1.102, $p = 1.40 \times 10^{-3}$), HF (OR = 1.050, 95% CI: 1.029-1.072, $p = 4.05 \times 10^{-6}$), HT (OR = 1.013, 95% CI: 1.010-1.015, $p = 6.28 \times 10^{-25}$), CA (OR = 1.005, 95% CI: 1.004-1.007, $p = 3.28 \times 10^{-16}$), MCHDE (OR = 1.003, 95% CI: 1.002-1.004, $p = 2.74 \times 10^{-11}$), and CM (OR = 1.001, 95% CI: 1.000-1.001, $p = 9.83 \times 10^{-6}$). We also found T2DM was causally related to lower levels of HDL-C (OR = 0.965, 95% CI: 0.958-0.973, $p = 2.13 \times 10^{-18}$) and apoA (OR = 0.982, 95% CI: 0.977-0.987, $p = 1.63 \times 10^{-11}$) but a higher level of triglycerides (OR = 1.060, 95% CI: 1.036-1.084, $p = 6.76 \times 10^{-7}$) (**Figure 3**).

### Causal Association of Glycemic Traits With CVDs and Lipid Traits

Genetically predicted FG was significantly associated with PAD (OR = 1.911, 95% CI: 1.309-2.790, $p = 7.89 \times 10^{-4}$) and CA (OR = 1.014, 95% CI: 1.005-1.023, $p = 2.64 \times 10^{-3}$). Additionally, a potential increased risk was observed for IHD (OR =1.187, 95% CI: 1.031-1.365, $p$ value = 0.017), MCHDE (OR =1.008, 95% CI: 1.001-1.015, $p$ value = 0.017), and CM (OR = 1.003, 95% CI: 1.001-1.005, $p = 3.61 \times 10^{-3}$) (**Figure 4**).

Genetically predicted FI was suggested to be positively corelated with PAD (OR = 2.804, 95% CI: 1.604-4.902, p = 2.97 × 10-4), IHD (OR = 2.020, 95% CI: 1.374-2.972, p = 3.53 × 10-4), MI (OR = 2.009, 95% CI: 1.317-3.064, $p = 1.20 \times 10^{-3}$) and HT (OR = 1.098, 95% CI: 1.054-1.144, $p = 8.31 \times 10^{-6}$) but negatively associated with HDL-C (OR = 0.644, 95% CI: 0.549-0.755, $p = 6.56 \times 10^{-8}$) and apoA (OR = 0.790, 95% CI: 0.713-0.874, $p = 5.37 \times 10^{-6}$). An indistinct relation to HF (OR = 1.442, 95% CI: 1.052-1.978, $p = 0.023$), stroke (OR = 1.421, 95% CI: 1.060-1.905, $p = 0.019$), IS (OR =1.480, 95% CI: 1.111-1.970, $p = 0.007$), CA (OR = 1.034, 95% CI: 1.006-1.064, $p = 0.019$), MCHDE (OR = 1.021, 95% CI: 1.001-1.042, $p = 0.036$) and lower level of Lp(a) (OR = 0.873, 95% CI: 0.780-0.978, $p = 0.019$) was also found (**Figure 5**).

Genetically predicted $HbA_{1c}$ was significantly associated with CA (OR = 1.019, 95% CI: 1.008-1.031, $p = 6.58 \times 10^{-4}$) and an increased level of LDL-C (OR =1.205, 95% CI: 1.157-1.256, $p = 2.81 \times 10^{-19}$). A suggestive causal effect was also observed for IHD (OR =1.277, 95% CI: 1.075-1.516, $p = 0.005$) and MI (OR =1.220, 95% CI: 1.002-1.484, $p = 0.047$), and increased level of apoB (OR =1.056, 95% CI: 1.011-1.104, $p = 0.015$) (**Figure 6**).

### Robustness of the Primary Analyses

In the univariable MR analysis, we observed significant heterogeneities in some estimates. We adopted a random-effects model to adjust the IVW estimates, as mentioned in the Methods section. The MR–Egger intercepts were mostly insignificantly larger or less than zero, eliminating part of the horizontal pleiotropy. Using the MR-PRESSO method, several outliers were identified during the analysis, and in most cases, the results

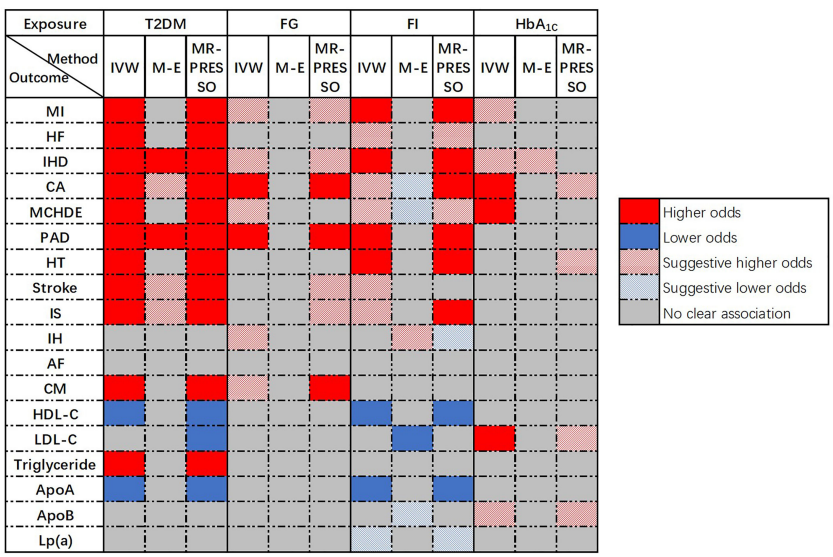


**FIGURE 2** | Overview of the main results of Univariable MR.

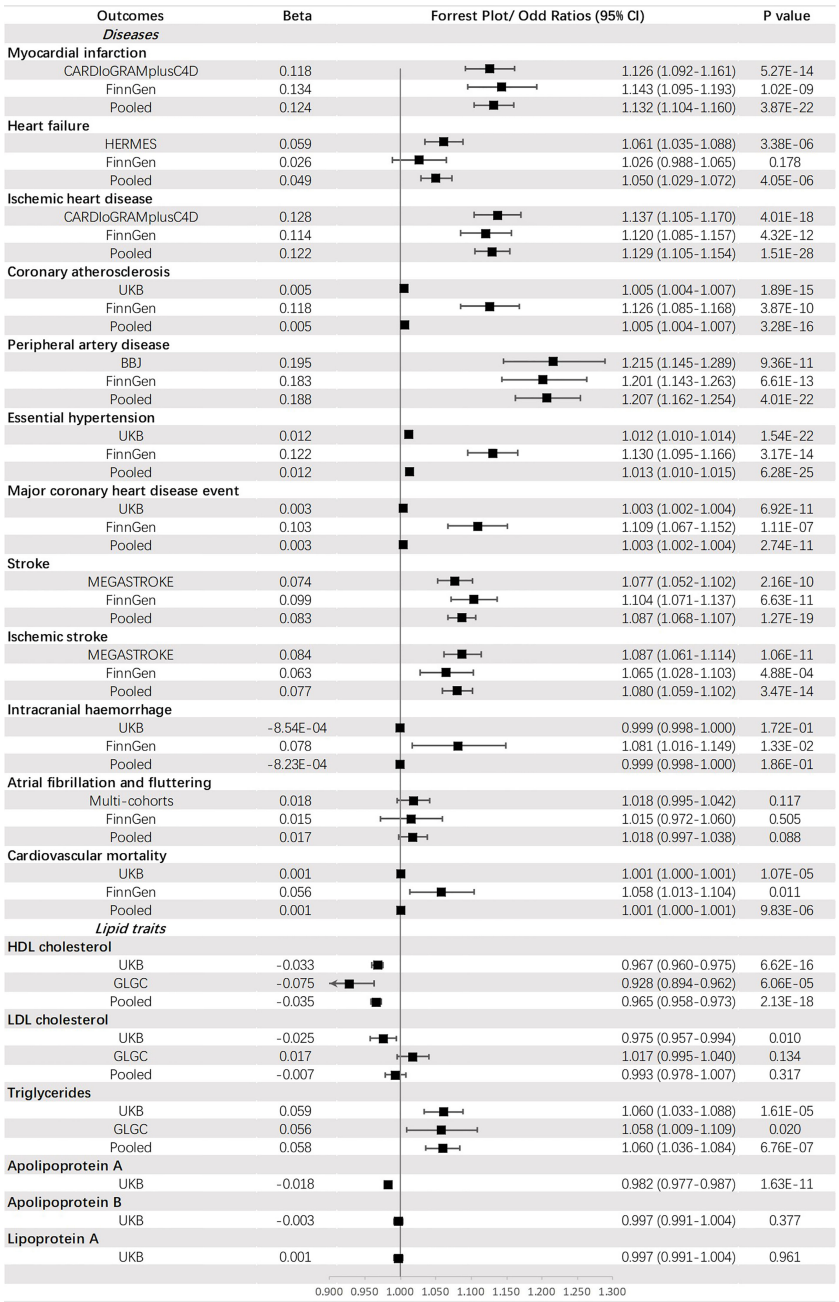


**FIGURE 3** | The association between type 2 diabetes mellitus and outcomes.

remained consistent with the original ones after removing these outliers. In addition, estimates using MR–Egger, weighted median, simple mode, and weighted mode were also calculated, and the results suggested relatively high robustness (**Supplementary Tables 4–7**).

## Mediation Analyses

We performed mediation analyses using two-step MR to clarify whether the causal effect of T2DM on the risk of CVDs was mediated by dyslipidemia. HDL-C, triglycerides and apoA were chosen as potential mediators since they showed a significant association with T2DM in the primary analyses. We found HDL-C explained a small part of the casual effects of T2DM on the risk of MI, CA, PAD, and HT, and the mediation proportions were 7.4%, 12.8%, 10.6%, and 5.9%, respectively (**Supplementary Table 8**). Triglycerides and apoA were also mediators of the causal association between T2DM and several types of CVDs (**Supplementary Tables 9, 10**).

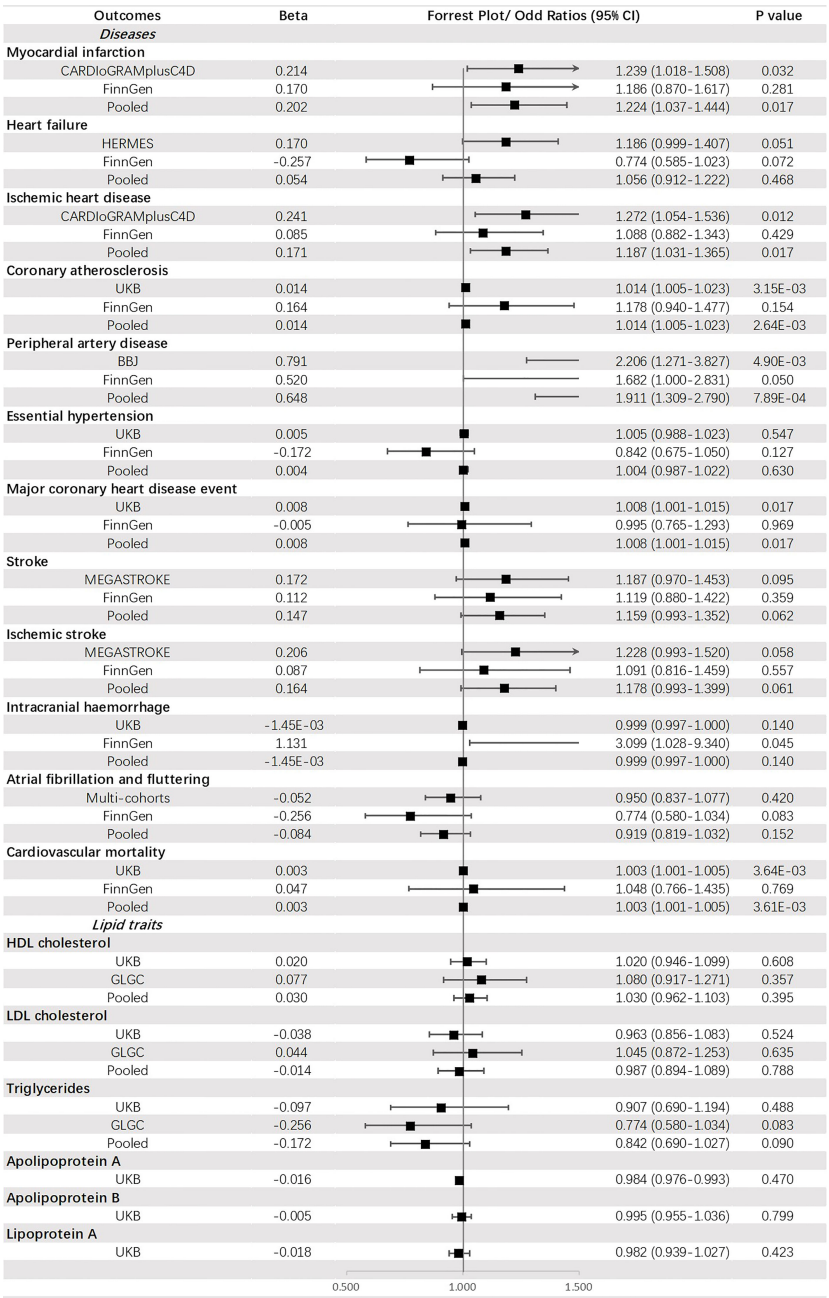


**FIGURE 4** | The association between fasting glucose (mmol/mol) and outcomes.

## Complementary Analyses

MVMR was conducted for outcomes with significant estimates in primary analyses. Most results of univariable MR were supported by MVMR. However, the causal effects of T2DM on HF, FI on IHD, FG on PAD, FG on CA, and $HbA_{1c}$ on CA were not found following adjustment for the other three exposures. An inverse association between T2DM and level of LDL-C was observed using multivariable analysis. Detailed results of MVMR were presented in **Supplementary Table 11**.

