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## Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Study

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**Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Study**

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**Abstract**

**Objectives:** Glycated albumin (GA) and body mass index (BMI) have been linked to atherosclerotic cardiovascular disease (ASCVD). However, the role of BMI in the correlation between GA and 10-year ASCVD risk is still not fully understood.

**Design:** A Retrospective Study

**Setting:** This study retrospectively obtained data from 2,107 participants who received health checkups at the Health Management Medicine Center, Third Xiangya Hospital of Central South University from January 1, 2022 to December 30, 2023.

**Participants:** The inclusion criteria for this study included: a) age is greater than or equal to 18 years old; and b) subjects were long-term residents of Hunan province.

**Primary and secondary outcome measures:** The 10-year ASCVD risk was evaluated via the China-PAR equation. The link between GA and 10-year ASCVD risk was examined through a multivariable logistic regression model and the dose-response relationship was demonstrated using the restricted cubic spline. The potential mediation effect of BMI on this association was explored and the differences in this mediation effect across age and metabolic-associated fatty liver disease (MAFLD) subgroups were analyzed.

**Results:** Elevated GA levels were positively linked to an intensified 10-year ASCVD risk (OR=1.160, 95% CI =1.055–1.276). Additionally, BMI was negatively linked to GA and 10-year ASCVD risk. BMI mediated 13.9% of the connection between GA and 10-year ASCVD risk. Specifically, the mediating effect of BMI remained significant in the 40–60 years age subgroup and non-MAFLD subgroup, with mediation ratios of 43.7% and 8.5%, respectively.

**Conclusions:** GA is a key predictor of 10-year ASCVD risk, and BMI partially mediates this relationship in healthy populations. Therefore, targeted weight

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management is recommended to reduce the adverse effect of GA on 10-year ASCVD risk in different populations.

**Strengths and limitations of this study**

1. This study firstly established body mass index (BMI) as a partial mediator in the association between Glycated albumin (GA) and 10-year atherosclerotic cardiovascular disease (ASCVD) risk using a mediation model.

2. We used multivariate logistic regression to explore the link between GA and 10-year ASCVD risk, developing three models to control confounding effects stepwise.

3. Restricted Cubic Splines (RCS) were applied to illustrate the dose-response relationship between GA and 10-year ASCVD risk.

4. Given the retrospective cross-sectional nature of this study, it is not feasible to establish the exact causality between GA and 10-year ASCVD risk.

**Keywords:** Glycated Albumin, Body Mass Index, Atherosclerotic Cardiovascular Disease Risk, Mediation, Metabolic Associated Fatty Liver Disease

**1. Introduction**

Cardiovascular diseases (CVDs) stand as primary culprits behind death and disability worldwide, presenting a substantial risk to human health. This category of diseases encompasses coronary artery disease (CAD), stroke, and myocardial infarction, which commonly arise due to arteriosclerosis, hypertension, hyperlipidemia, and diabetes[1-5]. Currently, glycated hemoglobin A1c (HbA1c) is recognized as a leading indicator in the clinical assessment of long-term blood sugar control. The latest KDIGO guidelines have recommended a target value of 6.5% to 8.0% for HbA1c in those with diabetes

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and chronic kidney disease (CKD) to reduce cardiovascular risk[6]. Nevertheless, HbA1c can be influenced by hemoglobin levels, red blood cell lifespan, anemia, liver dysfunction, and kidney disease[7]. Concurrently, there is increasing interest in an alternative indicator, glycated albumin (GA), a novel index for glucose monitoring, reflecting average blood sugar levels over the past 2 to 3 weeks[8, 9] GA is calculated as the ratio of GA to total albumin concentration[10]. Unlike HbA1c, GA has the advantage of being independent of hemoglobin and red blood cell turnover and less affected by red blood cell lifespan, thus serving as a good marker for assessing blood glucose levels[11].

Numerous guidelines have recommended that the prevention and treatment of atherosclerotic CVD (ASCVD) be based on risk assessment. Traditional risk assessment methods, including the Framingham D'Agostino 2008 model, PCE white model, SCORE model, and QRI SK model, are recognized for their reliability in Western populations. However, the Chinese population presents distinct disease patterns and risk factors, making these models less suitable. Particularly, the Framingham D'Agostino model overestimates the CVD risk in men and underestimates it in women, a trend similarly observed in the PCE model[12]. S Since 2003, several large-sample prediction models for CVD risk have been established for Chinese adults. Among them, the 10-year risk prediction models for Chinese CVD[13, 14] and the China-PAR model for ASCVD[15] are notable. China-PAR model, a CVD risk assessment tool developed by Chinese scholars based on large-sample cohort data, is most often externally validated and is considered potentially a better option for predicting CVD risk in China[16]. It ultimately provides a 10-year and lifetime risk assessment based on relevant metrics, which benefits primary prevention and health management of CVD.

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Obese people have a high prevalence of ASCVD, and body mass index (BMI), as an important indicator for assessing obesity in an individual, has a very close correlation with ASCVD risk. Similarly, the study of GA in predicting CVD risk has attracted much attention in recent years. GA, as an early glycated protein, may be more sensitive than other markers to reflect CVD and its complications[5, 17]. Several publications have found an link between GA and cardiovascular mortality in diabetes mellitus (DM) patients[9, 18, 19]. Notably, multiple studies confirm that GA is more strongly than HbA1C associated with CVD in CKD patients requiring dialysis[18, 20, 21]。 Several papers have noted a weak negative connection between GA and BMI in DM populations but with a narrow range for BMI or GA values[11, 22-27]. In contrast, an investigation of Caucasian populations with a median BMI of 38.4 (large body weight) similarly unveiled a marked negative link between BMI and GA[7]. Therefore, the association between GA and BMI in a wider population remains to be confirmed by further studies. There are few studies on the correlation among GA, BMI, and 10-year ASCVD risk in normal populations. Hence, this paper was to illustrate whether BMI mediates the link between GA and 10-year ASCVD risk in the normal population.

## 2.Methods

### 2.1 Participant Selection

This cross-sectional study obtained data from 4646 healthy subjects during health checkups at the Health Management Medicine Center, Third Xiangya Hospital of Central South University from January 1, 2022, to June 30, 2023. To control for potential biases, we included all health examination data during this period, rather than relying on random sampling.

The inclusion criteria for this study included: a) age is greater than or equal to 18 years

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old; and b) subjects were long-term residents of Hunan province. The exclusion criteria

of this study were a) subjects did not have complete data required for this study; and b)

subjects did not provide written informed consent; and c) Subjects reported established

family history and medical history of CVD and cerebrovascular diseases (including

coronary heart disease, stroke, congestive heart failure, myocardial infarction, and

angina) in questionnaires ;and d) Subjects was discovered severe liver or kidney

dysfunction (which may affect GA results) in health checkups. According to the

exclusion criteria, 84 participants under the age of 18 were excluded, 1237 participants

lacking GA, BMI, and China-PAR equation-related data were excluded, 276

participants with self-reported CVD and cerebrovascular diseases on the questionnaire

were excluded, 28 participants with severe liver or kidney dysfunction were excluded,

and 914 participants with missing information on cardiovascular risk factors were

excluded. Ultimately, 2107 participants were enrolled. The specific selection process is

displayed in Figure 1. This retrospective study adhered to the institution and national

research committee's ethical standards, and approval was obtained from the

institutional ethics committee of the Third Xiangya Hospital of Central South

University and review board (Ethics Approval Number: Quick24559).

**2.2 Measurements**

**2.2.1 GA measurement**

Compared to HbA1c testing, the detection methods for GA are not yet fully

standardized. In this study, enzyme-linked immunosorbent assay (ELISA), a widely

adopted and reliable technique[28] was employed to detect GA.

**2.2.2 Cardiovascular Risk Estimation Based on China-PAR Project**

The China-PAR score was employed to appraise ASCVD risk. The China-PAR model

encompassed sex, age, systolic blood pressure (SBP), total cholesterol (TC), high-

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density lipoprotein cholesterol (HDL-C), waist circumference (WC), smoking status, DM, region, urbanization, and family history of ASCVD. The participants were allocated into three risk subgroups based on the scores: <5% as low risk, 5−10% as medium risk, and ≥10% as high risk[15].

Health status and lifestyle information of the participants were collected through questionnaires during their health checkups. This questionnaire, designed based on the National Physical Examination Questionnaire[29], included details on the participants' and family history of CVDs and cerebrovascular diseases, and lifestyle information such as history of hypertension, CVDs, DM, smoking, alcohol consumption, and physical activity. Hypertension was diagnosed as SBP ≥140 mmHg and/or diastolic blood pressure (DBP)≥90 mmHg or receiving antihypertensive treatment. DM was set as fasting plasma glucose (FPG) ≥7.0 mmol/L, HbA1c ≥6.5%, or receiving hypoglycemic treatment. A family history of ASCVD was described as congestive heart failure, stroke, coronary heart disease, myocardial infarction, or angina in first-degree relatives. In this study, based on the three risk categories mentioned above, participants were allocated into medium-high risk group and low risk groups.

### 2.2.3 Covariate Assessment

Demographic data were obtained from physical examination reports. Age as a continuous variable was categorized into subgroups: 18-39 years, 40-60 years, or >60 years. During the physical examination, weight, height, WC, SBP, and DBP were gauged. BMI was presented as weight (kg) divided by height square (m²). Laboratory tests covered routine blood tests, liver function tests, GA, FBG, glycated hemoglobin, fasting insulin, and blood lipids. All participants underwent liver and cervical vascular ultrasonography performed by an ultrasonologist. These reports were reviewed and confirmed by another senior ultrasonologist. The diagnoses of fatty liver (hepatic

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steatosis) and cervical vascular plaques were based on liver and cervical vascular ultrasound scans using linear array or convex high-frequency probes with frequencies of 5-10 MHz. These scans evaluated the echo, size, and morphology of the liver, as well as intima-media thickness, plaque size, morphology, and location of cervical vessels. Furthermore, smoking history, alcohol consumption, and exercise frequency were recorded directly from the physical examination questionnaire. Smoking history was defined as smoking continuously or cumulatively for more than six months[29]. Alcohol consumption manifested as consuming over 10 grams of alcohol per day[30]. Physical activity represented moderate- or high-intensity physical activity at least three times per week, with each session lasting at least 30 minutes[31].

### 2.2.4 Diagnosis of MAFLD

According to Eslam et al.[32], metabolic-associated fatty liver disease (MAFLD) means steatosis in combination with metabolic dysfunction. The diagnostic criteria included hepatic steatosis through histology (liver biopsy), imaging techniques, or blood-based biomarkers and at least one of the following conditions: overweight/obesity, T2DM, and metabolic dysfunction. Metabolic dysfunction refers to at least two of the seven metabolic risk abnormalities (Figure 2).

### 2.3 Data Analysis

Continuous variables were denoted as median and interquartile spacing and compared utilizing the independent samples t-test. Categorical variables were depicted as unweighted frequencies, weighted percentages, and standard error (SE), and compared utilizing the chi-square test. Multiple regression analysis was used to test the correlation between GA (continuous exposure variable) and 10-year ASCVD risk (binary outcome variable). Variables showing between-group differences in the baseline information table (P < 0.05) were identified and included as potential confounders, serving as

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covariates in subsequent analyses. Three models were constructed to progressively control for the effects of confounders: Unadjusted crude Model 1; Model 2 adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, and DBP; and Model 3 adjusted for all covariates, such as albumin, platelet, TC, Triglyceride, HDL-C, Cervical vascular plaques, and MAFLD. Subsequently, restricted cubic spline (RCS) was adopted to demonstrate the dose-response relationship between GA and 10-year ASCVD risk. Finally, a logistic regression model was employed to illustrate the relationships among GA, BMI, and 10-year ASCVD risk. To analyze the mediation effect of BMI [(mediation effect/total effect) × 100%], a simple mediation model was used, with three paths (Figure 3). The total effect represented the impact of GA (exposure) on 10-year ASCVD risk (outcome). Path A assessed the effect of GA on BMI (mediator). Path B evaluated the link between BMI and 10-year ASCVD risk. Path C estimated the direct impact of GA on 10-year ASCVD risk. The mediated effect was calculated as (mediated effect/total effect) × 100%.

All statistical analyses, including mediation analysis, were done in the R statistical package. Statistical significance was delineated at a two-sided p-value of < 0.05.

## 3.Results

### 3.1. Characteristics of Participants

Table S1 displays the baseline characteristics of study participants. Based on the three risk categories mentioned above, participants were assigned to a medium-high-risk group (n=230) and a low-risk group (n=1877). The differences were statistically notable (p < 0.001) in age, BMI, WC, SBP, DBP, albumin, GA, platelet, FBG, TC, cervical vascular plaques, smoking status, and MAFLD.

Table S2 exhibits the multivariable logistic analysis results. In model 1, a higher GA

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was greatly connected with higher odds of 10-year ASCVD risk (OR =1.138, 95%

CI =1.094, 1.185). After adjusting for covariates (age, sex, smoking status, sport status,

BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular

plaques, and MAFLD), GA was positively connected with 10-year ASCVD risk

(OR =1.160, 95% CI =1.055, 1.276) in Model 3.

The RCS curve showed that increased GA levels were significantly linked to increased

10-year ASCVD risk (P for overall < 0.001), with a linear relationship (P for non-linear

=0.2616) (Figure 4). The red solid line indicates the point estimate of OR, and the pink

shaded area represents the 95% CI. The curve indicates that when GA levels are below

12.95%, the OR remains around 1, suggesting that GA does not significantly affect the

risk within this range. However, when GA levels > 12.95%, the OR starts to increase

significantly, indicating a marked correlation between higher GA levels and greater 10-

year ASCVD risk.

### 3.2 Mediation Analysis of BMI

We then delved into the mediation effect of BMI on the link between GA and 10-year

ASCVD risk. All mediation analyses were carried out after adjustment for age, sex,

smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride,

HDL-C, cervical vascular plaques, and MAFLD.

GA was negatively correlated with BMI (p < 0.001) and positively correlated with 10-

year ASCVD risk (p < 0.001). BMI was positively related to 10-year ASCVD risk (p <

0.001) (Table S3). It is estimated that BMI mediated 13.9% of the total link between

GA and 10-year ASCVD risk.

Given the effect of age and MAFLD, subgroup analyses were implemented(Table S4).

In the age group, BMI partially mediated the link between GA and 10-year ASCVD

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risk. In the age subgroup 40–60 years, there was a 43.7% mediating effect of BMI on the link between GA and 10-year ASCVD risk. However, in the age subgroup < 40 years and > 60 years, no mediating effect of BMI was observed on the correlation between GA and 10-year ASCVD risk. Similarly, BMI mediated 8.5% of the total link between GA and 10-year ASCVD risk in the non-MAFLD group. However, the mediating effect was not observed in the MAFLD group.

### 4.Discussion

This study elucidated the relationship among GA, BMI, and 10-year ASCVD risk in the normal population undergoing physical examination and estimated the mediating role of BMI. This is the first study on their relationship, and our results confirm: 1. BMI mediated the link between GA and 10-year ASCVD risk, with 13.9% of the association mediated by BMI. 2. The mediating role of BMI varies by age and MAFLD subgroups. BMI plays a partially mediating role of 8.5% in the 40-60 age group and no mediating role in the 30-44 and over 60 age groups. Similarly, BMI has an 8.5% partial mediating effect on the non-MAFLD group, but no mediating effect was revealed in the MAFLD group.

The present study examined a real-world normal population undergoing medical examinations and showed a positive correlation between GA and 10-year CVD risk. These associations remained after adjustment for established cardiovascular risk factors and socio-behavioral factors. Although GA is widely recognized to reflect blood glucose levels, relevant studies have focused on its application in diverse situations (e.g., anemia, abnormal liver function, and renal disease) where HbA1c accuracy may be impaired. However, there is controversy about the ability of GA to predict long-term ASCVD outcomes. Previous studies have manifested that GA upregulation is positively

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linked to CAD and its severity in DM patients, whereas HbA1c levels are not as strongly correlated with CAD[33, 34]. Similarly, several publications have highlighted that after adjustment for HbA1c levels, the higher GA in dialysis-requiring patients with CKD and DM, the higher risk of cardiovascular mortality, all-cause mortality, CAD, major adverse cardiovascular events, and stroke; no correlation was found in non-DM individuals[9, 18, 19]. However, Copur et al. revealed a prominent correlation of GA levels with all-cause mortality in DM patients undergoing dialysis, but not with CVD mortality[35]. Additionally, high GA levels were independently linked to unfavorable intermediate-term efficacy in low-risk populations undergoing percutaneous coronary intervention, but the prognostic role was only present in the DM subgroup, and in the non-DM individuals, this association was not supported by clear evidence[36]. It is well known that atherosclerosis is the most important cause of CVDs[37]. Significant glycemic excursions or fluctuations potentiate oxidative stress and drive atherosclerosis, which can lead to ASCVD[38, 39]. We hypothesized that relative to HbA1c, GA may better indicate glycemic fluctuations (eg, acute hyperglycemia or significant postprandial glycemic excursion) before the onset of acute coronary syndrome (within a short period[38, 40], thereby promoting oxidative stress and accelerating atherosclerosis in both DM and non-DM subjects[41, 42]. In non-DM subjects, short-term glycemic fluctuations, especially significant postprandial glycemic excursion, may be manifested as small but notable differences in GA. The effect of HbA1c on non-DM participants may be confounded by non-glycemic factors, like abnormalities in liver and kidney function and differences in red blood cell lifespan. Therefore, further investigations are warranted to elucidate the complex pathophysiologic mechanisms that link GA to ASCVD risk.

Our experimental results showed that GA was negatively connected with BMI.

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Research in normoglycemic populations, pre-DM patients, and T2DM patients has reported a link between BMI and HbA1c or GA/HbA1c[27, 43]. However, studies on the link between GA and BMI are very limited in normal populations in China. As mentioned earlier, several papers have demonstrated a weak negative connection between GA and BMI among DM populations, but mainly in normal-weight subjects with little fluctuation in BM[11, 22-27], or Caucasian populations with large body weights (mean BMI of 38.4). In contrast, the two papers that did not discover a negative correlation between GA and BMI were conducted in the Japanese population with type 1 DM with a mean BMI of 20.1[44] or without DM with a nearly constant GA[45]. Thereby, the negative link between BMI and GA may not be exclusively attributable to glycemic exposure, but that there are other factors. An increase in BMI increases renal blood flow, glomerular filtration rate, and tubular reabsorption, resulting in glomerular enlargement and obesity-associated glomerular disease[46, 47]. There is a difference in the rate of GA synthesis in participants with higher BMI and normal BMI[22]. BMI may affect GA clearance. A mild increment in BMI is related to an increased prevalence of microalbuminuria, which is a marker of early renal damage and is also closely related to cardiovascular prognosis[48]. Elevated GA levels may reflect the presence of microalbuminuria and cardiovascular events, but the exact mechanism is currently unknown. Therefore, future studies should refine the proteinuria and renal clearance of GA, which may influence the relationship between GA and BMI. Additional research utilizing labeled albumin in animal models of obesity and DM may offer valuable insight into this process.

Interestingly, the mediation analysis unraveled that BMI mediated 13.9% of the link between GA and 10-year ASCVD risk. This result emphasizes the importance of reducing BMI in lowering 10-year ASCVD risk, especially in high-GA populations,

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regardless of DM. Overweight and obesity are drivers of insulin resistance[49, 50]. Insulin resistance is the core connecting several events of cardiometabolic disorders[49-54], molecularly due to impaired insulin signaling transduction via the PI3K pathway and intact signaling transduction via the MAPK pathway, leading to altered glucose-insulin homeostasis and increased glucose fluctuations. Significant shifts in blood glucose values facilitate oxidative stress and atherosclerosis[38, 39]. Similarly, elevated serum GA levels are notably related to augmented carotid intima-media thickness and vascular endothelial dysfunction, a sign of early atherosclerosis[55]. Vascular smooth muscle cell proliferation, vascular endothelial dysfunction, and overproduction of collagen and inflammatory cytokines contribute to atherosclerosis and adverse cardiovascular events. Patients in the high GA group were more susceptible to metabolic disorders and severe CAD than those in the low and mediate GA groups and therefore had an intensified risk of CAD. When GA levels are substantially elevated (in the higher range, e.g., more than 17.1%), the CAD risk augments by approximately two folds[36]. Akane Mihara et al. disclosed findings from 10-year follow-up of the Hisayama study, suggesting that the highest quartile of GA levels (≥15.7%) was connected with a 2.2-fold increase in CAD incidence and a 2.5-fold increase in stroke risk[56]. The exact mechanism of the mediating effect of BMI is currently unclear and may be different in different populations. Additional research is warranted to illustrate the mechanisms.

Interestingly, we found age and MAFLD were involved in the mediating role of BMI in the association between GA and 10-year ASCVD risk. Age-stratified subgroup analyses found that BMI did not mediate the association in the <40 and >60 groups. This finding suggests that BMI reduction in the <40 and >60 groups with increased GA may not be the cause for reducing 10-year CVD risk compared with the partial mediation effect in the 40-60 group. We suggest that in the 40-60 group, BMI reduction

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will be the focus of reducing the 10-year CVD risk induced by increased GA. In addition, subgroup analyses by MAFLD discovered that the link between GA and 10-year CVD risk was partially mediated by BMI in the non-MAFLD group, whereas BMI had no mediating effect in the MAFLD group. This finding suggests that BMI reduction in the MAFLD group with increased GA may not be the cause for reducing 10-year CVD risk. In the non-MAFLD group, BMI reduction will be the focus of preventing 10-year CVD risk. Since GA is an indicator of short-term glucose fluctuations and insulin resistance is the main mechanism of MAFLD, it is primarily a long-term effect. Therefore, long-term monitoring of GA is of limited benefit in assessing 10-year CVD risk in MAFLD populations. This may provide new insights into assessing and reducing 10-year CVD risk in these two groups.

Our study first investigates the mediating role of BMI in the link between GA and 10-year ASCVD risk. Nevertheless, we must acknowledge certain inherent limitations of our study. Firstly, multiple multivariate models were adopted to unveil the independent link between GA and 10-year ASCVD risk. Essential confounding factors like alcohol consumption and smoking were obtained through self-reported data, which are potentially influenced by recall bias and misinterpretations and potentially affect the accuracy of our findings. Secondly, it is crucial to analyze the correlation between GA and proteinuria. However, since this study involved non-hospitalized population and the incidence of proteinuria among participants was exceedingly low, statistical analysis was not possible. This limitation underscores further investigations into the renal clearance of proteinuria and GA, which is pivotal in elucidating the link between BMI and GA. Lastly, given the cross-sectional nature, it is not feasible to establish the exact causality between GA and 10-year ASCVD risk. Thereby, prospective cohort studies are imperative to further unveil this relationship. Despite these limitations, our

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study, based on a real-world context and substantial sample size, has fully adjusted for confounders, so the conclusions drawn are relatively reliable.

In conclusion, our cross-sectional study of 2,107 participants demonstrates for the first time that BMI effectively mediates the impact of GA on the 10-year ASCVD risk. Consequently, healthy populations, especially those aged 40-60 without MAFLD, should realize the beneficial effects of GA in reducing their 10-year ASCVD risk through lowering BMI, rather than just preventing diabetes. Prospective studies are crucial to validate these findings and elucidate intrinsic mechanisms, thus paving the way for targeted therapeutic strategies.

## List of abbreviations

Glycated albumin (GA); atherosclerotic cardiovascular disease (ASCVD); Cardiovascular diseases (CVDs); coronary artery disease (CAD); hemoglobin A1c (HbA1c); chronic kidney disease (CKD); systolic blood pressure (SBP); waist circumference (WC); total cholesterol (TC); metabolic-associated fatty liver disease (MAFLD); standard error (SE)

## Declarations

### Ethics approval and consent to participate

This retrospective study adhered to the institution and national research committee's ethical standards, and approval was obtained from the institutional ethics committee of the Third Xiangya Hospital of Central South University and review board (Ethics Approval Number: Quick24559).

### Data availability statement

The original contributions presented in the study are included in the

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article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**Consent for publication**

Not applicable

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**Author contributions**

All authors contributed to the study conception and design. Writing - original draft preparation: XZ, YH, SX, NC, YZ, ML; Writing - review and editing: XZ; Conceptualization: YH, NC; Methodology: SX, YZ; Formal analysis and investigation: XZ, YH; Funding acquisition: ML, XZ; Resources: XZ; Supervision: ML, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Not applicable.