## DISCUSSION

In this study, a two-sample MR method utilizing GWAS summary-level data was applied to explore the causal association of T2DM and glycemic traits (FG, FI, and $HbA_{1c}$) with a wide range of CVDs as well as lipid traits [HDL-C, LDL-C, triglycerides, apoA, apoB, and Lp(a)]. The primary analyses found evidence that genetically predicted T2DM was associated with various types of CVDs including MI, HF, IHD, CA,

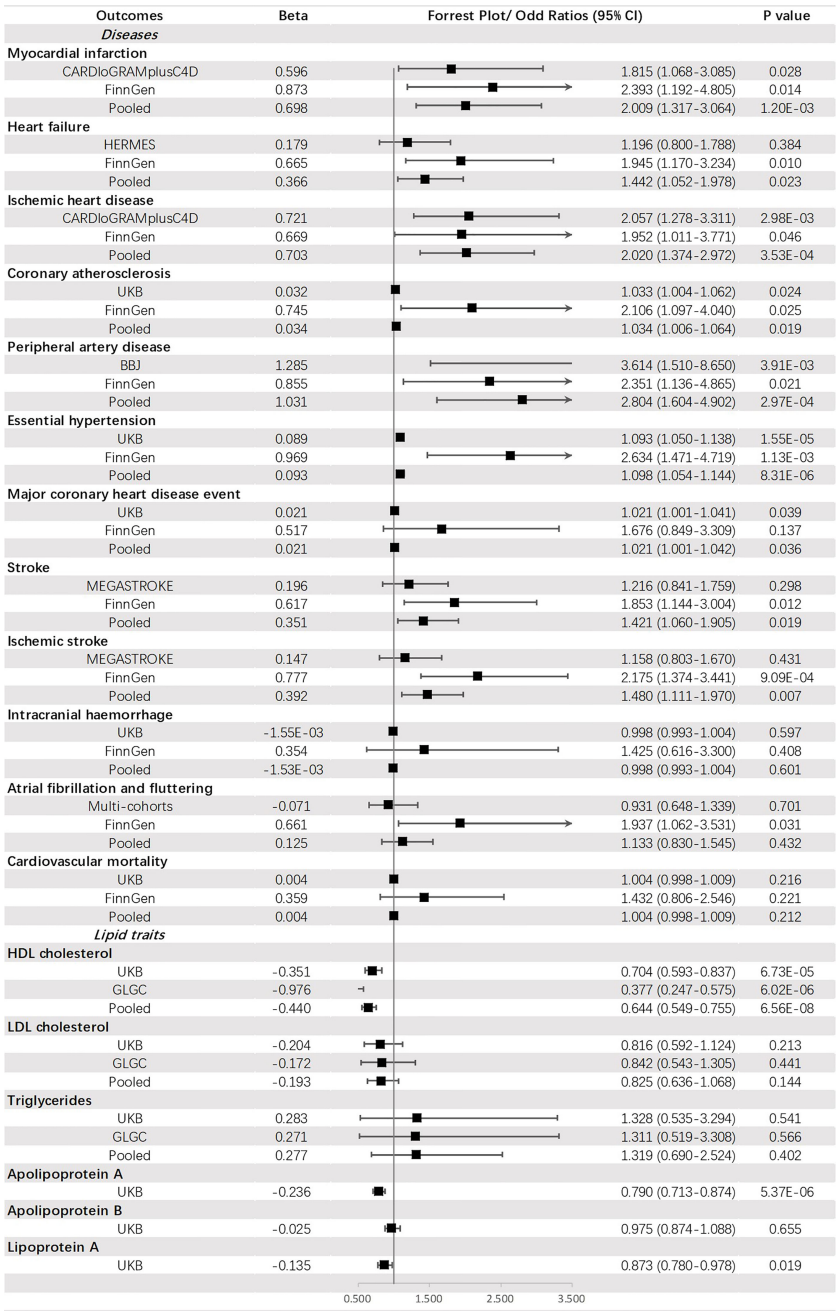


**FIGURE 5** | The association between fasting insulin (mmol/mol) and outcomes.

MCHDE, PAD, HT, stroke, IS, and CM. Additionally, T2DM was associated with a higher level of triglycerides but lower levels of HDL-C and apoA. Moreover, causal effect of glycemic traits on CVDs and lipid traits were also observed. FI was associated with higher levels of HDL-C and triglycerides, and $HbA_{1c}$ was associated with a higher level of LDL-C. Sensitivity analyses suggested the robustness of the causal effects. As a complementary analysis, MVMR was conducted which incorporated the four exposures into a model. Most results of univariable MR were supported by multivariable MR.

## T2DM and CVDs

Our findings are in line with the previous MR studies supporting casual effects of T2DM on various CVDs (14). We here provide

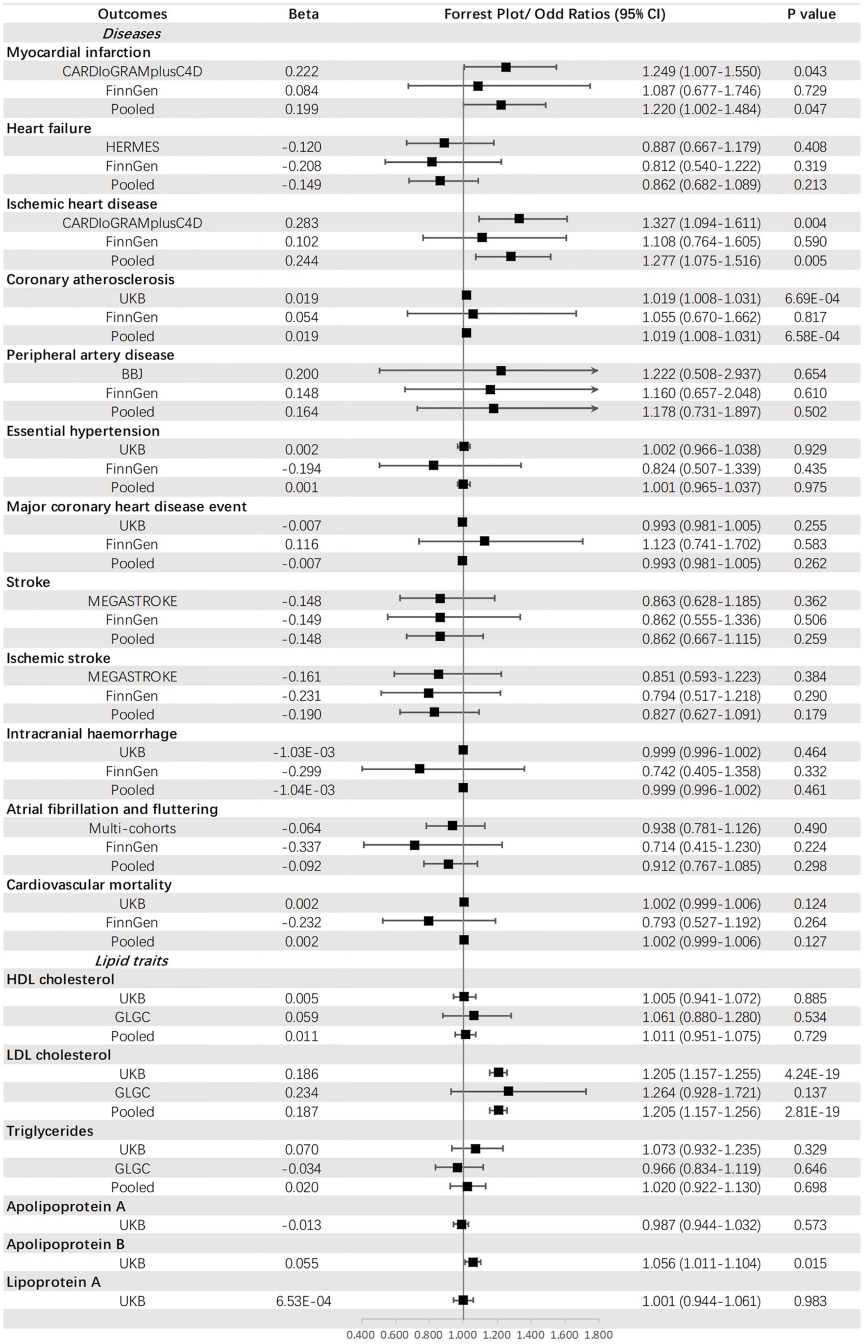


**FIGURE 6** | The association between $HbA_{1c}$ (mmol/mol) and outcomes.

evidence supporting additional effects of T2DM on HT, CA, and CM. However, in our MR study, the causal association between T2DM and HF disappeared after adjusting for multiple variables, which was inconsistent with the results of Liu et al. Moreover, multiple epidemiological studies had consistent results with ours (36–38). However, Wei et al. investigated the association between T2DM and several CVDs using phenotype and genetic predisposition data from the China Kadoorie Biobank. At the observational level, a significantly positive correlation was observed for all CVD outcomes but not for major coronary events, cardiovascular mortality, or total stroke at the genetic level (39). This discrepancy between observational and genetic

results suggests that the causal link between T2DM and CVDs remains largely to be determined. Fortunately, we found the causal effect of T2DM on these diseases.

Unfortunately, we failed to obtain a causal effect of T2DM on AF and IH. No association of T2DM with AF was also found by Hadi et al. using the MR approach (40). However, the Framingham Heart Study observed a 1.4- to 1.6-fold greater risk of AF in diabetic individuals after adjusting for age and other risk factors (41). One hypothesis about this inconsistency was that hypertension and obesity are the common comorbidities of T2DM, which could result in confounder bias in the observational studies, but not in the MR studies (40). In another MR study focusing on T2DM and cerebral disease, the researcher also found the null association between T2DM and IH even subdividing IH into lobar IH and deep IH (42).

## Glycemic Traits and CVDs

Previous MR results showed that $HbA_{1c}$ has a causal role in coronary artery disease but FG does not (43). However, in our study, FG was also shown to have a causal effect on several types of coronary artery diseases. Notably, with regard to glycemic traits, some epidemiological evidence did not support our findings of their causal effects on CVDs. The results derived from the Jackson Heart Study (JHS) revealed that dysglycemia, including higher levels of FG and $HbA_{1c}$, was associated with an increased risk of HF (44), which failed to reappear in our MR study. We inferred that ethnic variation may have led to the difference in results since the JHS recruited mainly Black participants from Mississippi. Justin et al. found that even stratifying the HF into HF with preserved ejection fraction and reduced ejection fraction, the causal chain was still there (45).

## T2DM-Related Traits and Lipid Traits

As suggested by our data, T2DM negatively affected HDL-C. Accordingly, a causal effect of T2DM on the decreased level of apoA (a main component of HDL-C) was also observed. Decreased HDL-C levels in T2DM patients was observed in a previous observational study (46). One explanation was that insulin resistance in T2DM patients might be responsible for the low level of HDL-C (47). As far as we know, our study is the first to provide evidence on the causal association between T2DM and a lower level of HDL-C from the genetic level. In our study, T2DM was also found to be causally associated with triglycerides, which is consistent with a previous MR study (48).

In our study, $HbA_{1c}$ was causally associated with an increased level of LDL-C. A transversal observational study found that oxidized LDL-C, rather than total LDL-C, was associated with $HbA_{1c}$ in the non-diabetic range (49). However, we could not obtain data to stratify LDL-C into subgroups to explore this relation further. In our study, FI was found to negatively affected on Lp(a), and it showed a potentially negative effect. Conversely, Buchmann et al. found no evidence of a causal effect of FI on Lp (a) using rs780094 and rs10195252 (SNPs associated with FI) as IVs through the MR method (50). The possible mechanism by which insulin modulates Lp(a) synthesis may be that increased insulin levels promote the progression of insulin resistance and, under these circumstances, reduce the synthesis of Lp(a) (51).

This study had several strengths. As we known, our study is the first to demonstrate a causal association of T2DM and the related glycemic traits with a broad spectrum of CVDs and dyslipidaemia using MR and employing large GWASs data. Two-sample MR method was utilized, which eliminated residual confounding as much as possible. For the outcomes of CVDs, we utilized two highly representative and independent cohorts to stabilize the results of our causal inference. Moreover, the F-statistics of the genetic variants were mostly more than 10, which indicated that the genetic variants were strong enough to be IVs for exposure. Since T2DM and glycemic traits may interact mutually from the pathogenesis of diseases, multivariable MR was applied to adjust the estimate for these exposures. Ultimately, in order to evaluate the robustness of MR results, different MR methods and tests of heterogeneity and pleiotropy were conducted as additional means of sensitivity analyses.