**References**

1.1. Improving Care and Promoting Health in Populations: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S7-s13.doi:10.2337/dc20-S001

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2.Health TWCotARoC, China Di. Interpretation of the Annual Report on Cardiovascular Health and Diseases in China 2020. Cardiology Discovery. 2022;2(4):269-85.doi:10.1097/cd9.0000000000000077

3.Zhao D, Liu J, Wang M, Zhang X, Zhou M. Epidemiology of cardiovascular disease in China: current features and implications. Nat Rev Cardiol. 2019;16(4):203-12.doi:10.1038/s41569-018-0119-4

4.Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM*, et al.* Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020;76(25):2982-3021.doi:10.1016/j.jacc.2020.11.010

5.Schram MT, Schalkwijk CG, Bootsma AH, Fuller JH, Chaturvedi N, Stehouwer CD. Advanced glycation end products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study. Hypertension. 2005;46(1):232-7.doi:10.1161/01.HYP.0000164574.60279.ba

6.KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. Kidney Int. 2022;102(5s):S1-s127.doi:10.1016/j.kint.2022.06.008

7.Selvin E, Rawlings AM, Lutsey PL, Maruthur N, Pankow JS, Steffes M*, et al.* Fructosamine and Glycated Albumin and the Risk of Cardiovascular Outcomes and Death. Circulation. 2015;132(4):269-77.doi:10.1161/circulationaha.115.015415

8.Takahashi S, Uchino H, Shimizu T, Kanazawa A, Tamura Y, Sakai K*, et al.* Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic

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control. Endocr J. 2007;54(1):139-44.doi:10.1507/endocrj.k06-103

9.Rooney MR, Daya N, Tang O, McEvoy JW, Coresh J, Christenson RH*, et al.* Glycated Albumin and Risk of Mortality in the US Adult Population. Clin Chem. 2022;68(3):422-30.doi:10.1093/clinchem/hvab232

10.Kouzuma T, Uemastu Y, Usami T, Imamura S. Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method. Clin Chim Acta. 2004;346(2):135-43.doi:10.1016/j.cccn.2004.02.019

11.Koga M, Matsumoto S, Saito H, Kasayama S. Body mass index negatively influences glycated albumin, but not glycated hemoglobin, in diabetic patients. Endocr J. 2006;53(3):387-91.doi:10.1507/endocrj.k05-137

12.Damen JA, Pajouheshnia R, Heus P, Moons KGM, Reitsma JB, Scholten R*, et al.* Performance of the Framingham risk models and pooled cohort equations for predicting 10-year risk of cardiovascular disease: a systematic review and meta-analysis. BMC Med. 2019;17(1):109.doi:10.1186/s12916-019-1340-7

13.Wu Y, Liu X, Li X, Li Y, Zhao L, Chen Z*, et al.* Estimation of 10-year risk of fatal and nonfatal ischemic cardiovascular diseases in Chinese adults. Circulation. 2006;114(21):2217-25.doi:10.1161/circulationaha.105.607499

14.Liu J, Hong Y, D'Agostino RB, Sr., Wu Z, Wang W, Sun J*, et al.* Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study. Jama. 2004;291(21):2591-9.doi:10.1001/jama.291.21.2591

15.Yang X, Li J, Hu D, Chen J, Li Y, Huang J*, et al.* Predicting the 10-Year Risks of

19

Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China). Circulation. 2016;134(19):1430-40.doi:10.1161/circulationaha.116.022367

16.Zhiting G, Jiaying T, Haiying H, Yuping Z, Qunfei Y, Jingfen J. Cardiovascular disease risk prediction models in the Chinese population- a systematic review and meta-analysis. BMC Public Health. 2022;22(1):1608.doi:10.1186/s12889-022-13995-z

17.Bunn HF, Gabbay KH, Gallop PM. The glycosylation of hemoglobin: relevance to diabetes mellitus. Science. 1978;200(4337):21-7.doi:10.1126/science.635569

18.Hoshino J, Abe M, Hamano T, Hasegawa T, Wada A, Ubara Y, *et al.* Glycated albumin and hemoglobin A1c levels and cause-specific mortality by patients' conditions among hemodialysis patients with diabetes: a 3-year nationwide cohort study. BMJ Open Diabetes Res Care. 2020;8(1).doi:10.1136/bmjdrc-2020-001642

19.Ciardullo S, Rea F, Perseghin G. Glycated albumin is associated with all-cause and cardiovascular mortality among U.S. adults with and without diabetes: A retrospective cohort study. Nutr Metab Cardiovasc Dis. 2022;32(10):2375-82.doi:10.1016/j.numecd.2022.07.008

20.Shafi T, Sozio SM, Plantinga LC, Jaar BG, Kim ET, Parekh RS *, et al.* Serum fructosamine and glycated albumin and risk of mortality and clinical outcomes in hemodialysis patients. Diabetes Care. 2013;36(6):1522-33.doi:10.2337/dc12-1896

21.Fukuoka K, Nakao K, Morimoto H, Nakao A, Takatori Y, Arimoto K *, et al.* Glycated albumin levels predict long-term survival in diabetic patients undergoing haemodialysis. Nephrology (Carlton). 2008;13(4):278-83.doi:10.1111/j.1440-

1797.2007.00864.x

22.Sullivan VK, Wallace AS, Rooney MR, Zhang S, Fang M, Christenson RH*, et al.* Inverse Associations between Measures of Adiposity and Glycated Albumin in US Adults, NHANES 1999-2004. J Appl Lab Med. 2023;8(4):751-62.doi:10.1093/jalm/jfad004

23.Sumner AE, Duong MT, Bingham BA, Aldana PC, Ricks M, Mabundo LS*, et al.* Glycated Albumin Identifies Prediabetes Not Detected by Hemoglobin A1c: The Africans in America Study. Clin Chem. 2016;62(11):1524-32.doi:10.1373/clinchem.2016.261255

24.Miyashita Y, Nishimura R, Morimoto A, Matsudaira T, Sano H, Tajima N. Glycated albumin is low in obese, type 2 diabetic patients. Diabetes Res Clin Pract. 2007;78(1):51-5.doi:10.1016/j.diabres.2007.02.021

25.Koga M, Otsuki M, Matsumoto S, Saito H, Mukai M, Kasayama S. Negative association of obesity and its related chronic inflammation with serum glycated albumin but not glycated hemoglobin levels. Clin Chim Acta. 2007;378(1-2):48-52.doi:10.1016/j.cca.2006.10.013

26.He X, Mo Y, Ma X, Ying L, Zhu W, Wang Y*, et al.* Associations of body mass index with glycated albumin and glycated albumin/glycated hemoglobin A(1c) ratio in Chinese diabetic and non-diabetic populations. Clin Chim Acta. 2018;484:117-21.doi:10.1016/j.cca.2018.05.044

27.Reynolds AN, Duncan A, Kruimer D, Venn BJ. Glycated albumin is associated with body mass index in euglycemic adults but is not predictive of postprandial blood

21

glucose response. J Clin Lab Anal. 2017;31(5).doi:10.1002/jcla.22085

28.Danese E, Montagnana M, Nouvenne A, Lippi G. Advantages and pitfalls of fructosamine and glycated albumin in the diagnosis and treatment of diabetes. J Diabetes Sci Technol. 2015;9(2):169-76.doi:10.1177/1932296814567227

29.Vejbjerg P, Knudsen N, Perrild H, Carlé A, Laurberg P, Pedersen IB, *et al.* The impact of smoking on thyroid volume and function in relation to a shift towards iodine sufficiency. Eur J Epidemiol. 2008;23(6):423-9.doi:10.1007/s10654-008-9255-1

30.Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, *et al.* Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 2018;391(10129):1513-23.doi:10.1016/s0140-6736(18)30134-x

31.van der Kolk NM, de Vries NM, Kessels RPC, Joosten H, Zwinderman AH, Post B, *et al.* Effectiveness of home-based and remotely supervised aerobic exercise in Parkinson's disease: a double-blind, randomised controlled trial. Lancet Neurol. 2019;18(11):998-1008.doi:10.1016/s1474-4422(19)30285-6

32.Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, *et al.* A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73(1):202-9.doi:10.1016/j.jhep.2020.03.039

33.Shen Y, Pu LJ, Lu L, Zhang Q, Zhang RY, Shen WF. Glycated albumin is superior to hemoglobin A1c for evaluating the presence and severity of coronary artery disease in type 2 diabetic patients. Cardiology. 2012;123(2):84-90.doi:10.1159/000342055

22

34.Pu LJ, Lu L, Shen WF, Zhang Q, Zhang RY, Zhang JS*, et al.* Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients. Circ J. 2007;71(7):1067-73.doi:10.1253/circj.71.1067

35.Copur S, Siriopol D, Afsar B, Comert MC, Uzunkopru G, Sag AA*, et al.* Serum glycated albumin predicts all-cause mortality in dialysis patients with diabetes mellitus: meta-analysis and systematic review of a predictive biomarker. Acta Diabetol. 2021;58(1):81-91.doi:10.1007/s00592-020-01581-x

36.Zhang J, Du Y, Hu C, Liu Y, Liu J, Gao A*, et al.* Elevated Glycated Albumin in Serum Is Associated with Adverse Cardiac Outcomes in Patients with Acute Coronary Syndrome Who Underwent Revascularization Therapy. J Atheroscler Thromb. 2022;29(4):482-91.doi:10.5551/jat.61358

37.Doménech M, Roman P, Lapetra J, García de la Corte FJ, Sala-Vila A, de la Torre R*, et al.* Mediterranean diet reduces 24-hour ambulatory blood pressure, blood glucose, and lipids: one-year randomized, clinical trial. Hypertension. 2014;64(1):69-76.doi:10.1161/hypertensionaha.113.03353

38.Oka S, Deyama J, Umetani K, Harama T, Shimizu T, Makino A*, et al.* Glycemic variability is associated with myocardial damage in nondiabetic patients with ST-elevation myocardial infarction. Cardiovasc Endocrinol Metab. 2018;7(2):47-53.doi:10.1097/xce.0000000000000145

39.Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI, Jr. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J.

23

2016;473(24):4527-50.doi:10.1042/bcj20160503c

40.Hashimoto K, Tanikawa K, Nishikawa J, Chen Y, Suzuki T, Koga M. Association of variation range in glycated albumin (GA) with increase but not decrease in plasma glucose: implication for the mechanism by which GA reflects glycemic excursion. Clin Biochem. 2015;48(6):397-400.doi:10.1016/j.clinbiochem.2014.12.021

41.Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. Jama. 2006;295(14):1707-8.doi:10.1001/jama.295.14.1707

42.Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, *et al.* Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes. 2008;57(5):1349-54.doi:10.2337/db08-0063

43.Selvin E, Warren B, He X, Sacks DB, Saenger AK. Establishment of Community-Based Reference Intervals for Fructosamine, Glycated Albumin, and 1,5-Anhydroglucitol. Clin Chem. 2018;64(5):843-50.doi:10.1373/clinchem.2017.285742

44.Hirata T, Koga M, Kasayama S, Morimoto J, Maruyama T. Glycated albumin is not significantly correlated with body mass index in patients with acute-onset type 1 diabetes. Clin Chim Acta. 2015;438:248-51.doi:10.1016/j.cca.2014.08.038

45.Furusyo N, Koga T, Ai M, Otokozawa S, Kohzuma T, Ikezaki H, *et al.* Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS). Diabetologia. 2011;54(12):3028-36.doi:10.1007/s00125-011-2310-6

24

46. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, *et al.* Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. Nat Rev Nephrol. 2016;12(8):453-71.doi:10.1038/nrneph.2016.75

47. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. Int J Gen Med. 2016;9:229-55.doi:10.2147/ijgm.S102819

48. Ren M, Sun K, Li F, Qi YQ, Lin DZ, Li N, *et al.* Association between obesity measures and albuminuria: A population-based study. J Diabetes Complications. 2016;30(3):451-6.doi:10.1016/j.jdiacomp.2015.12.007

49. Johnson AM, Olefsky JM. The origins and drivers of insulin resistance. Cell. 2013;152(4):673-84.doi:10.1016/j.cell.2013.01.041

50. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia. 2010;53(7):1270-87.doi:10.1007/s00125-010-1684-1

51. Pedersen DJ, Guilherme A, Danai LV, Heyda L, Matevossian A, Cohen J, *et al.* A major role of insulin in promoting obesity-associated adipose tissue inflammation. Mol Metab. 2015;4(7):507-18.doi:10.1016/j.molmet.2015.04.003

52. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;126(1):12-22.doi:10.1172/jci77812

53. Fryk E, Olausson J, Mossberg K, Strindberg L, Schmelz M, Brogren H, *et al.*

25

Hyperinsulinemia and insulin resistance in the obese may develop as part of a homeostatic response to elevated free fatty acids: A mechanistic case-control and a population-based cohort study. EBioMedicine. 2021;65:103264.doi:10.1016/j.ebiom.2021.103264

54.Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med. 2017;23(7):804-14.doi:10.1038/nm.4350

55.Mukai N, Ninomiya T, Hata J, Hirakawa Y, Ikeda F, Fukuhara M, *et al.* Association of hemoglobin A1c and glycated albumin with carotid atherosclerosis in community-dwelling Japanese subjects: the Hisayama Study. Cardiovasc Diabetol. 2015;14:84.doi:10.1186/s12933-015-0247-7

56.Mihara A, Ohara T, Hata J, Honda T, Chen S, Sakata S*, et al.* Association between serum glycated albumin and risk of cardiovascular disease in a Japanese community: The Hisayama Study. Atherosclerosis. 2020;311:52-9.doi:10.1016/j.atherosclerosis.2020.08.016

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**Figure legends**

**Figure 1:** Flowchart for study population selection.

**Figure 2:** Diagnostic standard flowchart of MAFLD.

**Figure 3:** Path diagram of the mediation analysis models

**Figure 4:** Restricted Cubic Spline Curve for association between odds ratio of GA and 10-year ASCVD risk

**Supplementary Materials**

**Table S1** Baseline characteristics of enrolled participants

**Table S2**. Logistic regression models of the link between GA and 10-year ASCVD risk

**Table S3.** Mediation effect of BMI on the association between GA and 10-year ASCVD risk

**Table S4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk by age and MAFLD
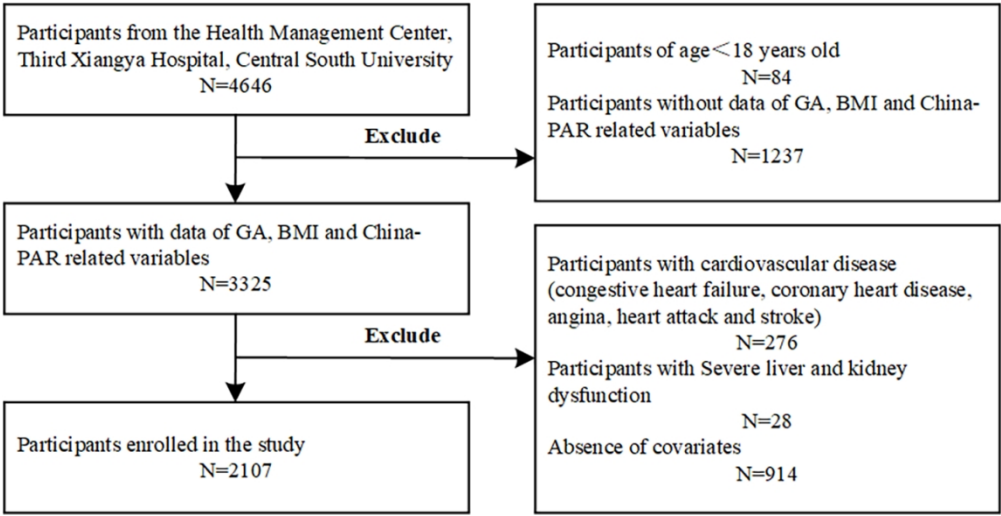
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Figure 1: Flowchart for study population selection.
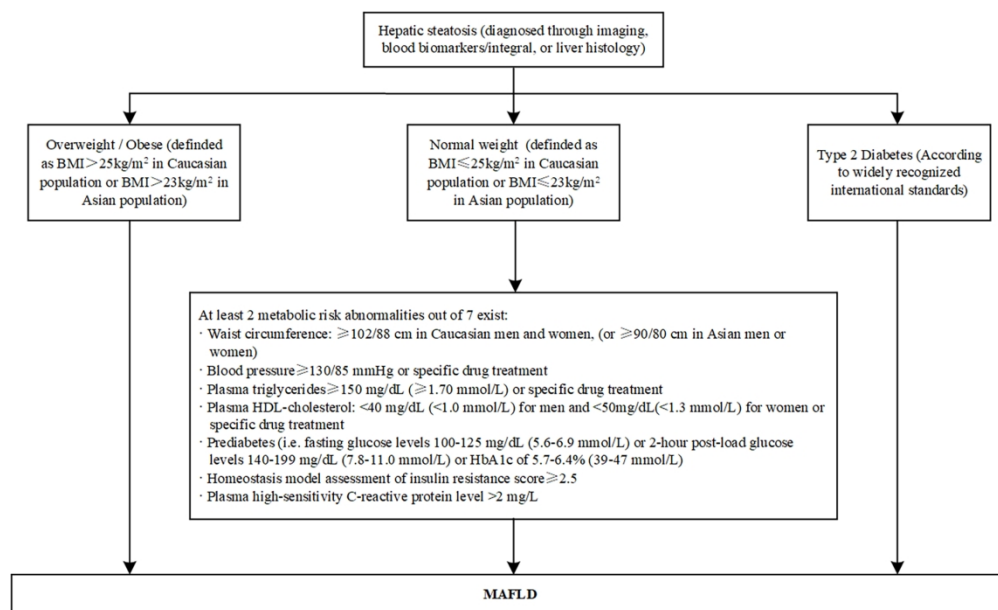
112x57mm (300 x 300 DPI)

Figure 2: Diagnostic standard flowchart of MAFLD.
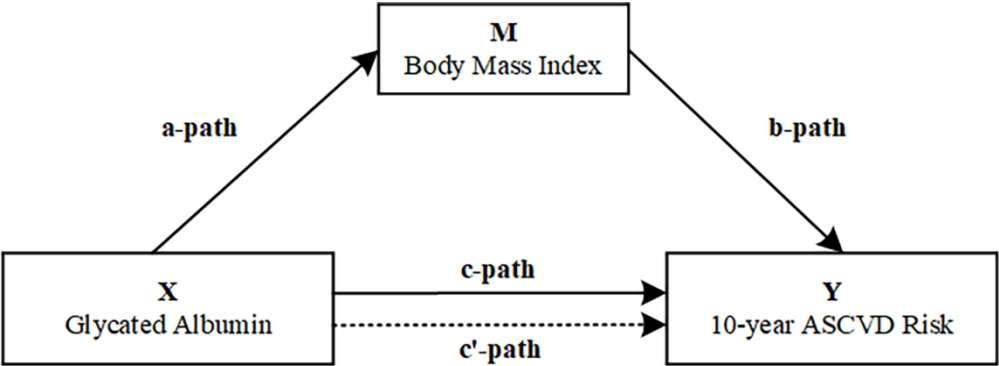
169x102mm (300 x 300 DPI)

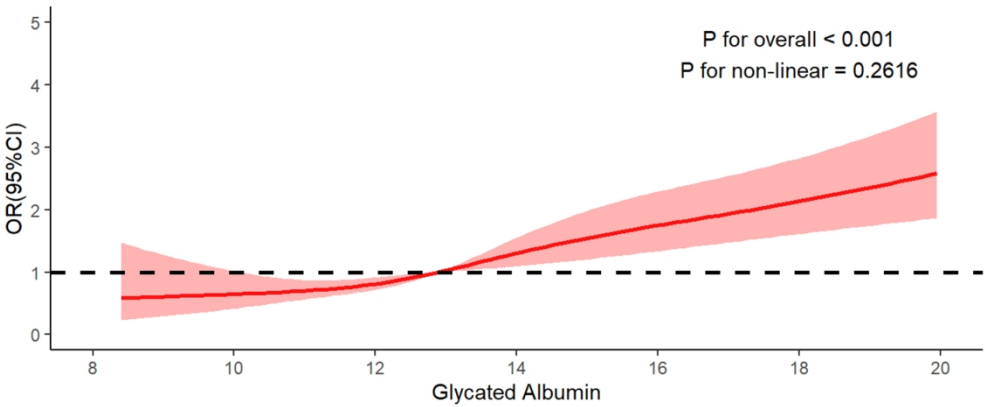Figure 3: Path diagram of the mediation analysis models

169x63mm (300 x 300 DPI)

Figure 4: Restricted Cubic Spline Curve for association between odds ratio of GA and 10-year ASCVD risk

169x75mm (300 x 300 DPI)

**Table 1** Baseline characteristics of enrolled participants

| | Total | China-PAR | | p-Value |
|---|---|---|---|---|
| | （n=2107） | Medium-high Risk Group (n=230) | Low Risk Group (n=1877) | |
| **Characteristics [median (25th, 75th)]** | | | | |
| Age (year) | 45.00 [36.00, 52.00] | 59.00 [56.00, 66.00] | 43.00 [35.00, 50.00] | <0.001 |
| BMI* | 24.20 [21.85, 26.50] | 25.20 [23.63, 27.90] | 24.00 [21.70, 26.20] | <0.001 |
| Waist circumference | 83.00 [74.00, 91.00] | 89.00 [83.00, 96.00] | 82.00 [73.00, 90.00] | <0.001 |
| Systolic Pressure | 119.00 [109.00, 130.00] | 138.00 [130.00, 147.75] | 118.00 [108.00, 127.00] | <0.001 |
| Diastolic Pressure | 73.00 [65.00, 81.00] | 83.00 [77.00, 89.00] | 71.00 [65.00, 80.00] | <0.001 |
| albumin | 45.50 [43.80, 47.60] | 44.60 [42.90, 46.10] | 45.70 [43.90, 47.70] | <0.001 |
| GA* | 12.90 [11.60, 14.20] | 13.60 [12.20, 15.40] | 12.80 [11.50, 14.10] | <0.001 |
| platelet | 227.00 [192.00, 261.00] | 208.00 [176.25, 245.75] | 229.00 [194.00, 262.00] | <0.001 |
| ALT* | 20.00 [14.00, 31.50] | 20.00 [15.00, 28.00] | 21.00 [14.00, 32.00] | 0.391 |
| AST* | 22.00 [18.00, 27.00] | 23.00 [20.00, 26.00] | 22.00 [18.00, 27.00] | 0.043 |
| Fasting blood glucose | 5.23 [4.90, 5.67] | 5.74 [5.20, 6.51] | 5.20 [4.87, 5.59] | <0.001 |
| Total cholesterol | 4.97 [4.42, 5.68] | 5.18 [4.59, 5.92] | 4.95 [4.41, 5.64] | 0.006 |
| triglyceride | 1.42 [0.92, 2.26] | 1.72 [1.05, 2.66] | 1.38 [0.90, 2.22] | <0.001 |
| HDL-C* | 1.25 [1.07, 1.47] | 1.21 [1.04, 1.40] | 1.25 [1.07, 1.48] | 0.006 |
| LDL-C* | 2.90 [2.39, 3.45] | 2.94 [2.41, 3.55] | 2.90 [2.39, 3.44] | 0.408 |
| **Characteristics [n (%)]** | | | | |
| Sex | | | | 0.001 |
| Female | 1131 (53.7) | 148 (64.3) | 983 (52.4) | |
| Male | 976 (46.3) | 82 (35.7) | 894 (47.6) | |
| Cervical vascular plaques | | | | <0.001 |
| No | 1632 (77.5) | 101 (43.9) | 1531 (81.6) | |
| Yes | 475 (22.5) | 129 (56.1) | 346 (18.4) | |
| Smoking status | | | | <0.001 |
| No | 1538 (73.0) | 131 (57.0) | 1407 (75.0) | |
| Yes | 569 (27.0) | 99 (43.0) | 470 (25.0) | |
| Drinking status | | | | 0.449 |
| No | 1344 (63.8) | 141 (61.3) | 1203 (64.1) | |
| Yes | 763 (36.2) | 89 (38.7) | 674 (35.9) | |
| Sport status | | | | 0.012 |
| No | 854 (40.5) | 75 (32.6) | 779 (41.5) | |
| Yes | 1253 (59.5) | 155 (67.4) | 1098 (58.5) | |
| MAFLD* | | | | <0.001 |
| No | 1116 (53.0) | 62 (27.0) | 1054 (56.2) | |
| Yes | 991 (47.0) | 168 (73.0) | 823 (43.8) | |

**Notes**: Values are median (25th, 75th), or n (%). P values were computed utilizing Student's t-test or the Mann-Whitney U test for continuous variables, and the chi-squared test was adopted for categorical variables.

**Table 2**. Logistic regression models of the link between GA and 10-year ASCVD risk

|     | Model 1 | | | Model 2 | | | Model 3 | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|     | OR | 95%CI | p | OR | 95%CI | p | OR | 95%CI | p |
| GA | 1.138 | (1.094, 1.185) | < 0.001 | 1.142 | (1.049, 1.240) | 0.002 | 1.160 | (1.055, 1.276) | 0.002 |

**Notes:** Model 1 = GA; Model 2 = Model 1 + Age + Sex + Smoking status + Sport status + BMI + WC + SBP +DBP; Model 3 = Model 2 + Albumin + Platelet + Total Cholesterol + Triglyceride + HDL-C + Cervical vascular plaques + MAFLD.

**Table 3.** Mediation effect of BMI on the association between GA and 10-year ASCVD risk

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| BMI | 2107 | -0.146 (0.026) p<0.001 p<0.001 | 0.190 (0.015) p<0.001 p<0.001 | 0.229 (0.040) p<0.001 p<0.001 | -0.028 (0.006) p<0.001 p<0.001 | 0.201 (0.040) p<0.001 p<0.001 | 13.9% |

**Notes:** Exposure: GA; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

**Table 4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk by age and MAFLD

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| | | | | **Stratification by Age** | | | |
| <40 | 743 | -0.388 (0.064) p < .001 | 0.056 (0.005) p < .001 | 0.030 (0.022) p =.177 | -0.022(0.007) 95%CI (-0.037, -0.011) p = .002 | 0.008 (0.023) p = .714 | - |
| 40-60 | 1214 | -0.147 (0.030) p < .001 | 0.210 (0.016) p < .001 | 0.102 (0.029) p < .001 | -0.031(0.007) 95%CI (-0.046, -0.020) p < .001 | 0.071 (0.030) p = .017 | **43.7** |
| >60 | 150 | 0.136 (0.075) p =.270 | 0.185 (0.093) p < .05 | 0.415 (0.121) p < .001 | 0.025(0.022) 95%CI (-0.016, 0.069) p = .245 | 0.440 (0.117) p < .001 | - |
| | | | | **Stratification by MAFLD** | | | |
| MALFD | 991 | -0.040 (0.029) p =.092 | 0.081 (0.029) p < .01 | 0.275 (0.043) p < .001 | -0.003(0.003) 95%CI (-0.010, 0.002) p = .310 | 0.272 (0.043) p < .001 | - |
| Non-MAFLD | 1116 | -0.107*** (0.029) p < .001 | 0.127*** (0.026) p < .001 | 0.178 (0.068) p =.009 | -0.014(0.005) 95%CI (-0.025, -0.006) p = .004 | 0.164 (0.067) p = .014 | **8.5** |

**Notes:** Exposure: GA; Mediator: BMI; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, total cholesterol, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

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## Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Cross-sectional Study

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**Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Cross-sectional Study**

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**Abstract**

**Objectives:** Glycated albumin (GA) and body mass index (BMI) have been linked to atherosclerotic cardiovascular disease (ASCVD). However, the role of BMI in the correlation between GA and 10-year ASCVD risk is still not fully understood.