Some limitations could not be ignored. First, most participants included in the GWASs were of European ancestry. Consequently, whether our findings are generalizable to other populations and regions remains to be determined. Second, it is scarcely possible to remove all pleiotropy in MR studies, and some undetected pathways may play a role as confounders between exposures and outcomes, biasing our results. Third, we could obtain only summary-level GWAS data, failing to conduct further investigation on the sex-, age-, and specific type of exposure-related effect on the outcomes. Moreover, the results of MVMR were possibly biased by overfitting from the multivariable model and attenuated or amplified the estimates of effect, which was also observed in this study as compared to the univariable MR. Last, since the glycemic and lipid traits were predisposed as continuous variables, we assumed that the relationship between T2DM or CVDs was linear, which could be inconsistent with the actual situation.

In conclusion, our MR study provides further evidence that T2DM and its related glycemic traits play a causal role in the increased risk of various CVDs and dyslipidemia. The findings should be interpreted to strengthen the awareness of early detection of T2DM and its related glycemic traits. Further work using individual-level data or basic science approaches to investigate the mechanisms mediating these causal associations is warranted.

# DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

# AUTHOR CONTRIBUTIONS

MH and LL designed the study. MH, LZ, and LL undertook the data analyses with feedback from TT and XC. MH and LL co-wrote the paper. TT and XC reviewed and provided important suggestions for the manuscript. TT and XC are the guarantors of

this study. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.840579/full#supplementary-material

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# Glycated Hemoglobin and Risk of Arterial Stiffness in a Chinese Han Population: A Longitudinal Study

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**Background and Aims:** Glycated hemoglobin (HbA1c) associates with the risk of arterial stiffness, and such association can be found between fasting blood glucose (FBG), postprandial blood glucose (PBG), triglyceride-glucose index (TyG index), and arterial stiffness. However, the results were inconsistent, longitudinal studies were sparse, and comparison of these glycemic parameters was less conducted. We aimed to explore the longitudinal relationship between HbA1c and arterial stiffness and compare the effect of the parameters.

**Methods:** Data were collected from 2011 to 2019 in Beijing Health Management Cohort (BHMC) study. Cox proportional hazard models were fitted to investigate the association between the parameters and arterial stiffness. A generalized estimation equation (GEE) analysis was conducted to investigate the effect of repeated measurements of glycemic parameters. A receiver operating characteristic (ROC) analysis was performed to compare the predictive value of glycemic parameters for arterial stiffness.

**Results:** Among 3,048 subjects, 591 were diagnosed as arterial stiffness during the follow-up. The adjusted hazard ratio (HR) [95% confidence interval (CI)] for arterial stiffness of the highest quartile group of HbA1c was 1.63 (1.22–2.18), which was higher than those of FBG, PBG, and TyG index. The nonlinear association of arterial stiffness with HbA1c and PBG was proved. The robust results of the sensitivity analysis were obtained.

**Conclusions:** HbA1c is an important risk factor of arterial stiffness compared with PBG, FBG, and TyG index, and has a strong predictive ability for arterial stiffness among non-diabetics and the general population.

**Keywords: glycated hemoglobin, arterial stiffness, longitudinal study, brachial-ankle pulse wave velocity, ankle brachial index**

## INTRODUCTION

Arterial stiffness, an important pre-stage status of disease, has a dramatic effect on the progression of severe vascular diseases (1). Many studies have investigated the risk factors of arterial stiffness (2). As one of the products in the process of long-term adverse glycation, glycated hemoglobin (HbA1c) would describe the risk of not only arterial stiffness, but also cardiovascular diseases (3). Many indicators diagnosing arterial stiffness are adopted (4–7). Brachial-ankle pulse wave velocity (baPWV) and ankle brachial index (ABI) are two effective methods for the definition of arterial stiffness. Many studies have demonstrated the effect (6–9) and discriminatory power (10) of HbA1c on arterial stiffness. However, in some other studies, such association did not exist (5, 11). There exists interaction between arterial stiffness and diabetes mellitus. Recently, many studies have investigated the relationship between glucose parameters and arterial stiffness (12–14), such as fasting blood glucose (FBG) and postprandial blood glucose (PBG). Compared with FBG and PBG, HbA1c is a predictive indicator of arterial stiffness among non-diabetics (15, 16). However, the results were inconsistent (12, 15, 17). Triglyceride-glucose index (TyG index) is an indicator of insulin resistance, and is closely associated with the progression of arterial stiffness and atherosclerosis (18, 19). However, to the best of our knowledge, there is no study about the comparison between these parameters with the risk of arterial stiffness.

Currently, many studies have explored the relationship between HbA1c and risk of arterial stiffness, but studies comparing the effect of HbA1c, FBG, PBG, and TyG index on arterial stiffness were sparse. Additionally, most of the studies were cross-sectional (15, 16), and the results were inconsistent (4, 20, 21). Repeated measurement of glucose parameters was not fully considered, and the predictive ability of these parameters for arterial stiffness was not clarified and compared.

In this cohort study, we aimed to explore the longitudinal relationship between HbA1c and the risk of arterial stiffness; compare the effect of HbA1c, PBG, FBG, and TyG index on arterial stiffness; and provide effective information for population with different levels of the parameters.

## MATERIALS AND METHODS

### Study Population

The Beijing Health Management Cohort (BHMC) study aims to explore the important chronic diseases among participants in Beijing. Participants were enrolled in 2011 and 2012, and followed until December 31, 2019, in this study. Of 8,917 participants, 2,555 participants with cardiovascular diseases, cancer, or atherosclerosis or without information about HbA1c were excluded. A total of 3,314 participants failed to take the final diagnostic test at the final survey. A total of 3,048 participants received at least one follow-up and were enrolled in the study (**Figure 1**).

### Data Collection

Physical and laboratory examination was conducted by trained medical professionals in the whole process based on the 1964 Declaration of Helsinki and the updated version.

Participants were required to take off their shoes and heavy clothes when taking their anthropometric measurements. Body mass index (BMI) is the measure of dividing the weight (kg) by the square of the height ($m^2$). After at least 5 min rest and 30 min interval with caffeine forbidden, mean arterial pressure (MAP) was calculated as diastolic blood pressure (DBP) plus one-third of pulse pressure, and the latter was measured as systolic blood pressure (SBP) minus DBP. Participants were supposed to keep calm in the sitting position, and the equipment should be placed at the height of the heart, as well as the tested right arm. Such test should be conducted at least three times with 1–2 min interval, and the mean of the records was applied. After at least 12 h overnight fasting, the blood samples were collected and the following main parameters were tested by high-performance liquid chromatography (HPLC) (chemistry analyzer: Beckman LX 20, Beckman, Brea, CA, USA): HbA1c, FBG, high-density lipoprotein cholesterol (HDL-C), hemoglobin, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG).

Demographic data were obtained according to the questionnaire. Briefly, information about age, gender, behavioral factors, including physical activity intensity (low, moderate, and high intensity), education level (lower than high-school education and over or equal to high-school education), excessive salt intake (>6 g/day), drinking status (past/current alcohol drinker), smoking status (past/current smoker), sleep duration (<6 h/day, 6–8 h/day and >8 h/day), and medication history of hypertension, diabetes, and hyperlipidemia was collected.

### Definition of Arterial Stiffness

We defined arterial stiffness as baPWV >1,800 m/s or ABI <0.9 (22–24) in this study. The above two indicators were tested with an automatic arterial stiffness analyzer. The requirement of the participants was similar with that when testing the blood pressure. We calculated ABI as the ratio of SBP at the ankle to that on the upper arm on each side, and the minimum ratio was applied. BaPWV was calculated based on pressure in ankles and upper arms and the distance tailored to the height of each participant by the analyzer automatically, and the maximum record was applied.

### Statistical Analysis

We stratified the study population into quartiles based on HbA1c level (Q1 group: participants with HbA1c ≤5.29%; Q2 group: participants with HbA1c in range of 5.30%–5.52%; Q3 group: participants with HbA1c in range of 5.53%–5.81%; Q4 group: participants with HbA1c >5.81%). TyG index was calculated as ln (fasting TG (mg/dl) × FBG (mg/dl)/2). We also

**Abbreviations:** HbA1c, glycated hemoglobin; FBG, fasting blood glucose; PBG, postprandial blood glucose; TyG Index, triglyceride-glucose index; BHMC, Beijing Health Management Cohort; baPWV, brachial-ankle pulse wave velocity; ABI, ankle brachial index; BMI, body mass index; MAP, mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HR, hazard ratio; IQR, interquartile range; CI, confidence interval; Q, quartile; HPLC, high-performance liquid chromatography; ROC, receiver operating characteristic; AUC, area under curve.

classified the participants into quartile groups based on the level of PBG, FBG, and TyG index. Continuous variables were summarized as mean ± standard deviation or median with interquartile range (IQR). Categorical variables were presented as numbers and proportions. We used ANOVA test for non-paired samples of normally distributed parameters and the Kruskal–Wallis test for non-parametric variables. The chi-squared test was applied for the comparison of categorical variables among 'four quartile groups of HbA1c.

Three-step stepwise multivariable-adjusted Cox proportional hazard regression models were conducted to explore the association between HbA1c, PBG, FBG, and TyG index and the risk of the arterial stiffness. Model 1 was adjusted for age and gender. Model 2 was adjusted for variables in model 1, as well as education level, smoking status, drinking status, physical activity intensity, sleep duration, excessive salt intake, anemia, and medication history. Model 3 was adjusted for variables in model 2 plus BMI, MAP, LDL-C, HDL-C, and TG. As for TyG index, we excluded TG from model 3. A multivariable adjusted restricted cubic spline model with 3 knots was used to assess the dose–response relationship between HbA1c, PBG, FBG, and TyG index and the risk of arterial stiffness. Receiver operating characteristic (ROC) analysis was performed to explore the predictive ability of HbA1c, PBG, FBG, and TyG index on arterial stiffness. Considering the effect of glycemic status, ROC curves were also obtained in both diabetic and non-diabetic populations.

We performed several sensitivity analyses. A generalized estimation equation (GEE) model was built to explore the association of repeated measurement of glycemic parameters and arterial stiffness. Cox regression analysis was further performed among diabetics and non-diabetics. Considering the effect of anemia on measurement of HbA1c, Cox regression analysis was conducted and the GEE model was used among individuals without anemia at baseline.

For all analyses, a two-tailed $p$-value <0.05 was considered to be statistically significant. All statistical analyses were performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), SAS version 9.4 (SAS Institute, Cary, North Carolina, USA) and Stata version 15 (College Station, TX, StataCorp LLC).

# RESULTS

## Baseline Characteristics of the Study Population

In this cohort study, 3,048 qualified participants finished the follow-up and were enrolled in the final analysis. Of the 3,048 participants, 1,939 participants took the baseline and final examination, and their repeated measurements of glycemic parameters and outcomes in 2013–2017 were also collected. Finally, 1,939 individuals were included to explore the impact of repeated measurement of the glycemic parameters on arterial stiffness. The baseline characteristics of the whole study population in different groups stratified by HbA1c quartiles were summarized in **Table 1**. In detail, 2,311 men and 737 women were investigated. Of the 3,048 participants, 14.93% of the participants were affected by diabetes mellitus and 141

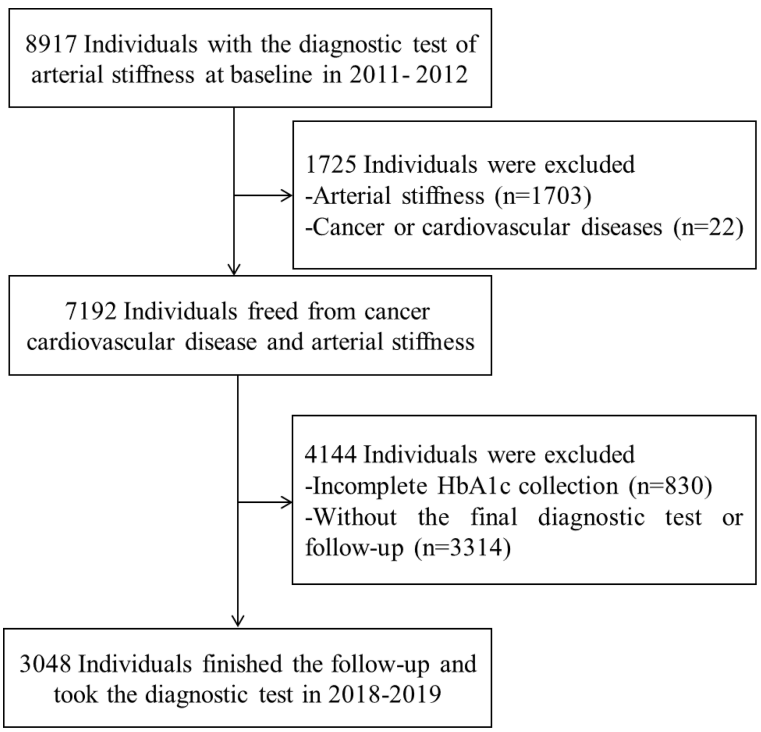


**FIGURE 1** | Flow table of the study population in the Beijing Health Management Cohort from 2011–2012 to 2018–2019. HbA1c, glycated hemoglobin.