**Design:** A Retrospective Cross-sectional Study

**Setting:** In this retrospective cross-sectional study, 4,646 healthy subjects who received a full health checkup at the Health Management Medical Center, Third Xiangya Hospital of Central South University from January 1, 2022 to December 30, 2023, were initially identified. After applying the exclusion criteria, 2,107 participants were included in the final analysis.

**Participants:** The inclusion criteria for this study included: a) age is greater than or equal to 18 years old; and b) subjects were long-term residents of Hunan province.

**Primary and secondary outcome measures:** The 10-year ASCVD risk was evaluated via the China-PAR equation. The link between GA and 10-year ASCVD risk was examined through a multivariable logistic regression model and the dose-response relationship was demonstrated using the restricted cubic spline. The potential mediation effect of BMI on this association was explored and the differences in this mediation effect across age and metabolic-associated fatty liver disease (MAFLD) subgroups were analyzed.

**Results:** Elevated GA levels were positively linked to an intensified 10-year ASCVD risk (OR=1.160, 95% CI =1.055–1.276). Additionally, BMI was negatively linked to GA and 10-year ASCVD risk. BMI mediated 13.9% of the connection between GA and 10-year ASCVD risk. Specifically, the mediating effect of BMI remained significant in the 40–60 years age subgroup and non-MAFLD subgroup, with mediation ratios of 43.7% and 8.5%, respectively.

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**Conclusions:** GA is a key predictor of 10-year ASCVD risk, and BMI partially mediates this relationship in healthy populations. Therefore, targeted weight management is recommended to reduce the adverse effect of GA on 10-year ASCVD risk in different populations.

**Strengths and limitations of this study**

1.We used a mediation model to identify BMI as a partial mediator in the association between GA and 10-year ASCVD risk.

2.We applied multivariate logistic regression to explore the link between GA and 10-year ASCVD risk, controlling confounding effects stepwise.A

3.Restricted Cubic Splines were used to illustrate the dose-response relationship between GA and 10-year ASCVD risk.

4.The study's retrospective cross-sectional design prevents establishing causality between GA and 10-year ASCVD risk and exploring the GA-BMI bidirectional relationship.

**Keywords:** Glycated Albumin, Body Mass Index, Atherosclerotic Cardiovascular Disease Risk, Mediation, Metabolic Associated Fatty Liver Disease

## 1. Introduction

Cardiovascular diseases (CVDs) stand as primary culprits behind death and disability worldwide, presenting a substantial risk to human health. This category of diseases encompasses coronary artery disease (CAD), stroke, and myocardial infarction, which commonly arise due to arteriosclerosis, hypertension, hyperlipidemia, and diabetes[1-5]. Currently, glycated hemoglobin A1c (HbA1c) is recognized as a leading indicator in

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the clinical assessment of long-term blood sugar control. The latest KDIGO guidelines have recommended a target value of 6.5% to 8.0% for HbA1c in those with diabetes and chronic kidney disease (CKD) to reduce cardiovascular risk[6]. Nevertheless, HbA1c can be influenced by hemoglobin levels, red blood cell lifespan, anemia, liver dysfunction, and kidney disease[7]. Concurrently, there is increasing interest in an alternative indicator, glycated albumin (GA), a novel index for glucose monitoring, reflecting average blood sugar levels over the past 2 to 3 weeks[8,9] GA is calculated as the ratio of GA to total albumin concentration[10]. Unlike HbA1c, GA has the advantage of being independent of hemoglobin and red blood cell turnover and less affected by red blood cell lifespan, thus serving as a good marker for assessing blood glucose levels[11].

Numerous guidelines have recommended that the prevention and treatment of atherosclerotic CVD (ASCVD) be based on risk assessment. Traditional risk assessment methods, including the Framingham D'Agostino 2008 model, PCE white model, SCORE model, and QRI SK model, are recognized for their reliability in Western populations. However, the Chinese population presents distinct disease patterns and risk factors, making these models less suitable. Particularly, the Framingham D'Agostino model overestimates the CVD risk in men and underestimates it in women, a trend similarly observed in the PCE model[12]. S Since 2003, several large-sample prediction models for CVD risk have been established for Chinese adults. Among them, the 10-year risk prediction models for Chinese CVD[13,14] and the China-PAR model for ASCVD[15] are notable. China-PAR model, a CVD risk assessment tool developed by Chinese scholars based on large-sample cohort data, is most often externally validated and is considered potentially a better option for predicting CVD risk in China[16]. It ultimately provides a 10-year and lifetime risk assessment based on

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relevant metrics, which benefits primary prevention and health management of CVD.

Obese people have a high prevalence of ASCVD, and body mass index (BMI), as an

important indicator for assessing obesity in an individual, has a very close correlation

with ASCVD risk. Similarly, the study of GA in predicting CVD risk has attracted

much attention in recent years. GA, as an early glycated protein, may be more sensitive

than other markers to reflect CVD and its complications[5,17]. Several publications have

found an link between GA and cardiovascular mortality in diabetes mellitus (DM)

patients[9,18,19]. Notably, multiple studies confirm that GA is more strongly than HbA1C

associated with CVD in CKD patients requiring dialysis[18,20,21] 。 Several papers have

noted a weak negative connection between GA and BMI in DM populations but with a

narrow range for BMI or GA values[11,22-27]. In contrast, an investigation of Caucasian

populations with a median BMI of 38.4 (large body weight) similarly unveiled a marked

negative link between BMI and GA[7]. Therefore, the association between GA and BMI

in a wider population remains to be confirmed by further studies. There are few studies

on the correlation among GA, BMI, and 10-year ASCVD risk in normal populations.

Hence, this paper was to illustrate whether BMI mediates the link between GA and 10-

year ASCVD risk in the normal population.

## 2.Methods

### 2.1 Participant Selection

This cross-sectional study obtained data from 4646 healthy subjects during health

checkups at the Health Management Medicine Center, Third Xiangya Hospital of

Central South University from January 1, 2022, to June 30, 2023. To control for

potential biases, we included all health examination data during this period, rather than

relying on random sampling.

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The inclusion criteria for this study included: a) age is greater than or equal to 18 years old; and b) subjects were long-term residents of Hunan province. The exclusion criteria of this study were a) subjects did not have complete data required for this study; and b) subjects did not provide written informed consent; and c) Subjects reported established family history and medical history of CVD and cerebrovascular diseases (including coronary heart disease, stroke, congestive heart failure, myocardial infarction, and angina) in questionnaires ;and d) Subjects was discovered severe liver or kidney dysfunction (which may affect GA results) in health checkups. According to the exclusion criteria, 84 participants under the age of 18 were excluded, 1237 participants lacking GA, BMI, and China-PAR equation-related data were excluded, 276 participants with self-reported CVD and cerebrovascular diseases on the questionnaire were excluded, 28 participants with severe liver or kidney dysfunction were excluded, and 914 participants with missing information on cardiovascular risk factors were excluded. Ultimately, 2107 participants were enrolled. The specific selection process is displayed in Figure 1. This retrospective study adhered to the institution and national research committee's ethical standards, and approval was obtained from the institutional ethics committee of the Third Xiangya Hospital of Central South University and review board (Ethics Approval Number: Quick24559).

**2.2 Measurements**

**2.2.1 GA measurement**

Compared to HbA1c testing, the detection methods for GA are not yet fully standardized. In this study, enzyme-linked immunosorbent assay (ELISA), a widely adopted and reliable technique[28] was employed to detect GA.

**2.2.2 Cardiovascular Risk Estimation Based on China-PAR Project**

The China-PAR score was employed to appraise ASCVD risk. The China-PAR model

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encompassed sex, age, systolic blood pressure (SBP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), waist circumference (WC), smoking status, DM, region, urbanization, and family history of ASCVD. The participants were allocated into three risk subgroups based on the scores: <5% as low risk, 5–10% as medium risk, and ≥10% as high risk[15].

Health status and lifestyle information of the participants were collected through questionnaires during their health checkups. This questionnaire, designed based on the National Physical Examination Questionnaire[29], included details on the participants' and family history of CVDs and cerebrovascular diseases, and lifestyle information such as history of hypertension, CVDs, DM, smoking, alcohol consumption, and physical activity. Hypertension was diagnosed as SBP ≥140 mmHg and/or diastolic blood pressure (DBP)≥90 mmHg or receiving antihypertensive treatment. DM was set as fasting plasma glucose (FPG) ≥7.0 mmol/L, HbA1c ≥6.5%, or receiving hypoglycemic treatment. A family history of ASCVD was described as congestive heart failure, stroke, coronary heart disease, myocardial infarction, or angina in first-degree relatives. In this study, based on the three risk categories mentioned above, participants were allocated into medium-high risk group and low risk groups.

**2.2.3 Covariate Assessment**

Demographic data were obtained from physical examination reports. Age as a continuous variable was categorized into subgroups: 18-39 years, 40-60 years, or >60 years. During the physical examination, weight, height, WC, SBP, and DBP were gauged. BMI was presented as weight (kg) divided by height square (m²). Laboratory tests covered routine blood tests, liver function tests, GA, FBG, HbA1c, fasting insulin, and blood lipids. It should be noted that, considering GA rather than HbA1C was the primary focus of this study, and most research subjects did not have both measurements

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above, HbA1C was not set as a mandatory enrollment criterion. All participants underwent liver and cervical vascular ultrasonography performed by an ultrasonologist after an overnight fast. These reports were reviewed and confirmed by another senior ultrasonologist. The diagnoses of fatty liver (hepatic steatosis) and cervical vascular plaques were based on liver and cervical vascular ultrasound scans using linear array or convex high-frequency probes with frequencies of 5-10 MHz. These scans evaluated the echo, size, and morphology of the liver, as well as intima-media thickness, plaque size, morphology, and location of cervical vessels. Furthermore, medication history, smoking history, alcohol consumption, and exercise frequency were recorded directly from the physical examination questionnaire. Medication history in this study was defined as medication use for diabetes mellitus, hypertension, and dyslipidemia. Smoking history was defined as smoking continuously or cumulatively for more than six months[29]. Alcohol consumption manifested as consuming over 10 grams of alcohol per day[30]. Physical activity represented moderate- or high-intensity physical activity at least three times per week, with each session lasting at least 30 minutes[31].

**2.2.4 Diagnosis of MAFLD**

According to Eslam et al.[32], metabolic-associated fatty liver disease (MAFLD) means steatosis in combination with metabolic dysfunction in the International Expert Consensus Statement on MAFLD. The diagnostic criteria included hepatic steatosis through histology (liver biopsy), imaging techniques, or blood-based biomarkers and at least one of the following conditions: overweight/obesity, T2DM, and metabolic dysfunction. Metabolic dysfunction refers to at least two of the seven metabolic risk abnormalities (Figure 2). In this study, all subjects were from a health - screening cohort. Thus, balancing feasibility, cost - effectiveness, and diagnostic accuracy in the screening population, we uniformly used liver ultrasonography after an overnight fast to assess

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hepatic steatosis in all subjects, instead of other imaging techniques (e.g., vibration - controlled transient elastography or MRI elastography), blood - based biomarkers (e.g., FIB - 4, APRI et.al ), or liver biopsy.

**2.3 Data Analysis**

Continuous variables were denoted as median and interquartile spacing and compared utilizing the independent samples t-test. Categorical variables were depicted as unweighted frequencies, weighted percentages, and standard error (SE), and compared utilizing the chi-square test. Multiple regression analysis was used to test the correlation between GA (continuous exposure variable) and 10-year ASCVD risk (binary outcome variable). Variables showing between-group differences in the baseline information table ($P < 0.05$) were identified and included as potential confounders, serving as covariates in subsequent analyses. Three models were constructed to progressively control for the effects of confounders: Unadjusted crude Model 1; Model 2 adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, and DBP; and Model 3 adjusted for all covariates, such as albumin, platelet, TC, Triglyceride, HDL-C, Cervical vascular plaques, and MAFLD. Subsequently, restricted cubic spline (RCS) was adopted to demonstrate the dose-response relationship between GA and 10-year ASCVD risk. Finally, a logistic regression model was employed to illustrate the relationships among GA, BMI, and 10-year ASCVD risk. To analyze the mediation effect of BMI [(mediation effect/total effect) × 100%], a simple mediation model was used, with three paths (Figure 3). The total effect represented the impact of GA (exposure) on 10-year ASCVD risk (outcome). Path A assessed the effect of GA on BMI (mediator). Path B evaluated the link between BMI and 10-year ASCVD risk. Path C estimated the direct impact of GA on 10-year ASCVD risk. The mediated effect was calculated as (mediated effect/total effect) × 100%.

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All statistical analyses, including mediation analysis, were done in the R statistical package. Statistical significance was delineated at a two-sided p-value of < 0.05.