**TABLE 1** | Baseline characteristics of the total participants.

| Variables | Total participants | Quartiles of HbA1c | | | | *p*-value |
|---|---|---|---|---|---|---|
| | | Q1 (≤5.29%) | Q2 (5.30%–5.52%) | Q3 (5.53%–5.81%) | Q4 (>5.81%) | |
| No. of participants, *n* | 3,048 | 774 | 772 | 748 | 754 | |
| Arterial stiffness, *n* (%) | 591 (19.39) | 90 (11.63) | 113 (14.64) | 149 (19.92) | 239 (31.70) | <0.0001 |
| Male, *n* (%) | 2,311 (75.82) | 582 (75.19) | 568 (73.58) | 547 (73.13) | 614 (81.43) | 0.0004 |
| Age (years), median (IQR) | 56 (48-63) | 45 (52-58) | 47 (54-61) | 51 (57-64) | 54 (59-67) | <0.0001 |
| Age ≥ 65, *n* (%) | 667 (21.88) | 103 (13.31) | 137 (17.75) | 182 (24.33) | 245 (32.49) | <0.0001 |
| Over or equal to high-school, *n* (%) | 2,995 (98.26) | 767 (99.10) | 762 (98.70) | 734 (98.13) | 732 (97.08) | 0.0165 |
| Physical activity intensity, *n* (%) | | | | | | 0.2358 |
| Low | 623 (20.47) | 143 (18.48) | 152 (19.69) | 169 (22.65) | 159 (21.14) | |
| Moderate | 2,358 (77.46) | 617 (79.72) | 599 (77.59) | 560 (75.07) | 582 (77.39) | |
| High | 63 (2.07) | 14 (1.81) | 21 (2.72) | 17 (2.28) | 11 (1.46) | |
| Past/current smoker, *n* (%) | 276 (9.06) | 67 (8.66) | 61 (7.90) | 78 (10.43) | 70 (9.28) | 0.3681 |
| Past/current alcohol drinker, *n* (%) | 374 (12.27) | 97 (12.53) | 90 (11.66) | 97 (12.97) | 90 (11.94) | 0.8651 |
| Excessive salt intake (>6 g/day), *n* (%) | 174 (5.71) | 45 (5.81) | 30 (3.89) | 53 (7.09) | 46 (6.10) | 0.0543 |
| Sleep duration, *n* (%) | | | | | | 0.3240 |
| <6 h/day | 72 (2.36) | 16 (2.07) | 21 (2.72) | 22 (2.94) | 13 (1.72) | |
| 6–8 h/day | 2,868 (94.36) | 731 (94.44) | 729 (94.43) | 702 (93.85) | 706 (93.63) | |
| >8 h/day | 108 (3.54) | 27 (3.49) | 22 (2.85) | 24 (3.21) | 35 (4.64) | |
| Diabetes, *n* (%) | 455 (14.93) | 11 (1.42) | 22 (2.85) | 53 (7.09) | 369 (48.94) | <0.0001 |
| Anemia, *n* (%) | 141 (5.82) | 24 (3.98) | 34 (5.50) | 36 (6.15) | 47 (7.62) | 0.0550 |
| Medication history of diabetes, *n* (%) | 266 (8.73) | 6 (0.78) | 12 (1.55) | 33 (4.41) | 215 (28.51) | <0.0001 |
| Medication history of hyperlipidemia, *n* (%) | 218 (7.15) | 25 (3.23) | 38 (4.92) | 55 (7.35) | 100 (13.26) | <0.0001 |
| Medication history of hypertension, *n* (%) | 924 (30.31) | 183 (23.64) | 196 (25.39) | 223 (29.81) | 322 (42.71) | <0.0001 |
| MAP (mmHg), median (IQR) | 92.67 (84.67–100.33) | 92.33 (84.00–100.00) | 91.33 (83.67–99.33) | 92.00 (84.33–100.00) | 94.33 (86.67–102.00) | <0.0001 |
| BMI (kg/m$^2$), median (IQR) | 25.51 (23.62–27.54) | 24.91 (23.26–26.82) | 25.25 (23.26–27.19) | 25.47 (23.48–27.56) | 26.40 (24.55–28.50) | <0.0001 |
| LDL-C (mmol/L) median (IQR), | 3.08 (2.52–3.70) | 3.01 (2.51–3.59) | 3.14 (2.57–3.74) | 3.20 (2.64–3.82) | 2.98 (2.38–3.65) | <0.0001 |
| TG (mmol/L) median (IQR) | 1.39 (1.01–2.05) | 1.29 (0.95–1.89) | 1.38 (0.98–2.00) | 1.42 (1.03–2.06) | 1.56 (1.11–2.22) | <0.0001 |
| HDL-C (mmol/L), median (IQR) | 1.20 (1.05–1.40) | 1.23 (1.07–1.46) | 1.22 (1.08–1.41) | 1.22 (1.06–1.39) | 1.12 (1.04–1.30) | <0.0001 |

*HbA1c, glycated hemoglobin; IQR, interquartile range; Q: quartile; MAP, mean arterial pressure; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.*

(5.82%) participants were affected by anemia at baseline. The distribution of HbA1c, PBG, FBG, and TyG index at baseline among individuals grouped by the occurrence of arterial stiffness during the follow-up or at the end point is shown in **Figure 2**. The outcome of each participant in different quartile groups of HbA1c, PBG, FBG, and TyG index at baseline is shown in **Figure 3**. The distribution of age, education level, diabetes status, and medication history of hypertension, diabetes, and hyperlipidemia among the quartile groups of HbA1c was significantly different. Individuals in the higher HbA1c quartile group had a significantly higher level of LDL-C, TG, MAP, and BMI and a lower level of HDL-C. A significant difference was not observed among different HbA1c quartile groups for physical activity intensity, smoking status, drinking status, excessive salt intake, anemia, and sleep duration.

## Association Between Arterial Stiffness and Glucose Parameters

The results indicated that compared with the lowest quartile group of HbA1c, people in higher quartile groups had higher HR (**Figure 4**). The HRs (95% CI, *p*-value) were 1.13 (0.83–1.54, $p = 0.434$) for the Q2 group, 1.53 (1.14–2.04, $p = 0.005$) for the Q3 group and 1.63 (1.22–2.18, $p = 0.001$) for the Q4 group with the covariates in Model 3 controlled (**Table 2**).

## Dose–Response Association Between Four Glucose Parameters and Arterial Stiffness

The result of the restricted cubic spline model showed that the level of HbA1c from 5.71% to 6.95% had a significantly nonlinear relationship with the risk of arterial stiffness. The nonlinear relationship between arterial stiffness and PBG level higher than or equal to 7.70 mmol/L was observed. However, we did not find such relationship between FBG and TyG index and the risk of arterial stiffness (**Figure 5**).

## Predictive Ability of Four Glucose Parameters for Arterial Stiffness

HbA1c had powerful predictive ability on arterial stiffness among non-diabetics and the whole study population (**Figure 6**). Although PBG presented a better predictive performance, statistical significance was not observed when comparing areas under curve (AUCs) between PBG and HbA1c (**Supplementary Table S1**).

## Sensitivity Analysis

HbA1c was the strongest risk factor for arterial stiffness (HR: 1.47, 95% CI: 1.25–1.72) in comparison to other parameters (**Supplementary Table S2**) based on GEE analysis. The

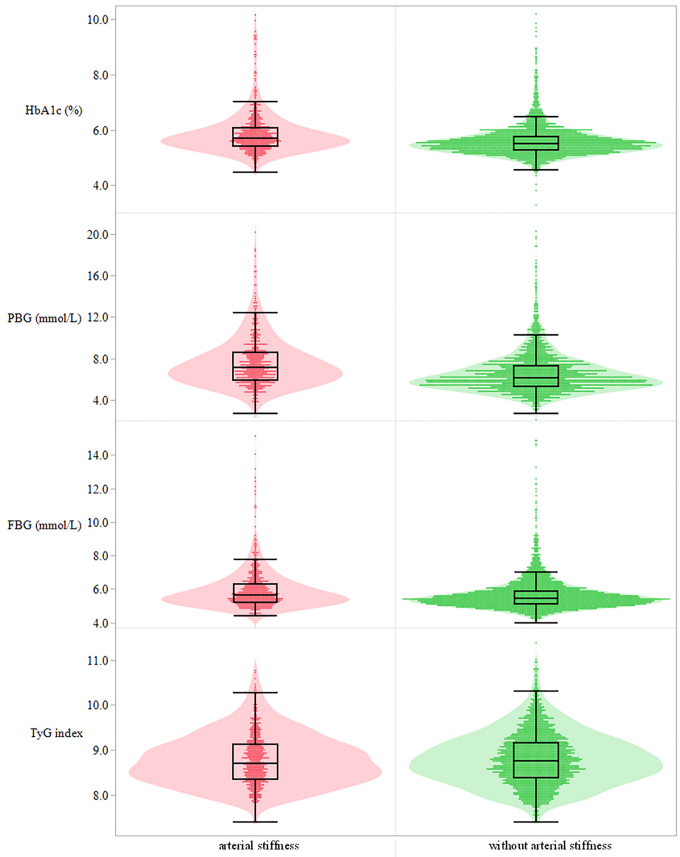


**FIGURE 2** | Distribution of the four glucose parameters at baseline among individuals with and without arterial stiffness. Sequentially presented in the picture from top to bottom is HbA1c (%), PBG (mmol/L), FBG (mmol/L), and TyG index. HbA1c, glycated hemoglobin; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index.

significant association was found between HbA1c and the risk of arterial stiffness among non-diabetics (**Supplementary Table S3**), but such association was not found among diabetics (**Supplementary Table S4**). When individuals with an anemic status at baseline were excluded, the robust results were obtained. Individuals in the higher quartile group of HbA1c had a higher risk for arterial stiffness (**Supplementary Table S5**), and the association between repeated measurement of HbA1c and the higher risk of arterial stiffness was statistically significant (**Supplementary Table S6**). Such significant association was observed among the non-diabetic population without anemia at baseline (**Supplementary Table S7**), but not in the diabetic population without anemia at baseline (**Supplementary Table S8**).

## DISCUSSION

In our longitudinal study, HbA1c had a significantly positive relationship with the risk of arterial stiffness compared with PBG, FBG, and TyG index. The nonlinear relationship was found between HbA1c in the level from 5.71% to 6.95% and the risk of arterial stiffness. HbA1c and PBG had better predictive performance of arterial stiffness among the whole and non-diabetic population. The robust results were obtained.

Our result illustrated the important effect of HbA1c on arterial stiffness partly because of the glycation process (25). Hyperglycemia and insulin resistance could accelerate the progression of arterial lesions (26, 27). Insulin resistance can be regarded as treating target of arterial stiffness with the significant reduction of HbA1c among diabetics (28). Moreno (29) found that HbA1c could independently express the differences in the risk of arterial stiffness between groups with controlled and uncontrolled diabetes mellitus. Johansen (28) demonstrated that changes in HbA1c over time or measurement at baseline both had an impact on aortic stiffness. HbA1c is an indicator of long-term variation of blood glucose and advanced glycation products. Chronic hyperglycemia with long-term high level of HbA1c can promote protein glycosylation and accelerate the progression of arterial stiffness (29). In the long process of glycation, intermediate and end glycation products would result in the pathological change of arterial wall and endothelial dysfunction, and accelerate the progression of arterial stiffness (30).

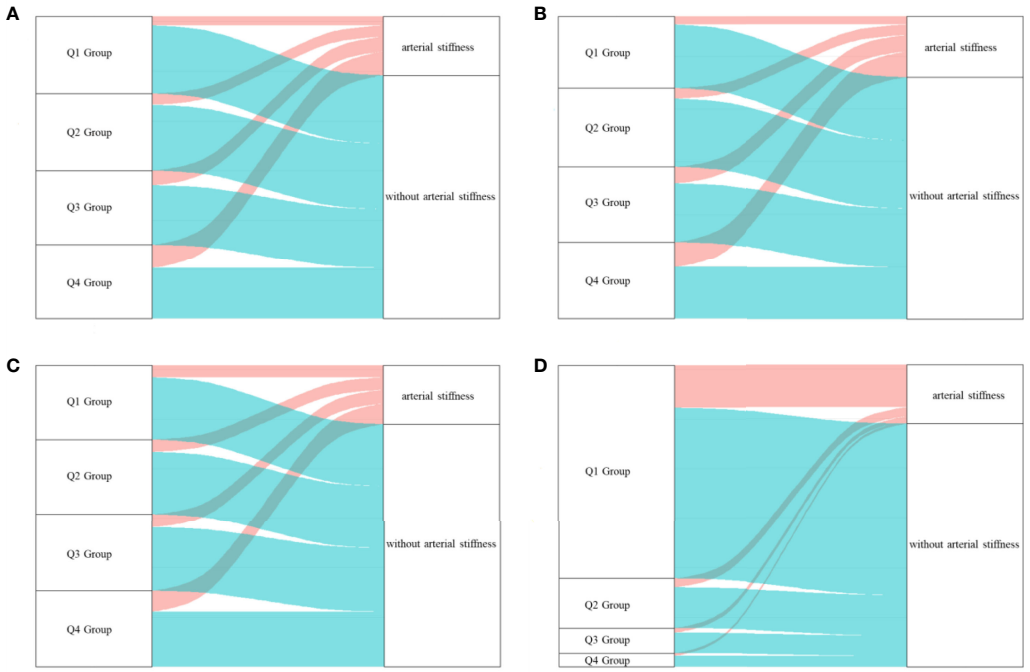


**FIGURE 3** | The outcome of participants in different quartile groups of the four glucose parameters at baseline. **(A)** HbA1c, **(B)** PBG, **(C)** FBG, **(D)** TyG index. A large proportion of individuals in higher quartile groups of HbA1c obtained a higher risk of arterial stiffness. HbA1c, glycated hemoglobin; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index; Q, quartile.

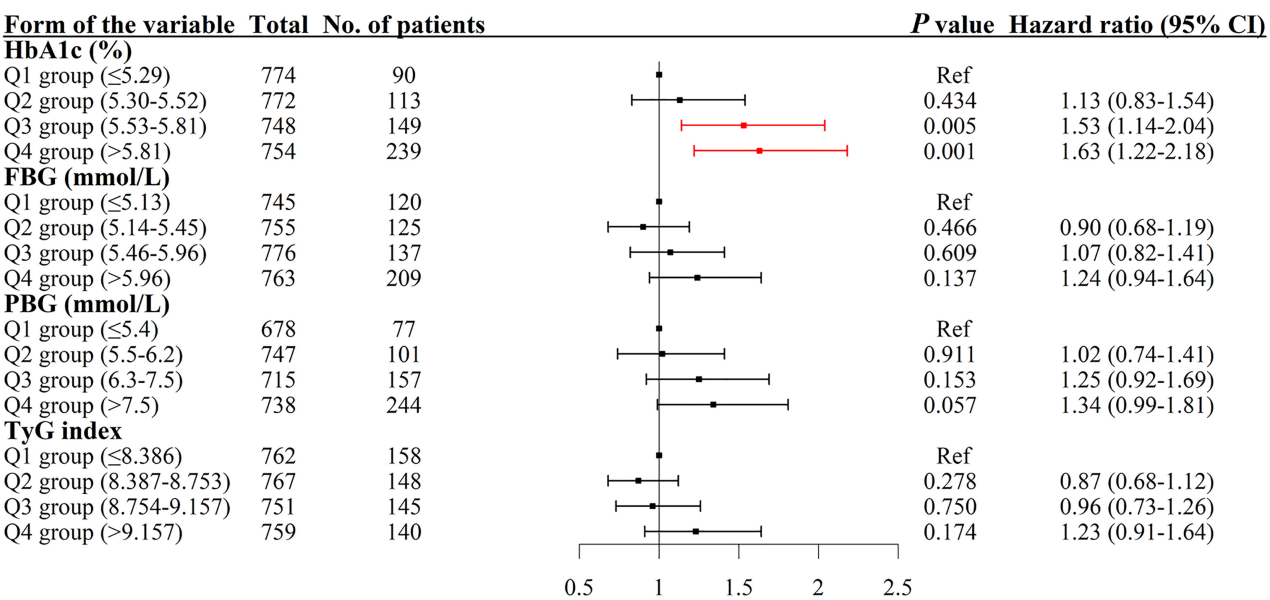


**FIGURE 4** | Association between HbA1c, PBG, FBG, and TyG index and the risk of arterial stiffness. The results were robust when more covariates were entered into the model. HbA1c, glycated hemoglobin; Q, quartile; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index; CI, confidence interval.

**TABLE 2 |** Association between glycemic parameters and arterial stiffness among the total population.

| Variables | Model 1: HR (95% CI: Lower–Upper) | *p*-value | Model 2: HR (95% CI: Lower–Upper) | *p*-value | Model 3: HR (95% CI: Lower–Upper) | *p*-value |
|---|---|---|---|---|---|---|
| HbA1c (%) | | | | | | |
| Q1 (≤5.29) | Reference | | Reference | | Reference | |
| Q2 (5.30–5.52) | 1.04 (0.79–1.37) | 0.777 | 1.11 (0.81–1.51) | 0.526 | 1.13 (0.83–1.54) | 0.434 |
| Q3 (5.53–5.81) | 1.31 (1.01–1.70) | 0.045 | 1.48 (1.11–1.98) | 0.008 | 1.53 (1.14–2.04) | 0.005 |
| Q4 (>5.81) | 1.69 (1.32–2.16) | <0.001 | 1.67 (1.25–2.22) | 0.001 | 1.63 (1.22–2.18) | 0.001 |
| FBG (mmol/L) | | | | | | |
| Q1 (≤5.13) | Reference | | Reference | | Reference | |
| Q2 (5.14–5.45) | 1.01 (0.79–1.30) | 0.917 | 0.95 (0.72–1.26) | 0.733 | 0.90 (0.68–1.19) | 0.466 |
| Q3 (5.46–5.96) | 1.09 (0.86–1.40) | 0.476 | 1.14 (0.88–1.49) | 0.319 | 1.07 (0.82–1.41) | 0.609 |
| Q4 (>5.96) | 1.56 (1.25–1.96) | <0.001 | 1.45 (1.11–1.90) | 0.006 | 1.24 (0.94–1.64) | 0.137 |
| PBG (mmol/L) | | | | | | |
| Q1 (≤5.4) | Reference | | Reference | | Reference | |
| Q2 (5.5–6.2) | 1.06 (0.78–1.42) | 0.722 | 1.10 (0.80–1.51) | 0.555 | 1.02 (0.74–1.41) | 0.911 |
| Q3 (6.3–7.5) | 1.36 (1.03–1.79) | 0.031 | 1.34 (0.99–1.81) | 0.056 | 1.25 (0.92–1.69) | 0.153 |
| Q4 (>7.5) | 1.68 (1.29–2.18) | <0.001 | 1.49 (1.11–2.00) | 0.007 | 1.34 (0.99–1.81) | 0.057 |
| TyG index | | | | | | |
| Q1 (≤8.386) | Reference | | Reference | | Reference | |
| Q2 (8.387–8.753) | 1.05 (0.84–1.31) | 0.679 | 0.97 (0.76–1.24) | 0.819 | 0.87 (0.68–1.12) | 0.278 |
| Q3 (8.754–9.157) | 1.20 (0.95–1.50) | 0.122 | 1.07 (0.83–1.37) | 0.592 | 0.96 (0.73–1.26) | 0.750 |
| Q4 (>9.157) | 1.64 (1.29–2.08) | <0.001 | 1.48 (1.14–1.92) | 0.003 | 1.23 (0.91–1.64) | 0.174 |

*Model 1: adjusted for age and gender.*

*Model 2: adjusted for variables in model 1, as well as education level, smoking status, drinking status, physical activity intensity, sleep duration, anemia, excessive salt intake, and medication history of hypertension, diabetes, and hyperlipidemia.*

*Model 3: adjusted for variables in model 2 plus BMI, MAP, LDL-C, HDL-C, and TG.*

*HbA1c, glycated hemoglobin; Q, quartile; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index; HR, hazard ratio; CI, confidence interval.*

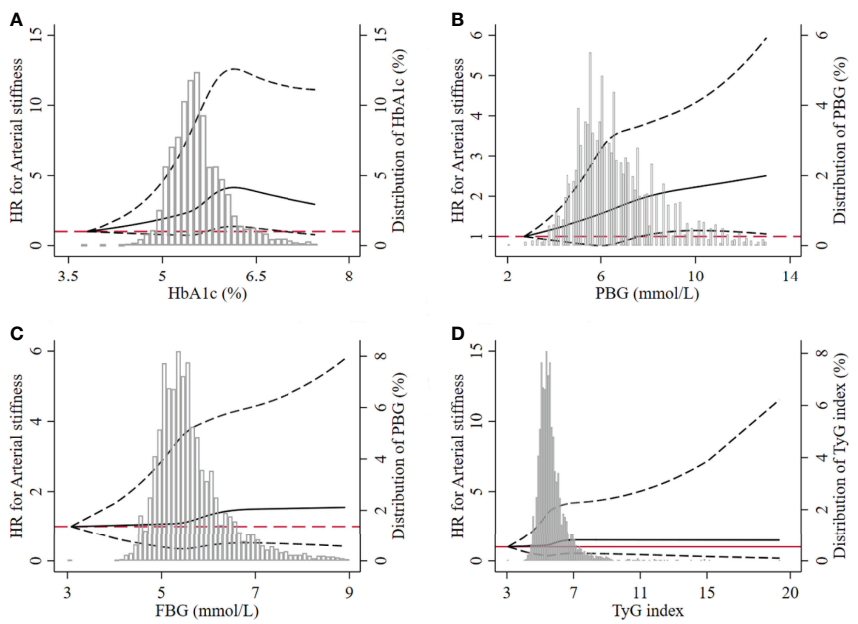


**FIGURE 5** | Dose–response relationship between the four glucose parameters and the risk of arterial stiffness. **(A)** HbA1c, **(B)** PBG, **(C)** FBG, **(D)** TyG index. The dotted lines represented the lower and upper limit of 95% CI at each dot. HbA1c, glycated hemoglobin; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index; HR, hazard ratio.

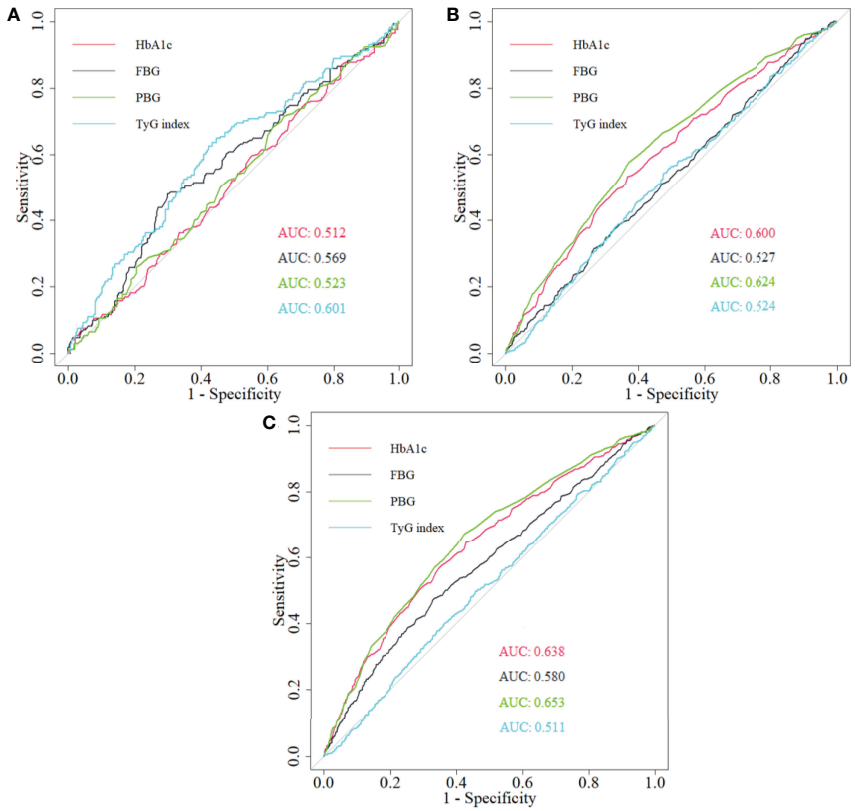


**FIGURE 6** | The comparison of the predictive ability of HbA1c, PBG, FBG, and TyG index for arterial stiffness among **(A)** diabetics, **(B)** non-diabetics, and **(C)** total population. HbA1c, glycated hemoglobin; PBG, postprandial blood glucose; FBG, fasting blood glucose; TyG index, triglyceride-glucose index; ROC, receiver operating characteristic; AUC, area under curve.

In our study, we found that HbA1c is an effective risk factor of arterial stiffness compared with three other glucose parameters. Some previous studies had investigated the effect of TyG index, an essential indicator of insulin-resistance, PBG, and FBG on arterial stiffness in different groups of population (31–33). However, comparison between these parameters was seldom conducted, and HbA1c had not been intensively explored. Our study supplies practical information about comparison of the risk among these glucose parameters on arterial stiffness based on existing mechanisms (34, 35). PBG level higher than or equal to 7.70 mmol/L and the level of HbA1c from 4% to 6% can be treated as abnormal glycemic status. Thus, our results of the nonlinear relationship of PBG ≥7.70 mmol/L and HbA1c from 5.71% to 6.95% with arterial stiffness were reliable.

The predictive role of HbA1c for arterial stiffness was proved previously (36), and the better predictive ability of HbA1c was found compared with FBG (10) in both diabetic and non-diabetic groups. The result was similar with ours. The better predictive ability of TyG index on arterial stiffness among diabetics was obtained, because TyG index was strongly associated with glycemic status and insulin resistance and was a better indicator of diabetic status (37–39). The better predictive role of PBG among non-diabetics and the whole study population was found compared with HbA1c; however, a significant difference was not found. Moreover, PBG was affected by personal diet pattern and not easily accessible. Although the significant association between HbA1c and arterial stiffness was not observed among diabetics, it is acknowledged that HbA1C is a strong prospective tool to assess the risk of diabetic complications and an effective indicator of treatment of diabetes (40, 41). Thus, the predictive role of HbA1c on arterial stiffness should not be ignored.

In many studies, the positive association between HbA1c and arteriosclerosis disappeared (42) after adjusting for general confounding factors. The endpoint of our study is the occurrence of arterial stiffness diagnosed by baPWV and ABI. Many studies had diagnosed arterial stiffness by angiography examination. Angiography examination can express the progression and condition of arteriosclerosis (43), but its measurement was not sensitive at the initial stage of arteriosclerosis. BaPWV and ABI are simple and harmless tests and can serve as a routine clinical examination for measuring arterial stiffness (24). The differences in diagnosis criteria among these studies would cause contradictory results. On the other hand, measurement of HbA1c was different, the sample sizes of the study populations were distinct (11), and the study

population was composed of individuals with different characteristics (44). Health condition and medication history of the study population also had an impact. Statin therapy, one of the most widely applied dyslipidemia drugs (45), would improve endothelial function (46) and further improve the condition of vascular sclerosis. Diabetes drugs would preserve anti-oxidant function, and further decrease platelet activation and aggregation (47). In our analysis, medication history was taken into consideration as a confounding factor. Thus, the confounding effects of medication history were properly adjusted. More detailed information about medication history, such as the specific type, will be further investigated.