### 3.Results

### 3.1. Characteristics of Participants

Table S1 displays the baseline characteristics of study participants. Based on the three risk categories mentioned above, participants were assigned to a medium-high-risk group (n=230) and a low-risk group (n=1877). The differences were statistically notable (p < 0.001) in age, BMI, WC, SBP, DBP, albumin, GA, platelet, FBG, TC, cervical vascular plaques, smoking status, and MAFLD.

Table S2 exhibits the multivariable logistic analysis results. In model 1, a higher GA was greatly connected with higher odds of 10-year ASCVD risk (OR =1.138, 95% CI =1.094, 1.185). After adjusting for covariates (age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD), GA was positively connected with 10-year ASCVD risk (OR =1.160, 95% CI =1.055, 1.276) in Model 3.

The RCS curve showed that increased GA levels were significantly linked to increased 10-year ASCVD risk (P for overall < 0.001), with a linear relationship (P for non-linear =0.2616) (Figure 4). The red solid line indicates the point estimate of OR, and the pink shaded area represents the 95% CI. The curve indicates that when GA levels are below 12.95%, the OR remains around 1, suggesting that GA does not significantly affect the risk within this range. However, when GA levels > 12.95%, the OR starts to increase significantly, indicating a marked correlation between higher GA levels and greater 10-year ASCVD risk.

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### 3.2 Mediation Analysis of BMI

We then delved into the mediation effect of BMI on the link between GA and 10-year ASCVD risk. All mediation analyses were carried out after adjustment for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

GA was negatively correlated with BMI ($p < 0.001$) and positively correlated with 10-year ASCVD risk ($p < 0.001$). BMI was positively related to 10-year ASCVD risk ($p < 0.001$) (Table S3). It is estimated that BMI mediated 13.9% of the total link between GA and 10-year ASCVD risk.

Given the effect of age and MAFLD, subgroup analyses were implemented (Table S4). In the age group, BMI partially mediated the link between GA and 10-year ASCVD risk. In the age subgroup 40–60 years, there was a 43.7% mediating effect of BMI on the link between GA and 10-year ASCVD risk. However, in the age subgroup < 40 years and > 60 years, no mediating effect of BMI was observed on the correlation between GA and 10-year ASCVD risk. Similarly, BMI mediated 8.5% of the total link between GA and 10-year ASCVD risk in the non-MAFLD group. However, the mediating effect was not observed in the MAFLD group.

### 3.3 Sensitivity Analysis

In sensitivity analyses, we adjusted for three medication status variables (antidiabetic, antihypertensive, and lipid-lowering drugs) as potential confounders. After excluding 23 cases with missing data, the analysis of 2,084 eligible participants demonstrated that the positive association between GA and 10-year cardiovascular risk remained highly consistent (OR = 1.153, 95% CI = 1.033–1.277; Table S5). Detailed results of the adjusted model are provided (Table S5). This finding further supported the robustness

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of our primary analysis, indicating that GA maintained its independent predictive value

for 10-year cardiovascular risk even after controlling for the influence of

pharmacological interventions.

**4.Discussion**

This study elucidated the relationship among GA, BMI, and 10-year ASCVD risk in

the normal population undergoing physical examination and estimated the mediating

role of BMI. This is the first study on their relationship, and our results confirm: 1. BMI

mediated the link between GA and 10-year ASCVD risk, with 13.9% of the association

mediated by BMI. 2. The mediating role of BMI varies by age and MAFLD subgroups.

BMI plays a partially mediating role of 8.5% in the 40-60 age group and no mediating

role in the 30-44 and over 60 age groups. Similarly, BMI has an 8.5% partial mediating

effect on the non-MAFLD group, but no mediating effect was revealed in the MAFLD

group.

The present study examined a real-world normal population undergoing medical

examinations and showed a positive correlation between GA and 10-year CVD risk.

These associations remained after adjustment for established cardiovascular risk factors

and socio-behavioral factors. Although GA is widely recognized to reflect blood

glucose levels, relevant studies have focused on its application in diverse situations (e.g.,

anemia, abnormal liver function, and renal disease) where HbA1c accuracy may be

impaired. However, there is controversy about the ability of GA to predict long-term

ASCVD outcomes. Previous studies have manifested that GA upregulation is positively

linked to CAD and its severity in DM patients, whereas HbA1c levels are not as

strongly correlated with CAD[33,34]. Similarly, several publications have highlighted that

after adjustment for HbA1c levels, the higher GA in dialysis-requiring patients with

CKD and DM, the higher risk of cardiovascular mortality, all-cause mortality, CAD,

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major adverse cardiovascular events, and stroke; no correlation was found in non-DM individuals[9,18,19]. Zhao et al. had found in the meta - analysis that GA had a stronger association with CVD outcomes (including cardiovascular mortality in non - dialysis patients and all - cause mortality in dialysis patients) than HbA1c, and its link with MACCE was independent of traditional risk factors and HbA1c levels[35]. However, Copur et al. revealed a prominent correlation of GA levels with all-cause mortality in DM patients undergoing dialysis, but not with CVD mortality[36]. Additionally, high GA levels were independently linked to unfavorable intermediate-term efficacy in low-risk populations undergoing percutaneous coronary intervention, but the prognostic role was only present in the DM subgroup, and in the non-DM individuals, this association was not supported by clear evidence[37]. It is well known that atherosclerosis is the most important cause of CVDs[38]. Significant glycemic excursions or fluctuations potentiate oxidative stress and drive atherosclerosis, which can lead to ASCVD[39,40]. We hypothesized that relative to HbA1c, GA may better indicate glycemic fluctuations (eg, acute hyperglycemia or significant postprandial glycemic excursion) before the onset of acute coronary syndrome (within a short period[39,41], thereby promoting oxidative stress and accelerating atherosclerosis in both DM and non-DM subjects[42,43]. In non-DM subjects, short-term glycemic fluctuations, especially significant postprandial glycemic excursion, may be manifested as small but notable differences in GA. The effect of HbA1c on non-DM participants may be confounded by non-glycemic factors, like abnormalities in liver and kidney function and differences in red blood cell lifespan. In summary, studies comparing GA and HbA1c for long - term ASCVD prediction had mainly focused on diabetic patients and those with renal dysfunction, and there had been a lack of research on the longitudinal comparison of GA and HbA1c changes in the normal population. Therefore, further investigations are warranted to elucidate the

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complex pathophysiologic mechanisms that link GA to ASCVD risk.

Our experimental results showed that GA was negatively connected with BMI. Research in normoglycemic populations, pre-DM patients, and T2DM patients has reported a link between BMI and HbA1c or GA/HbA1c[27,44]. However, studies on the link between GA and BMI are very limited in normal populations in China. As mentioned earlier, several papers have demonstrated a weak negative connection between GA and BMI among DM populations, but mainly in normal-weight subjects with little fluctuation in BM[11,22-27], or Caucasian populations with large body weights (mean BMI of 38.4). In contrast, the two papers that did not discover a negative correlation between GA and BMI were conducted in the Japanese population with type 1 DM with a mean BMI of 20.1[45] or without DM with a nearly constant GA[46]. Thereby, the negative link between BMI and GA may not be exclusively attributable to glycemic exposure, but that there are other factors. An increase in BMI increases renal blood flow, glomerular filtration rate, and tubular reabsorption, resulting in glomerular enlargement and obesity-associated glomerular disease[47,48]. There is a difference in the rate of GA synthesis in participants with higher BMI and normal BMI[22]. BMI may affect GA clearance. A mild increment in BMI is related to an increased prevalence of microalbuminuria, which is a marker of early renal damage and is also closely related to cardiovascular prognosis[49]. Elevated GA levels may reflect the presence of microalbuminuria and cardiovascular events, but the exact mechanism is currently unknown. Therefore, future studies should refine the proteinuria and renal clearance of GA, which may influence the relationship between GA and BMI. Additional research utilizing labeled albumin in animal models of obesity and DM may offer valuable insight into this process.

Interestingly, the mediation analysis unraveled that BMI mediated 13.9% of the link

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between GA and 10-year ASCVD risk. This result emphasizes the importance of reducing BMI in lowering 10-year ASCVD risk, especially in high-GA populations, regardless of DM. Overweight and obesity are drivers of insulin resistance[50,51]. Insulin resistance is the core connecting several events of cardiometabolic disorders[50-55], molecularly due to impaired insulin signaling transduction via the PI3K pathway and intact signaling transduction via the MAPK pathway, leading to altered glucose-insulin homeostasis and increased glucose fluctuations. Significant shifts in blood glucose values facilitate oxidative stress and atherosclerosis[39,40]. Our study results supported this hypothesis. Although antihypertensive, lipid-lowering, and antidiabetic drugs might influence ASCVD risk through different pathways, sensitivity analysis showed that GA's independent predictive value remained unweakened after adjusting for these clinical interventions. Similarly, elevated serum GA levels are notably related to augmented carotid intima-media thickness and vascular endothelial dysfunction, a sign of early atherosclerosis[56]. Vascular smooth muscle cell proliferation, vascular endothelial dysfunction, and overproduction of collagen and inflammatory cytokines contribute to atherosclerosis and adverse cardiovascular events. Patients in the high GA group were more susceptible to metabolic disorders and severe CAD than those in the low and mediate GA groups and therefore had an intensified risk of CAD. When GA levels are substantially elevated (in the higher range, e.g., more than 17.1%), the CAD risk augments by approximately two folds[37]. Akane Mihara et al. disclosed findings from 10-year follow-up of the Hisayama study, suggesting that the highest quartile of GA levels (≥15.7%) was connected with a 2.2-fold increase in CAD incidence and a 2.5-fold increase in stroke risk[57]. The exact mechanism of the mediating effect of BMI is currently unclear and may be different in different populations. Additional research is warranted to illustrate the mechanisms.

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Interestingly, we found age and MAFLD were involved in the mediating role of BMI in the association between GA and 10-year ASCVD risk. Age-stratified subgroup analyses found that BMI did not mediate the association in the <40 and >60 groups. This finding suggests that BMI reduction in the <40 and >60 groups with increased GA may not be the cause for reducing 10-year CVD risk compared with the partial mediation effect in the 40-60 group. We suggest that in the 40-60 group, BMI reduction will be the focus of reducing the 10-year CVD risk induced by increased GA. In addition, subgroup analyses by MAFLD discovered that the link between GA and 10-year CVD risk was partially mediated by BMI in the non-MAFLD group, whereas BMI had no mediating effect in the MAFLD group. This finding suggests that BMI reduction in the MAFLD group with increased GA may not be the cause for reducing 10-year CVD risk. In the non-MAFLD group, BMI reduction will be the focus of preventing 10-year CVD risk. Since GA is an indicator of short-term glucose fluctuations and insulin resistance is the main mechanism of MAFLD, it is primarily a long-term effect. Therefore, long-term monitoring of GA is of limited benefit in assessing 10-year CVD risk in MAFLD populations. This may provide new insights into assessing and reducing 10-year CVD risk in these two groups.

Our study first investigates the mediating role of BMI in the link between GA and 10-year ASCVD risk. Nevertheless, we must acknowledge certain inherent limitations of our study. Firstly, multiple multivariate models were adopted to unveil the independent link between GA and 10-year ASCVD risk. Essential confounding factors like alcohol consumption and smoking were obtained through self-reported data, which are potentially influenced by recall bias and misinterpretations and potentially affect the accuracy of our findings. Secondly, it is crucial to analyze the correlation between GA and proteinuria. However, since this study involved non-hospitalized population and

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the incidence of proteinuria among participants was exceedingly low, statistical analysis was not possible. This limitation underscores further investigations into the renal clearance of proteinuria and GA, which is pivotal in elucidating the link between BMI and GA. Lastly, given the cross-sectional nature, it is not feasible to establish the exact causality as well as the bidirectional association between GA and 10-year ASCVD risk. Thereby, prospective cohort studies are imperative to further unveil this relationship. Despite these limitations, our study, based on a real-world context and substantial sample size, has fully adjusted for confounders, so the conclusions drawn are relatively reliable.

In conclusion, our cross-sectional study of 2,107 participants demonstrates for the first time that BMI effectively mediates the impact of GA on the 10-year ASCVD risk. Consequently, healthy populations, especially those aged 40-60 without MAFLD, should realize the beneficial effects of GA in reducing their 10-year ASCVD risk through lowering BMI, rather than just preventing diabetes. Prospective studies are crucial to validate these findings and elucidate intrinsic mechanisms, thus paving the way for targeted therapeutic strategies.

**List of abbreviations**

Glycated albumin (GA); atherosclerotic cardiovascular disease (ASCVD); cardiovascular diseases (CVDs); coronary artery disease (CAD); hemoglobin A1c (HbA1c); chronic kidney disease (CKD); systolic blood pressure (SBP); waist circumference (WC); total cholesterol (TC); metabolic-associated fatty liver disease (MAFLD); standard error (SE)

**Declarations**

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**Ethics approval and consent to participate**

This retrospective study adhered to the institution and national research committee's ethical standards, and approval was obtained from the institutional ethics committee of the Third Xiangya Hospital of Central South University and review board (Ethics Approval Number: Quick24559).