There are several strengths of this study. It should be emphasized that this is a longitudinal study investigating the positive association of baseline and repeated measurement of HbA1c and arterial stiffness. Besides HbA1c, we also compared the impact of PBG, FBG, and TyG index on arterial stiffness. ROC analysis was performed, and the results illustrated the highest risk of abnormal level of HbA1c and its strong predictive ability on arterial stiffness. Multiple sensitivity analyses are also the strength, and the robust results were obtained.

The present study has some limitations. First, we included all qualified individuals to make our conclusion accessible to the general population. However, more participants from different nationalities or races should be enrolled. Second, although HPLC is an effective method to differentiate HbA1c and some types of Hb variants, the influence of Hb variants on measurement of HbA1c should not be ignored. Many glycemic parameters, such as glycemic variability and time in range, have the potential to reflect the dynamic change in blood glucose (48, 49). Such parameters would better reflect glycemic status. Thus, we can investigate the impact of these parameters on the progression of arterial stiffness in future studies.

## CONCLUSIONS

In this longitudinal study, the association between HbA1c and the higher risk of arterial stiffness among the general or non-diabetic population was observed compared with PBG, FBG, and TyG index assessed by baPWV and ABI among a Chinese Han population. This study suggests the necessity for the early detection and management of arterial stiffness among a population with a high abnormal level of HbA1c, especially among non-diabetics.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Capital Medical University (number 2013SY26). The patients/participants provided written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

ZH, LT, and SC came up with the idea and made the design of the study. XK contributed to the data. ZH and JW conducted the statistical analysis. ZH drafted the primary manuscript. ZH, JL, JZ, JW, XZ, YL, and ZW revised the manuscript and provided important advice for modification. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.854875/full#supplementary-material

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# Sexual Dimorphism in the Association of Serum Retinol-Binding Protein-4 With Long-Term Dynamic Metabolic Profiles in Non-Diabetes

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**Objectives:** We aimed to investigate the association of circulating retinol-binding protein-4 (RBP4) levels with long-term cardiometabolic risk profiles and whether sex disparity mattered.

**Methods:** We included 784 non-diabetic participants aged 40 years and above from a well-defined community-based cohort at baseline in 2005 and they were invited to attend the on-site follow-up examination for two consecutive times with 3-year intervals in 2008 and 2011, respectively. Serum RBP4 was measured at baseline, and the anthropometry and biochemical measurements were performed at each visit. Generalized estimating equation models were used to assess the association of serum RBP4 levels with the dynamic changes in adiposity and glucolipid profile.

**Results:** Based on all the baseline and the 3- and 6-year follow-up data, baseline serum RBP4 levels (each 1-unit of $\log_{10}$RBP4) were significantly associated with waist circumference [β=3.12, 95% confidence interval (CI) (0.77, 5.47), P=0.01], fasting, and 2-h post-loading glucose [β=0.26 (0.05, 0.47), P=0.02, and 1.70 (1.29, 2.12), P< 0.0001], serum triglycerides [β=0.75, 95% CI (0.54, 0.96), P< 0.0001], total cholesterol [β=0.47, 95% CI [0.23 0.70], P<0.0001), and marginally with body mass index (β=0.97, 95% CI (0.02, 1.93), P=0.046], in total participants, after adjusting potential confounders. The association of RBP4 with 2-h post-loading glucose was stronger in women than that in men [β=1.99, 95% CI (1.49, 2.50) vs. 0.61 (-0.14, 1.36), P for interaction=0.001]. The analysis of change in Z-score of cardiometabolic profiles corresponding to each 1-unit increment in $\log_{10}$RBP4 showed consistent results.

**Conclusions:** Higher RBP4 levels are associated with longitudinal increase in adiposity and deteriorated glucolipid profile defined by repeated measurements. The associations differ in sex regarding to the 2-h post-loading glucose.

**Keywords: retinol-binding protein-4, long-term follow-up, dynamic change, adiposity, metabolic profile**

# INTRODUCTION

Retinol-binding protein 4 (RBP4), a retinol transporter in circulation belonging to the lipocalins family (1), has been reported to be associated with type 2 diabetes, obesity, insulin resistance, and other cardiometabolic disorders (2–5). RBP4 levels are higher in obese and/or diabetic individuals in cross-sectional studies (2, 3), and moderate weight reduction can lower serum RBP4 levels in nondiabetic subjects (3). In our previous study, we also found that serum RBP4 was associated with a higher risk for impaired glucose regulation (5). Several prospective cohort studies reported that serum RBP4 levels were associated with increased risk of diabetes and cardiovascular mortality in men with type 2 diabetes (6, 7). However, the results were not consistent. RBP4 concentrations are not increased in children as they are in obese adults with long-standing severe insulin resistance and type 2 diabetes (8). Recent research reported a U-shaped relationship between serum RBP4 levels and the risk of incident type 2 diabetes in subjects with prediabetes (9). The long-term effect of RBP4 on obesity, glucose, and other cardiometabolic risk needs to be further clarified.

Sexual dimorphisms in cardiometabolic disease features including prevalence, progression, and outcome were reported in the epidemiological and clinical setting. Recently, sex difference has been reported to play a pivotal role in the pathogenesis of several cardiometabolic disease (10–13). A recent nested case-control study performed in Singapore Chinese has reported a sex-specific association of RBP4 with risk of type 2 diabetes (14). However, whether sex disparities matter in the association of RBP4 levels with metabolic profiles remains underexplored.

In current longitudinal cohort study with metabolic traits repeatedly measured and followed up for two consecutive times with 3-years' interval, we investigated the association of baseline serum RBP4 with long-term dynamic metabolic profile changes including adiposity, blood glucose, and lipid metabolism. Meanwhile, we lay a particular emphasis on sex disparities in the relationship between baseline RBP4 levels and the metabolic profiles.

# METHODS

## Study Participants

The study participants were from a two-step blood glucose survey started at 2004, performed in an urban community of Shanghai. The study protocols were approved by the Institutional Review Board of the Ruijin Hospital, and written informed consent was provided by each participant. The detailed study design and protocols of this community-based cohort have been described in previous literature (5, 15). All local permanent residents aged 40 or older were invited and, in total, 9219 individuals attended the first step of this survey. After excluding those with previously diagnosed diabetes, remaining screened participants were classified into three groups according to capillary glucose concentrations: 7.0 mmol/L or above; 5.6–6.9 mmol/L; or below 5.6 mmol/L. There were 631 people with fasting capillary glucose levels of 7.0 mmol/L or above, 757 people with levels 5.6–6.9 mmol/L, and 909 individuals with levels below 5.6 mmol/L selected for further investigation, which were sex- and age-matched and based on a ratio of 1:1.2:1.44, in consideration of individuals with lower glucose levels might be less likely to participate than those with higher glucose concentrations. A total of 1835 individuals (491 with fasting capillary glucose levels of 7.0 mmol/L or above; 594 with levels at 5.6–6.9 mmol/L; and 750 with levels below 5.6 mmol/L) participated in the second step of the survey, with an overall recall rate of 80%. During the second step survey, a 75-g oral glucose tolerance test (OGTT) was performed, and both overnight fasting and 2 h OGTT venous blood samples were collected.

All the participants were invited to attend the follow-up visits for two consecutive times with a 3-year interval. Similarly, a 75-g OGTT and blood sampling were also performed in the subsequent follow-up visits. There were 1267 participants without diabetes at baseline, 918 and 784 individuals attended the follow-up visit and with blood sampled in 2008 and 2011, respectively. For the present study, 784 individuals without diabetes at baseline and attended the baseline and the two follow-up visits during a period over 6 years were included in the final analysis. A flow chart showing the study procedure is shown in **Figure 1**.

## Data Collection

Information on sociodemographic characteristics, lifestyle, disease, and medication history was collected by questionnaire at baseline and the subsequent visits. Current smokers or drinkers were defined as individuals who smoked or drank regularly in the past 6 months. Anthropometry traits like body height, body weight, and waist and hip circumferences were measured by well-trained examiners, when participants were dressed in light clothes and with bare feet. Body mass index (BMI) was calculated as body weight in kilograms divided by height squared in meters. Blood pressure was measured at the right arm three times consecutively with 1 min intervals after at least 5 min of rest in the seated position, with an automated electronic device (OMRON Model1 Plus; Omron Company, Kyoto, Japan).

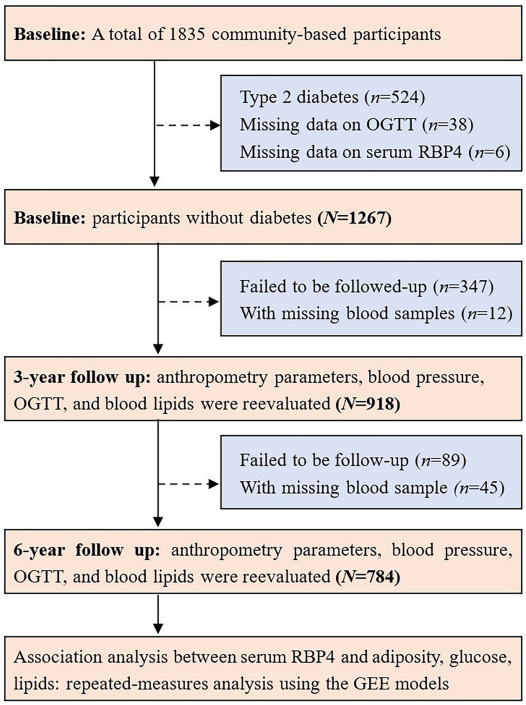


**FIGURE 1** | Flow chart of study population.

## Clinical and Biochemical Measurements

Overnight fasting and 2-h post glucose-load plasma glucose concentrations and fasting serum concentrations of triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, uric acid, and creatinine were measured using an automatic analyzer (Beckman CX-7 Biochemical Autoanalyzer, Brea, CA). Serum insulin concentrations were measured by radioimmunoassay (Sangon Company, Shanghai, China). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the following formula (16): HOMA-IR=fasting insulin (uU/L) ×fasting glucose (mmol/L)/22.5. HOMA-β was calculated as: HOMA-β=20×fasting insulin (uU/L)/[(fasting glucose (mmol/L)-3.5].

Serum RBP4 levels were measured at baseline in duplicate by enzyme linked immunosorbent assay developed in-house, using polyclonal and monoclonal antibodies generated against recombinant human RBP4 protein and purified using affinity chromatography (4, 5). The assay system has good precision with both inter-assay variation coefficient and intra-assay variation coefficient below 20%, and it was further cross-validated by Western blotting. The specific steps of serum RBP4 measurement were given previously.

## Statistical Analysis

Continuous variables in normal distribution were reported as means ± SDs, while the skewed ones are presented as medians (interquartile ranges). Categorical variables are presented as frequencies (%). The comparison of the metabolic traits for the three measurements at baseline, 3-year, and 6-year follow-up was conducted by using the analysis of covariance after adjustments for age and sex.

Generalized estimating equations (GEE) model, accounting for correlation over time instead of assuming independence, is often used to analyze longitudinal data (17). The GEE model was used to reveal the associations of baseline serum RBP4 levels and adiposity index and glucolipid profile in total participants. The analysis was adjusted for potential confounders. Model 1, adjusted for age and sex; model 2, further adjusted for BMI, and smoking and drinking habits based on model 1; and model 3, further adjusted for anti-diabetes drug usage in glucose metabolism traits or lipid-lowering drug usage in lipid profile. We also performed the association analysis between baseline RBP4 and the metabolic traits at each examination visit separately and compared the trajectory consistence of the associations.

To further detect whether sex affects the association between serum RBP4 levels and plasma glucolipid profile, we performed a subgroup analysis according to men vs. women and tested the significance of the multiply interaction with sex. Metabolic traits were also standardized to Z score to facilitate comparison of RBP4 effect sizes.

*P* values of less than 0.05 were considered statistically significant for all the analyses. SAS software version 9.4 (SAS Institute, Cary, NC) was used to perform statistical analysis.

# RESULTS

## Characteristics of Participants

The general characteristics of 784 participants who attended three visits are summarized in **Table 1**. The mean age at baseline was 61.5 ± 9.0 years, and women accounted for 65.3% of the overall population. During the entire follow-up period, most of the cardiometabolic traits including adiposity index (BMI and waist circumference) and glucolipid profiles showed a modest variation (**Figure 2**). Men have a similar trend of cardiometabolic traits during the entire follow-up period, as compared with women. Age adjusted waist circumference, systolic blood pressure, fasting insulin, HOMA-IR, total cholesterol, and LDL cholesterol experienced the undulation of rising first and then falling. During the first 3-year follow up, age adjusted BMI, diastolic blood pressure, fasting, and OGTT 2-h plasma glucose all showed signs of stabilization, but OGTT 2-h plasma glucose showed increased levels and the other three traits showed decreased levels during the next 3 years. HDL cholesterol showed a continued momentum of decline from baseline, and triglycerides showed a minimum of change during the entire follow-up period.

The median RBP4 level at baseline was 16.4 (inter-quartile range 12.1-21.7) ug/mL. Notably, women had lower serum RBP4 levels compared with men [15.8 ug/mL (11.3, 21.0) vs. 17.5 ug/mL (13.3, 23.9), P <0.05].

**TABLE 1** | Characteristics of participants in the longitudinal study with follow-up at 3 and 6 years, respectively (n = 784).

| Characteristics | Baseline | 3 years of follow-up | 6 years of follow-up | *P* value | SNK test |
|---|---|---|---|---|---|
| Age (year) | 61.5 ± 9.0 | 65.1 ± 9.0 | 67.8 ± 9.0 | < 0.0001 | C, B, A |
| Women [n (%)] | 512 (65.3) | 512 (65.3) | 512 (65.3) | / | / |
| Body mass index (kg/m$^2$) | 25.0 ± 3.2 | 25.3 ± 3.2 | 24.6 ± 3.3 | < 0.0001 | (B, A), C |
| Waist circumference (cm) | 83.6 ± 8.4 | 85.2 ± 9.1 | 83.4 ± 8.8 | < 0.0001 | B, (A, C) |
| Current smoking [n (%)] | 109 (13.9) | 99 (12.6) | 78 (10.0) | 0.05 | / |
| Current drinking [n (%)] | 94 (12.0) | 106 (13.5) | 105 (13.4) | 0.68 | / |
| Systolic BP (mm Hg) | 137.5 ± 22.6 | 142.2 ± 19.7 | 135.6 ± 19.3 | < 0.0001 | B, (A, C) |
| Diastolic BP (mm Hg) | 79.5 ± 10.5 | 80.0 ± 10.2 | 76.4 ± 9.5 | < 0.0001 | (B, A), C |
| Hypertension [n (%)] | 416 (53.1) | 532 (68.0) | 564 (72.0) | < 0.0001 | (C, B), A |
| Lower-BP therapy [n (%)] | 246 (31.4) | 277 (35.4) | 330 (42.1) | 0.0001 | C, (B, A) |
| Total cholesterol (mmol/L) | 4.8 ± 0.9 | 5.0 ± 0.8 | 4.3 ± 1.1 | < 0.0001 | B, A, C |
| Triglycerides (mmol/L) | 1.3 (0.9, 1.8) | 1.2 (0.8, 1.7) | 1.3 (0.9, 1.7) | 0.17 | / |
| HDL cholesterol (mmol/L) | 1.5 ± 0.4 | 1.4 ± 0.3 | 1.2 ± 0.4 | < 0.0001 | A, B, C |
| LDL cholesterol (mmol/L) | 2.7 ± 0.7 | 3.3 ± 0.8 | 2.5 ± 0.8 | < 0.0001 | A, B, C |
| FPG (mmol/L) | 5.6 ± 0.6 | 5.6 ± 0.9 | 5.5 ± 0.6 | < 0.0001 | (B, A), C |
| OGTT 2-h PPG (mmol/l) | 7.0 ± 1.6 | 7.0 ± 2.1 | 7.8 ± 2.3 | < 0.0001 | C, (A, B) |
| Fasting insulin (pmol/L) | 4.9 (2.5, 9.0) | 6.9 (4.7, 9.9) | 5.7 (4.2, 7.7) | < 0.0001 | B, (A, C) |
| HOMA-IR | 1.2 (0.6, 2.3) | 1.7 (1.1, 2.7) | 1.4 (1.0, 1.9) | < 0.0001 | B, (A, C) |
| HOMA-β | 49.5 (23.3, 87.0) | 65.1 (44.9, 95.8) | 60.1 (42.6, 83.6) | < 0.0001 | (B, C), A |
| RBP4 (ug/mL) | 16.4 (12.1, 21.7) | / | / | / | / |

*Data are presented as mean ± standard deviation (SD), median (interquartile range), or number (percentage). Multiple comparisons were performed by Student-Newman-Keuls (SNK) test. BP, blood pressure; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; OGTT 2-h PPG, oral glucose tolerate test 2-h post load plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cell function; RBP4, retinol binding protein 4; SNK test, Student-Newman-Keuls test. A, B, and C represent baseline, 3-year follow-up, and 6-year follow-up, respectively. Letters within brackets indicate means in different clusters are not significantly different.*

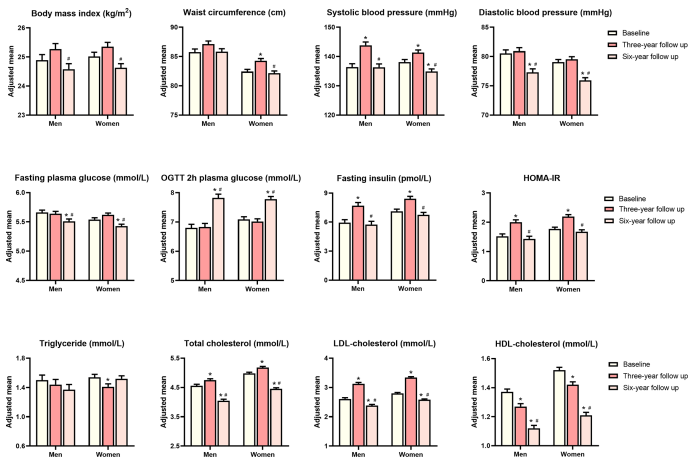


**FIGURE 2** | Age adjusted means of metabolic traits at baseline, 3-year follow-up and 6-year follow-up. P values were calculated from analysis of covariance. *P value significant compared with baseline, #P value significant compared with 3-year follow-up.

## Association of Serum RBP4 Levels With Adiposity Index and Glucolipid Metabolism Traits

The associations between baseline serum RBP4 levels and adiposity indexes, glucose metabolism traits, and lipid profile are presented in **Table 2**. In the age and sex adjusted model (model 1), baseline serum RBP4 levels were positively associated with BMI, waist circumference, fasting, and OGTT 2-h plasma glucose concentrations, serum triglycerides concentrations, and total cholesterol concentrations. When smoking status and drinking status were further adjusted (model 2), per 1-unit increase in log10-RBP4 was associated with 0.97 kg/m$^2$ [95% CI (0.02, 1.93), P=0.046] increment of BMI and 3.12 cm increment of waist circumference [95% CI (0.77, 5.47), P=0.01]. Additional adjustment for BMI, smoking status, and drinking status did not change the associations between baseline serum RBP4 levels and fasting plasma glucose, OGTT 2-h plasma glucose, serum triglycerides, and total cholesterol concentrations. When further adjusted for anti-diabetes drug usage in glucose metabolism traits or lipid-lowering drug usage

**TABLE 2 |** Adjusted changes (95% CI) in metabolic traits per 1-unit increase in $log_{10}$-RBP4 by using the GEE model.

| | *Model 1* | | *Model 2* | | *Model 3* | |
|---|---|---|---|---|---|---|
| | **Unit change (95% CI)** | ***P* value** | **Unit change (95% CI)** | ***P* value** | **Unit change (95% CI)** | ***P* value** |
| **Adiposity** | | | | | | |
| Body mass index, kg/m$^2$ | 0.98 (0.03, 1.93) | 0.04 | 0.97 (0.02, 1.93) | 0.046 | / | / |
| Waist circumference, cm | 3.00 (0.64, 5.35) | 0.01 | 3.12 (0.77, 5.47) | 0.01 | / | / |
| **Glucose metabolism** | | | | | | |
| Fasting plasma glucose, mmol/L | 0.26 (0.05, 0.47) | 0.01 | 0.24 (0.03, 0.45) | 0.02 | 0.26 (0.05, 0.47) | 0.02 |
| OGTT 2-h plasma glucose, mmol/L | 1.64 (1.22, 2.05) | < 0.0001 | 1.59 (1.16, 2.01) | < 0.0001 | 1.70 (1.29, 2.12) | < 0.0001 |
| $Log_{10}$ (fasting insulin), pmol/L | -0.01 (-0.14, 0.11) | 0.85 | -0.06 (-0.22, 0.09) | 0.43 | -0.07 (-0.23, 0.09) | 0.39 |
| $Log_{10}$ (HOMA-IR) | 0.03 (-0.07, 0.12) | 0.56 | -0.01 (-0.12, 0.09) | 0.81 | -0.02 (-0.13, 0.09) | 0.70 |
| $Log_{10}$ (HOMA-β) | 0.02 (-0.04, 0.09) | 0.48 | -0.002 (-0.07, 0.06) | 0.97 | -0.005 (-0.07, 0.06) | 0.93 |
| **Lipids profile** | | | | | | |
| Triglycerides, mmol/L | 0.78 (0.56, 1.00) | < 0.0001 | 0.75 (0.54, 0.96) | < 0.0001 | 0.75 (0.54, 0.96) | < 0.0001 |
| Total cholesterol, mmol/L | 0.48 (0.25, 0.71) | < 0.0001 | 0.45 (0.21, 0.68) | 0.0002 | 0.47 (0.23, 0.70) | < 0.0001 |
| LDL cholesterol, mmol/L | -0.006 (-0.01, 0.0002) | 0.06 | 0.19 (-0.002, 0.37) | 0.053 | 0.02 (-0.03, 0.07) | 0.43 |
| HDL cholesterol, mmol/L | -0.04 (-0.13, 0.05) | 0.41 | -0.01 (-0.1, 0.08) | 0.83 | -0.02 (-0.10, 0.07) | 0.74 |

*Data are changes and 95% confidence intervals (CI) of cardiometabolic traits per 1-unit increase in log10-RBP4. In the generalized estimating equation (GEE) model, the exposure was baseline blood RBP4 levels, the dependent variables were repeated evaluation levels of cardiometabolic traits. Model 1, adjusted for age and sex; model 2, further adjusted for BMI, and smoking and drinking habits based on model 1, while BMI was not adjusted for the adiposity index BMI and waist circumference; model 3, further adjusted for anti-diabetes drug usage based on model 2 for glucose metabolism traits, further adjusted for lipid-lowering drug usage based on model 2 for lipid profile.*

in the lipid profile (model 3), RBP4 was significantly associated with fasting and 2-h post-loading glucose [β=0.26 (0.05, 0.47), P=0.02, and β=1.70 (1.29, 2.12), P< 0.0001], serum triglycerides [β=0.75, 95% CI (0.54, 0.96), P<0.0001], and total cholesterol [β=0.47, 95% CI (0.23 0.70), P<0.0001]. We did not find any significant associations between serum RBP4 levels and fasting insulin, HOAM-IR, HOMA-β, LDL cholesterol, and HDL cholesterol.

To track the change in association between RBP4 and metabolic traits, multiple linear regression analyses were performed at baseline and two follow-up visits. The dynamic *P* values for significance between RBP4 and metabolic traits were presented in **Supplementary Figure S1**.

## Sex-Specific Association of Serum RBP4 Levels With Adiposity Index and Glucolipid Metabolism Traits

**Table 3** shows the sex-specific associations of serum RBP4 levels with adiposity indexes and glucolipid metabolism traits. Baseline serum RBP4 levels (indicated as each 1-unit of $log_{10}$-RBP4) were significantly associated with waist circumference in men [β=4.80, 95% CI (0.43, 9.17), P=0.03] but not in women [β=2.13, 95% CI (-0.57, 4.84), P=0.12] (P for interaction=0.31). Baseline serum RBP4 was significantly associated with OGTT 2-h plasma glucose in women [β=1.99, 95% CI (1.49, 2.50), P <0.0001] but not in men [β=0.61, 95% CI (-0.14, 1.36), P=0.11] (P for interaction=0.001). Both in men and in women, baseline serum

**TABLE 3 |** Adjusted changes (95% CI) in metabolic traits per 1-unit increase in $log_{10}$-RBP4 in participants stratified according to sex by using the GEE model.

| | **Men (*n* = 272)** | | **Women (*n* = 512)** | | ***P* for interaction** |
|---|---|---|---|---|---|
| | **Change (95% CI)** | ***P* value** | **Change (95% CI)** | ***P* value** | |
| **Adiposity** | | | | | |
| Body mass index, kg/m$^2$ | 0.95 (-0.73, 2.63) | 0.27 | 0.91 (-0.25, 2.07) | 0.12 | 0.93 |
| Waist circumference, cm | 4.80 (0.43, 9.17) | 0.03 | 2.13 (-0.57, 4.84) | 0.12 | 0.31 |
| **Glucose metabolism** | | | | | |
| Fasting plasma glucose, mmol/L | 0.19 (-0.08, 0.45) | 0.16 | 0.01 (-0.23, 0.25) | 0.96 | 0.52 |
| OGTT 2h plasma glucose, mmol/L | 0.61 (-0.14, 1.36) | 0.11 | 1.99 (1.49, 2.50) | < 0.0001 | 0.001 |
| $Log_{10}$ (fasting insulin), pmol/L | 0.03 (-0.09, 0.16) | 0.61 | -0.09 (-0.28, 0.10) | 0.33 | 0.23 |
| $Log_{10}$ (HOMA-IR) | 0.05 (-0.08) 0.19) | 0.44 | -0.05 (-0.18, 0.09) | 0.50 | 0.25 |
| $Log_{10}$ (HOMA-β) | -0.01 (-0.14, 0.12) | 0.84 | 0.00 (-0.08, 0.08) | 0.98 | 0.91 |
| **Lipids profile** | | | | | |
| Triglycerides, mmol/L | 0.49 (0.13, 0.85) | 0.007 | 0.82 (0.56, 1.07) | < 0.0001 | 0.20 |
| Total cholesterol, mmol/L | 0.54 (0.11, 0.97) | 0.01 | 0.45 (0.17, 0.73) | 0.002 | 0.68 |
| LDL cholesterol, mmol/L | 0.28 (-0.07, 0.62) | 0.12 | 0.19 (-0.04, 0.42) | 0.10 | 0.65 |
| HDL cholesterol, mmol/L | 0.10 (-0.05, 0.26) | 0.20 | -0.07 (-0.17, 0.04) | 0.22 | 0.07 |

*Data are changes and 95% confidence intervals (CI) of cardiometabolic traits per 1-unit increase in log10-RBP4. In the generalized estimating equation (GEE) model, the exposure was baseline blood RBP4 levels, the dependent variables were repeated evaluation levels of cardiometabolic traits. The adjustments of adiposity index included age, smoking status, drinking status; the adjustments of glucose metabolism traits included age, BMI, smoking status, drinking status, and anti-diabetes drug usage; the adjustments of lipid profile included age, BMI, smoking status, drinking status, and lipid-lowering drug usage. For the interaction test, sex, log10(RBP4), and sex*log10(RBP4) were simultaneously added to the model.*

RBP4 was significantly associated with serum triglycerides and total cholesterol (both P ≤0.01).