**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.

**Consent for publication**

Not applicable

**Funding**

**Author contributions**

All authors contributed to the study conception and design. Writing - original draft preparation: XZ, YH, SX, NC, YZ, ML; Writing - review and editing: XZ; Conceptualization: YH, NC; Methodology: SX, YZ; Formal analysis and investigation: XZ, YH; Funding acquisition: ML, XZ; Resources: XZ; the guarantor: ML; Supervision: ML, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial

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or financial relationships that could be construed as a potential conflict of interest.

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**Patient and Public Involvement**

Not applicable

**References**

1.  1. Improving Care and Promoting Health in Populations: Standards of Medical Care in Diabetes-2020. *Diabetes Care*. Jan 2020;43(Suppl 1):S7-s13. doi:10.2337/dc20-S001

2.  Health TWCotARoC, China Di. Interpretation of the Annual Report on Cardiovascular Health and Diseases in China 2020. *Cardiology Discovery*. 2022;2(4):269-285. doi:10.1097/cd9.0000000000000077

3.  Zhao D, Liu J, Wang M, Zhang X, Zhou M. Epidemiology of cardiovascular disease in China: current features and implications. *Nat Rev Cardiol*. Apr 2019;16(4):203-212. doi:10.1038/s41569-018-0119-4

4.  Roth GA, Mensah GA, Johnson CO, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. *J Am Coll Cardiol*. Dec 22 2020;76(25):2982-3021. doi:10.1016/j.jacc.2020.11.010

5.  Schram MT, Schalkwijk CG, Bootsma AH, Fuller JH, Chaturvedi N, Stehouwer CD. Advanced glycation end products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study. *Hypertension*. Jul 2005;46(1):232-7. doi:10.1161/01.HYP.0000164574.60279.ba

6.  KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. *Kidney Int*. Nov 2022;102(5s):S1-s127.

19

doi:10.1016/j.kint.2022.06.008

7.  Selvin E, Rawlings AM, Lutsey PL, et al. Fructosamine and Glycated Albumin and the Risk of Cardiovascular Outcomes and Death. *Circulation*. Jul 28 2015;132(4):269-77. doi:10.1161/circulationaha.115.015415

8.  Takahashi S, Uchino H, Shimizu T, et al. Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J*. Feb 2007;54(1):139-44. doi:10.1507/endocrj.k06-103

9.  Rooney MR, Daya N, Tang O, et al. Glycated Albumin and Risk of Mortality in the US Adult Population. *Clin Chem*. Mar 4 2022;68(3):422-430. doi:10.1093/clinchem/hvab232

10. Kouzuma T, Uemastu Y, Usami T, Imamura S. Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method. *Clin Chim Acta*. Aug 16 2004;346(2):135-43. doi:10.1016/j.cccn.2004.02.019

11. Koga M, Matsumoto S, Saito H, Kasayama S. Body mass index negatively influences glycated albumin, but not glycated hemoglobin, in diabetic patients. *Endocr J*. Jun 2006;53(3):387-91. doi:10.1507/endocrj.k05-137

12. Damen JA, Pajouheshnia R, Heus P, et al. Performance of the Framingham risk models and pooled cohort equations for predicting 10-year risk of cardiovascular disease: a systematic review and meta-analysis. *BMC Med*. Jun 13 2019;17(1):109. doi:10.1186/s12916-019-1340-7

13. Wu Y, Liu X, Li X, et al. Estimation of 10-year risk of fatal and nonfatal ischemic cardiovascular diseases in Chinese adults. *Circulation*. Nov 21 2006;114(21):2217-25. doi:10.1161/circulationaha.105.607499

20

14. Liu J, Hong Y, D'Agostino RB, Sr., et al. Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study. *Jama*. Jun 2 2004;291(21):2591-9. doi:10.1001/jama.291.21.2591

15. Yang X, Li J, Hu D, et al. Predicting the 10-Year Risks of Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China). *Circulation*. Nov 8 2016;134(19):1430-1440. doi:10.1161/circulationaha.116.022367

16. Zhiting G, Jiaying T, Haiying H, Yuping Z, Qunfei Y, Jingfen J. Cardiovascular disease risk prediction models in the Chinese population- a systematic review and meta-analysis. *BMC Public Health*. Aug 24 2022;22(1):1608. doi:10.1186/s12889-022-13995-z

17. Bunn HF, Gabbay KH, Gallop PM. The glycosylation of hemoglobin: relevance to diabetes mellitus. *Science*. Apr 7 1978;200(4337):21-7. doi:10.1126/science.635569

18. Hoshino J, Abe M, Hamano T, et al. Glycated albumin and hemoglobin A1c levels and cause-specific mortality by patients' conditions among hemodialysis patients with diabetes: a 3-year nationwide cohort study. *BMJ Open Diabetes Res Care*. Oct 2020;8(1)doi:10.1136/bmjdrc-2020-001642

19. Ciardullo S, Rea F, Perseghin G. Glycated albumin is associated with all-cause and cardiovascular mortality among U.S. adults with and without diabetes: A retrospective cohort study. *Nutr Metab Cardiovasc Dis*. Oct 2022;32(10):2375-2382. doi:10.1016/j.numecd.2022.07.008

20. Shafi T, Sozio SM, Plantinga LC, et al. Serum fructosamine and glycated albumin and risk of mortality and clinical outcomes in hemodialysis patients. *Diabetes*

21

*Care*. Jun 2013;36(6):1522-33. doi:10.2337/dc12-1896

21. Fukuoka K, Nakao K, Morimoto H, et al. Glycated albumin levels predict long-term survival in diabetic patients undergoing haemodialysis. *Nephrology (Carlton)*. Jun 2008;13(4):278-83. doi:10.1111/j.1440-1797.2007.00864.x

22. Sullivan VK, Wallace AS, Rooney MR, et al. Inverse Associations between Measures of Adiposity and Glycated Albumin in US Adults, NHANES 1999-2004. *J Appl Lab Med*. Jul 5 2023;8(4):751-762. doi:10.1093/jalm/jfad004

23. Sumner AE, Duong MT, Bingham BA, et al. Glycated Albumin Identifies Prediabetes Not Detected by Hemoglobin A1c: The Africans in America Study. *Clin Chem*. Nov 2016;62(11):1524-1532. doi:10.1373/clinchem.2016.261255

24. Miyashita Y, Nishimura R, Morimoto A, Matsudaira T, Sano H, Tajima N. Glycated albumin is low in obese, type 2 diabetic patients. *Diabetes Res Clin Pract*. Oct 2007;78(1):51-5. doi:10.1016/j.diabres.2007.02.021

25. Koga M, Otsuki M, Matsumoto S, Saito H, Mukai M, Kasayama S. Negative association of obesity and its related chronic inflammation with serum glycated albumin but not glycated hemoglobin levels. *Clin Chim Acta*. Mar 2007;378(1-2):48-52. doi:10.1016/j.cca.2006.10.013

26. He X, Mo Y, Ma X, et al. Associations of body mass index with glycated albumin and glycated albumin/glycated hemoglobin A(1c) ratio in Chinese diabetic and non-diabetic populations. *Clin Chim Acta*. Sep 2018;484:117-121. doi:10.1016/j.cca.2018.05.044

27. Reynolds AN, Duncan A, Kruimer D, Venn BJ. Glycated albumin is associated with body mass index in euglycemic adults but is not predictive of postprandial blood glucose response. *J Clin Lab Anal*. Sep 2017;31(5)doi:10.1002/jcla.22085

28. Danese E, Montagnana M, Nouvenne A, Lippi G. Advantages and pitfalls of

22

fructosamine and glycated albumin in the diagnosis and treatment of diabetes. *J Diabetes Sci Technol*. Mar 2015;9(2):169-76. doi:10.1177/1932296814567227

29. Vejbjerg P, Knudsen N, Perrild H, et al. The impact of smoking on thyroid volume and function in relation to a shift towards iodine sufficiency. *Eur J Epidemiol*. 2008;23(6):423-9. doi:10.1007/s10654-008-9255-1

30. Wood AM, Kaptoge S, Butterworth AS, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet*. Apr 14 2018;391(10129):1513-1523. doi:10.1016/s0140-6736(18)30134-x

31. van der Kolk NM, de Vries NM, Kessels RPC, et al. Effectiveness of home-based and remotely supervised aerobic exercise in Parkinson's disease: a double-blind, randomised controlled trial. *Lancet Neurol*. Nov 2019;18(11):998-1008. doi:10.1016/s1474-4422(19)30285-6

32. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol*. Jul 2020;73(1):202-209. doi:10.1016/j.jhep.2020.03.039

33. Shen Y, Pu LJ, Lu L, Zhang Q, Zhang RY, Shen WF. Glycated albumin is superior to hemoglobin A1c for evaluating the presence and severity of coronary artery disease in type 2 diabetic patients. *Cardiology*. 2012;123(2):84-90. doi:10.1159/000342055

34. Pu LJ, Lu L, Shen WF, et al. Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients. *Circ J*. Jul 2007;71(7):1067-73. doi:10.1253/circj.71.1067

35. Zhao H, Hu Q, Chen J, et al. Glycated albumin and risk of cardiovascular diseases and mortality in patients with and without dialysis: A meta-analysis. *Diabetes*

23

*Obes Metab*. Aug 2023;25(8):2203-2217. doi:10.1111/dom.15097

36. Copur S, Siriopol D, Afsar B, et al. Serum glycated albumin predicts all-cause mortality in dialysis patients with diabetes mellitus: meta-analysis and systematic review of a predictive biomarker. *Acta Diabetol*. Jan 2021;58(1):81-91. doi:10.1007/s00592-020-01581-x

37. Zhang J, Du Y, Hu C, et al. Elevated Glycated Albumin in Serum Is Associated with Adverse Cardiac Outcomes in Patients with Acute Coronary Syndrome Who Underwent Revascularization Therapy. *J Atheroscler Thromb*. Apr 1 2022;29(4):482-491. doi:10.5551/jat.61358

38. Doménech M, Roman P, Lapetra J, et al. Mediterranean diet reduces 24-hour ambulatory blood pressure, blood glucose, and lipids: one-year randomized, clinical trial. *Hypertension*. Jul 2014;64(1):69-76. doi:10.1161/hypertensionaha.113.03353

39. Oka S, Deyama J, Umetani K, et al. Glycemic variability is associated with myocardial damage in nondiabetic patients with ST-elevation myocardial infarction. *Cardiovasc Endocrinol Metab*. Jun 2018;7(2):47-53. doi:10.1097/xce.0000000000000145

40. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI, Jr. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J*. Dec 15 2016;473(24):4527-4550. doi:10.1042/bcj20160503c

41. Hashimoto K, Tanikawa K, Nishikawa J, Chen Y, Suzuki T, Koga M. Association of variation range in glycated albumin (GA) with increase but not decrease in plasma glucose: implication for the mechanism by which GA reflects glycemic excursion. *Clin Biochem*. Apr 2015;48(6):397-400. doi:10.1016/j.clinbiochem.2014.12.021

24

42. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *Jama*. Apr 12 2006;295(14):1707-8. doi:10.1001/jama.295.14.1707

43. Ceriello A, Esposito K, Piconi L, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. May 2008;57(5):1349-54. doi:10.2337/db08-0063

44. Selvin E, Warren B, He X, Sacks DB, Saenger AK. Establishment of Community-Based Reference Intervals for Fructosamine, Glycated Albumin, and 1,5-Anhydroglucitol. *Clin Chem*. May 2018;64(5):843-850. doi:10.1373/clinchem.2017.285742

45. Hirata T, Koga M, Kasayama S, Morimoto J, Maruyama T. Glycated albumin is not significantly correlated with body mass index in patients with acute-onset type 1 diabetes. *Clin Chim Acta*. Jan 1 2015;438:248-51. doi:10.1016/j.cca.2014.08.038

46. Furusyo N, Koga T, Ai M, et al. Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS). *Diabetologia*. Dec 2011;54(12):3028-36. doi:10.1007/s00125-011-2310-6

47. D'Agati VD, Chagnac A, de Vries AP, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol*. Aug 2016;12(8):453-71. doi:10.1038/nrneph.2016.75

48. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *Int J Gen Med*. 2016;9:229-55. doi:10.2147/ijgm.S102819

25

49. Ren M, Sun K, Li F, et al. Association between obesity measures and albuminuria: A population-based study. *J Diabetes Complications*. Apr 2016;30(3):451-6. doi:10.1016/j.jdiacomp.2015.12.007

50. Johnson AM, Olefsky JM. The origins and drivers of insulin resistance. *Cell*. Feb 14 2013;152(4):673-84. doi:10.1016/j.cell.2013.01.041

51. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia*. Jul 2010;53(7):1270-87. doi:10.1007/s00125-010-1684-1

52. Pedersen DJ, Guilherme A, Danai LV, et al. A major role of insulin in promoting obesity-associated adipose tissue inflammation. *Mol Metab*. Jul 2015;4(7):507-18. doi:10.1016/j.molmet.2015.04.003

53. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest*. Jan 2016;126(1):12-22. doi:10.1172/jci77812

54. Fryk E, Olausson J, Mossberg K, et al. Hyperinsulinemia and insulin resistance in the obese may develop as part of a homeostatic response to elevated free fatty acids: A mechanistic case-control and a population-based cohort study. *EBioMedicine*. Mar 2021;65:103264. doi:10.1016/j.ebiom.2021.103264

55. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. *Nat Med*. Jul 11 2017;23(7):804-814. doi:10.1038/nm.4350

56. Mukai N, Ninomiya T, Hata J, et al. Association of hemoglobin A1c and glycated albumin with carotid atherosclerosis in community-dwelling Japanese subjects: the Hisayama Study. *Cardiovasc Diabetol*. Jun 24 2015;14:84. doi:10.1186/s12933-015-0247-7

57. Mihara A, Ohara T, Hata J, et al. Association between serum glycated albumin

26

and risk of cardiovascular disease in a Japanese community: The Hisayama Study.