For adiposity indexes, Z score standardized analysis revealed a little bit stronger association of baseline serum RBP4 levels with waist circumference than BMI (**Table 4**). There was a 0.34-units [95% CI (0.07, 0.61), P=0.01] increase in Z score of waist circumference corresponding to a 1-unit increase in log10-RBP4, while a 0.29-units [95% CI (-0.01, 0.59), P=0.051] increase in Z score of BMI. Compared with fasting plasma glucose concentrations, there was a stronger association between baseline serum RBP4 levels and OGTT 2-h plasma glucose concentrations in total participants (**Table 4**). There was only a 0.33-units [95% CI (0.04, 0.62), P=0.03] increase in Z score of fasting plasma glucose concentrations corresponding to a 1-unit increase in log10-RBP4, while a 0.82-units [95% CI (0.62, 1.03), P<0.0001] increase in Z score of OGTT 2-h plasma glucose concentrations (**Table 4**). After stratified by sex, the similar results were found as compared to that in **Table 3**.

## DISCUSSION

In the present study, we systematically assessed the association of baseline RBP4 levels with a longitudinal dynamic cardiometabolic profiles which were evaluated at baseline and two consecutive times of follow-up with 3-years' interval. We found that in the middle aged and elderly population without diabetes at baseline, baseline higher RBP4 levels were associated with increased BMI, waist circumference, fasting and OGTT 2-h plasma glucose triglyceride, and total cholesterol. Furthermore, sex disparities matter in the relationship of RBP4 with OGTT 2-h plasma glucose, in which RBP4 was associated with OGTT 2-h plasma glucose in women, but not in men.

Elevated circulation RBP4 levels were identified in obese subjects. However, RBP4 levels were associated with BMI in few epidemiological studies. Instead, more studies indicated RBP4 levels were positively associated with visceral body fat or abdominal fat mass (18–21) in individuals with or without diabetes (19) or in healthy women 21 to 67 years old (20). Serum RBP4 was found more highly expressed in visceral fat compared with subcutaneous fat (22), and to be a marker of intra-abdominal fat mass. Similarly, in our present study, we found that RBP4 was significantly associated with a long-term waist circumference level in non-diabetes, a commonly used proxy for visceral fat. Though the change pattern of BMI and waist circumference was similar in that they both increased first at the first 3-years' follow-up with an average age of 65 years and decreased at the second 3-years' follow-up with an average age of 68 years, and the effect estimates of waist circumference associated with RBP4 were stronger than that with BMI.

In the current study, we found plasma glucose concentrations in non-diabetes were associated with RBP4 levels as expected. Interestingly, OGTT 2-h plasma glucose concentrations had a more robust association with RBP4 levels than fasting plasma glucose concentrations, which might suggest OGTT 2-h plasma glucose as one main contribution through which increased RBP4 levels confer a higher risk of type 2 diabetes. It has been reported that OGTT 2-h plasma glucose level was regulated by intestine-derived incretin hormone besides glucose-stimulated insulin secretion (23). Intestine-derived incretin hormone can be a potential target for the regulatory role of RBP4. Liraglutide, one of the glucagon-like peptide-1 (GLP-1) analogs that could decrease post-prandial glucose level by regulating incretin in type 2 diabetes, reduced RBP4 gene expression in adipose tissue in mice, which might contribute to the reduction of cardiovascular risk in diabetes (24).

Rare literature reported sexual difference in the association between baseline RBP4 levels and glucose metabolism (14). In the nested case-cohort study from Singapore Chinese, higher risk of type 2 diabetes corresponding to increased plasma RBP4 levels was only observed in women but not in men, the results were still consistent after pooled with two prior studies. In a case-cohort design in which 1080 subjects from the Atherosclerosis Risk in Communities cohort were followed up for 9 years, women in the

**TABLE 4** | Adjusted changes (95% CI) in Z-score of metabolic traits per 1-unit increase in log10-RBP4 by using the GEE model.

| | Total sample (*n* = 784) | | Men (*n* = 272) | | Women (*n* = 512) | | *P* for interaction |
|---|---|---|---|---|---|---|---|
| | Change in Z-score (95% CI) | *P* value | Change in Z-score (95% CI) | *P* value | Change in Z-score (95% CI) | *P* value | |
| **Adiposity** | | | | | | | |
| Body mass index | 0.29 (-0.01, 0.59) | 0.051 | 0.30 (-0.24, 0.84) | 0.27 | 0.27 (-0.09, 0.62) | 0.14 | 0.87 |
| Waist circumference | 0.34 (0.07, 0.61) | 0.01 | 0.56 (0.05, 1.08) | 0.03 | 0.22 (-0.09, 0.52) | 0.17 | 0.26 |
| **Glucose metabolism** | | | | | | | |
| Fasting plasma glucose | 0.33 (0.04, 0.62) | 0.03 | 0.29 (-0.12, 0.71) | 0.16 | 0.01 (-0.32, 0.33) | 0.96 | 0.34 |
| OGTT 2-h plasma glucose | 0.82 (0.62, 1.03) | < 0.0001 | 0.29 (-0.07, 0.65) | 0.11 | 0.96 (0.72, 1.21) | < 0.0001 | 0.001 |
| **Lipids profile** | | | | | | | |
| Triglycerides | 0.73 (0.53, 0.94) | < 0.0001 | 0.40 (0.11, 0.69) | 0.007 | 0.84 (0.58, 1.10) | < 0.0001 | 0.04 |
| Total cholesterol | 0.49 (0.25, 0.74) | < 0.0001 | 0.59 (0.12, 1.06) | 0.01 | 0.45 (0.17, 0.74) | 0.002 | 0.58 |

*Data are changes and 95% confidence intervals (CI) of Z score transformed cardiometabolic traits per 1-unit increase in log10-RBP4. In the generalized estimating equation (GEE) model, the exposure was baseline blood RBP4 levels, the dependent variables were Z score transformed cardiometabolic traits. The adjustments of adiposity index included age, sex, smoking status, and drinking status; the adjustments of glucose metabolism traits included age, sex, BMI, smoking status, drinking status, and anti-diabetes drug usage; the adjustments of lipid profile included age, sex, BMI, smoking status, drinking status, and lipid-lowering drug usage. For the interaction test, sex, log10(RBP4), and sex*log10(RBP4) were simultaneously added to the model.*

highest tertile of RBP4 had 74% greater risk of developing diabetes, while no association between RBP4 levels and incident diabetes was found in men (25). The potential mechanisms for the observed sexual difference have not been fully understood. The sex hormone is a possible explanation for the observed sexual differences in association with baseline RBP4 levels and glucose metabolism. On the one hand, RBP4 levels were associated with estrogen levels. A cross-sectional study including 87 Chinese women found a negative association between estrogen levels and RBP4 levels in those who were obese (26). Besides, postmenopausal women have higher RBP4 levels compared to premenopausal women (27). On the other hand, elevated RBP4 levels were reported to be associated with higher free testosterone levels or estimation values of free testosterone in postmenopausal women (26, 27), despite some controversies. It is well established that higher free testosterone is associated with higher risk of disordered glucose metabolism in women, but lower risk in men (28). Therefore, both free testosterone and estrogen are a likely bridge for the sex disparity in glucose regulation.

Sex hormones have pronounced effects on body composition, adipose tissue mass distribution, and metabolic homeostasis. Men have relatively more energy storage in the abdominal and intra-abdominal adipose tissue depots, while premenopausal women store the excess energy in subcutaneous adipose tissue (29, 30). This sex dimorphism may explain the stronger association of the RBP4 level with waist circumference, a simple and practical index for assessing visceral fat.

In this study, there was no association for RBP4 and fasting insulin levels, HOMA-IR, or HOMA-β. These discrepancies within literature may lie in several aspects including that the study populations varied widely in study type, sample size, health condition, and ethnicity. Several studies also declared null association between RBP4 and insulin resistance (31–33). Another more important reason was that the indexes like HOMA-IR and HOMA-β and fasting insulin levels were not adequate to reflect the insulin resistance or β-cell function. In a study including 291 subjects across the spectrum of glycemia, circulating RBP4 levels were significantly and inversely associated with β-cell function indicated by the Stumvoll first-phase and second-phase insulin secretion indices, but not with HOMA-β (34).

RBP4 has also related to an unfavorable lipid metabolism (32, 35–37). But its role in lipid metabolism remains unclear, we know little about what kinds of lipids were regulated and how they were regulated. Most of the previous studies demonstrated that RBP4 was positively associated with triglycerides, while few studies manifested a significant association of RBP4 with other lipids like total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (36, 37). The current study presented positive association of RBP4 with triglyceride levels and total cholesterol levels no matter in total population or in sex subgroups, but not with low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. Our results added more evidence for RBP4 and lipid metabolism. Both triglycerides and total cholesterol were positively associated with RBP4, but triglycerides and total cholesterol have different change patterns, total cholesterol decreased significantly while triglycerides remained almost unchanged. In our previous repeated measures study including 1872 participants aged 40 years or older and free of dyslipidemia at baseline, lipid level was measured at baseline and at 4-year follow-up, results showed stable triglyceride levels and increased total cholesterol levels during a 4-year follow-up (38). In another longitudinal study aimed to study changes in participants' diabetes risk and anthropometrics from baseline to 60 months follow-up, measurements were performed annually up to 60 months after inclusion, both triglycerides and total cholesterol had no significant change (39). However, the result was based on small sample size data, only 120 participants were included in the final analysis. The different study population, sample size, follow-up time, and the usage of statins may be possible explanations for the different change trend in triglycerides and total cholesterol.

The main strength of this study is the longitudinal design with systematic and repeated monitoring for metabolic traits. Systematic metabolic traits including adiposity index, glucose metabolic traits, and blood lipid profile made it practicable to get a comprehensive knowledge about the association of RBP4 on cardiometabolic risk profile. Besides, the repeated measurements of metabolic traits also reduced the bias derived from a single cross-sectional survey. Another strength is the novelty of the current study, as we identified the sexual difference in the association between RBP4 levels and metabolic traits, especially for the 2-h post-loading glucose, which implies an import issue of sex disparities in the disease pathophysiology.

Severe limitations should be acknowledged. First, serum RBP4 was not repeatedly measured at follow-up, which prohibited us to track a long-term trajectory of circulating RBP4 levels. One previous study aimed to assess the biological variation of adipokines RBP4 within individuals by measuring the level of RBP4 twice, approximately 4 months apart (40). The intraclass correlation coefficient for repeated RBP4 measurements was 0.77 (95% CI 0.71, 0.82), indicating excellent reliability. However, the longitudinal changes in blood levels of RBP4 needs further investigation. Second, an imbalanced sex ratio existed in our population. That was partly because women live longer than men, another explanation for that is women retired 5 years earlier than men and have more time to participate in epidemiological investigation. However, we have statistical power to detect the interaction with sex and get the sex-specific conclusions. Third, compared with several previous studies from China (4, 9), the serum RBP4 level in the current study is much lower. Different health conditions of participants is the main explanation for the variance in serum RBP4 level. In the current study, we included non-diabetes participants in relatively good health. Besides, men have higher serum RBP4 levels than women (4, 25), sex distribution difference may be another explanation for the variance in serum RBP4 level. Fourth, we did not test the sex hormone levels for participants in the current study. We could not provide the interaction effects of RBP4 levels with sex hormone levels in the associations with the metabolic changes. Finally, any causal

inference could not be established as this was an observational study. Despite its observational nature, this study adds evidence of serum RBP4 with a long-term metabolic profile. Future large scale Mendelian randomization studies or animal models would help to clarify the role of RBP4 in metabolic regulation.

In conclusion, the baseline serum RBP4 level is robustly associated with a longitudinal profile of waist circumference, fasting and OGTT 2-h plasma glucose, triglyceride and total cholesterol, and marginally associated with BMI. The association of RBP4 levels on metabolic traits differ in sex, with stronger association with OGTT 2-h plasma glucose level in women. The results imply an import issue of sex disparities in the disease pathophysiology. Further experimental investigations are required to better understand the molecular mechanisms underlying the sex-specific deterioration of metabolic traits due to elevated RBP4 levels.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Each patient/participant provided the written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MX, YB, and JW contributed to the concept and design. JX, HD, YH, and QW contributed to the analysis of data. JX drafted the manuscript. MX edited the manuscript. TW, ML, ZZ, JL, MD, DZ, YX, GN, WW, YB, and MX contributed to collecting and the acquisition of data. All authors made important contributions to critically revising the manuscript for important intellectual content. MX guarantees this work, has full access to the data, and takes responsibility for the integrity of the data. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.880467/full#supplementary-material

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