*Atherosclerosis*. Oct 2020;311:52-59. doi:10.1016/j.atherosclerosis.2020.08.016

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**Figure legends**

**Figure 1:** Flowchart for study population selection.

**Figure 2:** Diagnostic standard flowchart of MAFLD.

**Figure 3:** Path diagram of the mediation analysis models

**Figure 4:** Restricted Cubic Spline Curve for association between odds ratio of GA and

10-year ASCVD risk

**Supplementary Materials**

**Table S1** Baseline characteristics of enrolled participants

**Table S2**. Logistic regression models of the link between GA and 10-year ASCVD risk

**Table S3.** Mediation effect of BMI on the association between GA and 10-year

ASCVD risk

**Table S4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk

by age and MAFLD

**Table S5.** Sensitivity Analysis Adjusted for Pharmacotherapy (Model 4)

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Figure 1: Flowchart for study population selection.

112x57mm (300 x 300 DPI)

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Hepatic steatosis (diagnosed through liver ultrasonography)

Overweight / Obese (definded as BMI＞25kg/m² in Caucasian population or BMI＞23kg/m² in Asian population)

Normal weight (definded as BMI≤25kg/m² in Caucasian population or BMI≤23kg/m² in Asian population)

Type 2 Diabetes (According to widely recognized international standards)

At least 2 metabolic risk abnormalities out of 7 exist:
· Waist circumference: ≥102/88 cm in Caucasian men and women, (or ≥90/80 cm in Asian men or women)
· Blood pressure≥130/85 mmHg or specific drug treatment
· Plasma triglycerides≥150 mg/dL (≥1.70 mmol/L) or specific drug treatment
· Plasma HDL-cholesterol: <40 mg/dL (<1.0 mmol/L) for men and <50mg/dL(<1.3 mmol/L) for women or specific drug treatment
· Prediabetes (i.e. fasting glucose levels 100-125 mg/dL (5.6-6.9 mmol/L) or 2-hour post-load glucose levels 140-199 mg/dL (7.8-11.0 mmol/L) or HbA1c of 5.7-6.4% (39-47 mmol/L)
· Homeostasis model assessment of insulin resistance score≥2.5
· Plasma high-sensitivity C-reactive protein level >2 mg/L

**MAFLD**

Diagnostic standard flowchart of MAFLD.
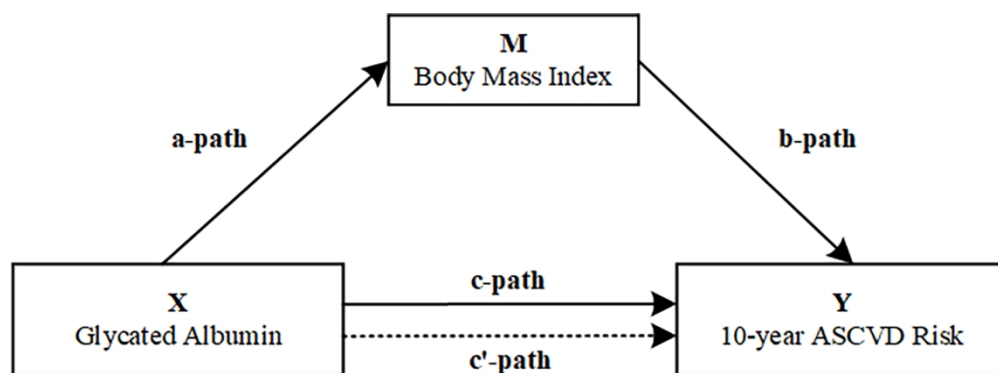
169x102mm (300 x 300 DPI)

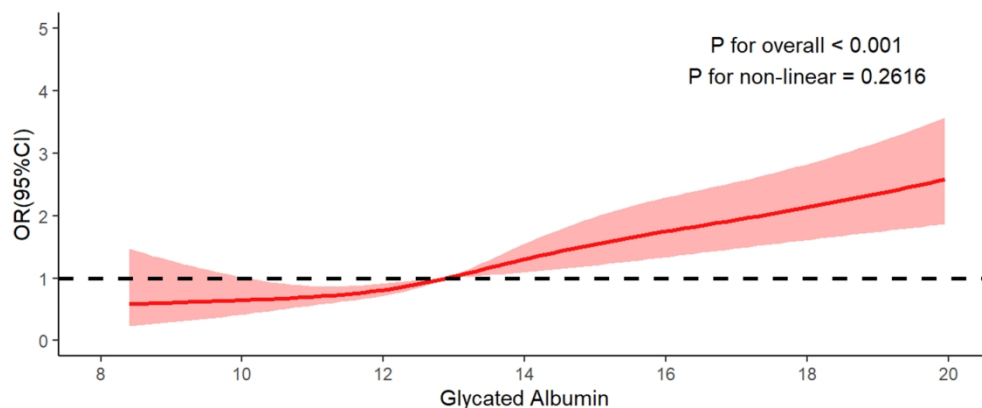Figure 3: Path diagram of the mediation analysis models

169x63mm (300 x 300 DPI)

Figure 4: Restricted Cubic Spline Curve for association between odds ratio of GA and 10-year ASCVD risk

169x75mm (300 x 300 DPI)

**Table S1** Baseline characteristics of enrolled participants

| | Total（n=2107） | China-PAR | | p-Value |
| --- | --- | --- | --- | --- |
| | | Medium-high Risk Group (n=230) | Low Risk Group (n=1877) | |
| **Characteristics [median (25th, 75th)]** | | | | |
| Age (year) | 45.00 [36.00, 52.00] | 59.00 [56.00, 66.00] | 43.00 [35.00, 50.00] | <0.001 |
| BMI*(kg/m$^2$) | 24.20 [21.85, 26.50] | 25.20 [23.63, 27.90] | 24.00 [21.70, 26.20] | <0.001 |
| Waist circumference(cm) | 83.00 [74.00, 91.00] | 89.00 [83.00, 96.00] | 82.00 [73.00, 90.00] | <0.001 |
| Systolic Pressure(mmHg) | 119.00 [109.00, 130.00] | 138.00 [130.00, 147.75] | 118.00 [108.00, 127.00] | <0.001 |
| Diastolic Pressure(mmHg) | 73.00 [65.00, 81.00] | 83.00 [77.00, 89.00] | 71.00 [65.00, 80.00] | <0.001 |
| albumin(g/L) | 45.50 [43.80, 47.60] | 44.60 [42.90, 46.10] | 45.70 [43.90, 47.70] | <0.001 |
| GA*(%) | 12.90 [11.60, 14.20] | 13.60 [12.20, 15.40] | 12.80 [11.50, 14.10] | <0.001 |
| platelet(10$^9$/L) | 227.00 [192.00, 261.00] | 208.00 [176.25, 245.75] | 229.00 [194.00, 262.00] | <0.001 |
| ALT*(U/L) | 20.00 [14.00, 31.50] | 20.00 [15.00, 28.00] | 21.00 [14.00, 32.00] | 0.391 |
| AST*(U/L) | 22.00 [18.00, 27.00] | 23.00 [20.00, 26.00] | 22.00 [18.00, 27.00] | 0.043 |
| Fasting blood glucose(mmol/L) | 5.23 [4.90, 5.67] | 5.74 [5.20, 6.51] | 5.20 [4.87, 5.59] | <0.001 |
| Total cholesterol(mmol/L) | 4.97 [4.42, 5.68] | 5.18 [4.59, 5.92] | 4.95 [4.41, 5.64] | 0.006 |
| triglyceride(mmol/L) | 1.42 [0.92, 2.26] | 1.72 [1.05, 2.66] | 1.38 [0.90, 2.22] | <0.001 |
| HDL-C*(mmol/L) | 1.25 [1.07, 1.47] | 1.21 [1.04, 1.40] | 1.25 [1.07, 1.48] | 0.006 |
| LDL-C*(mmol/L) | 2.90 [2.39, 3.45] | 2.94 [2.41, 3.55] | 2.90 [2.39, 3.44] | 0.408 |
| **Characteristics [n (%)]** | | | | |
| Sex | | | | 0.001 |
| Female | 1131 (53.7) | 148 (64.3) | 983 (52.4) | |
| Male | 976 (46.3) | 82 (35.7) | 894 (47.6) | |
| Cervical vascular plaques | | | | <0.001 |
| No | 1632 (77.5) | 101 (43.9) | 1531 (81.6) | |
| Yes | 475 (22.5) | 129 (56.1) | 346 (18.4) | |
| Smoking status | | | | <0.001 |
| No | 1538 (73.0) | 131 (57.0) | 1407 (75.0) | |
| Yes | 569 (27.0) | 99 (43.0) | 470 (25.0) | |
| Drinking status | | | | 0.449 |
| No | 1344 (63.8) | 141 (61.3) | 1203 (64.1) | |
| Yes | 763 (36.2) | 89 (38.7) | 674 (35.9) | |
| Sport status | | | | 0.012 |
| No | 854 (40.5) | 75 (32.6) | 779 (41.5) | |
| Yes | 1253 (59.5) | 155 (67.4) | 1098 (58.5) | |
| MAFLD* | | | | <0.001 |
| No | 1116 (53.0) | 62 (27.0) | 1054 (56.2) | |
| Yes | 991 (47.0) | 168 (73.0) | 823 (43.8) | |

**Notes**: Values are median (25th, 75th), or n (%). P values were computed utilizing Student's t-test or the Mann-Whitney U test for continuous variables, and the chi-squared test was adopted for categorical variables. *BMI: body mass index; GA: glycated albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MAFLD: metabolic associated fatty liver disease.

**Table S2**. Logistic regression models of the link between GA and 10-year ASCVD risk

| | Model 1 | | | Model 2 | | | Model 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | OR | 95%CI | p | OR | 95%CI | p | OR | 95%CI | p |
| GA | 1.138 | (1.094, 1.185) | < 0.001 | 1.142 | (1.049, 1.240) | 0.002 | 1.160 | (1.055, 1.276) | 0.002 |

**Notes:** Model 1 = GA; Model 2 = Model 1 + Age + Sex + Smoking status + Sport status + BMI + WC + SBP +DBP; Model 3 = Model 2 + Albumin + Platelet + Total Cholesterol + Triglyceride + HDL-C + Cervical vascular plaques + MAFLD.

**Table S3.** Mediation effect of BMI on the association between GA and 10-year ASCVD risk

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| BMI | 2107 | -0.146 (0.026) p<0.001 | 0.190 (0.015) p<0.001 | 0.229 (0.040) p<0.001 | -0.028 (0.006) p<0.001 | 0.201 (0.040) p<0.001 | 13.9% |

**Notes:** Exposure: GA; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

**Table S4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk by age and MAFLD

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| | | | | Stratification by Age | | | |
| <40 | 743 | -0.388 (0.064) p < .001 | 0.056 (0.005) p < .001 | 0.030 (0.022) p =.177 | -0.022(0.007) 95%CI (-0.037, -0.011) p = .002 | 0.008 (0.023) p = .714 | - |
| 40-60 | 1214 | -0.147 (0.030) p < .001 | 0.210 (0.016) p < .001 | 0.102 (0.029) p < .001 | -0.031(0.007) 95%CI (-0.046, -0.020) p < .001 | 0.071 (0.030) p = .017 | **43.7** |
| >60 | 150 | 0.136 (0.075) p =.270 | 0.185 (0.093) p < .05 | 0.415 (0.121) p < .001 | 0.025(0.022) 95%CI (-0.016, 0.069) p = .245 | 0.440 (0.117) p < .001 | - |
| | | | | Stratification by MAFLD | | | |
| MALFD | 991 | -0.040 (0.029) p =.092 | 0.081 (0.029) p < .01 | 0.275 (0.043) p < .001 | -0.003(0.003) 95%CI (-0.010, 0.002) p = .310 | 0.272 (0.043) p < .001 | - |
| Non-MAFLD | 1116 | -0.107*** (0.029) p < .001 | 0.127*** (0.026) p < .001 | 0.178 (0.068) p =.009 | -0.014(0.005) 95%CI (-0.025, -0.006) p = .004 | 0.164 (0.067) p = .014 | **8.5** |

**Notes:** Exposure: GA; Mediator: BMI; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, total cholesterol, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

**Table S5**. Sensitivity Analysis Adjusted for Pharmacotherapy (Model 4)

| | Model 4 | | |
|---|---|---|---|
| | **OR*** | **95%CI*** | **p** |
| GA* | 1.153 | (1.033, 1.277) | 0.008 |

**Notes:** Model 4 adjusted for antihypertensive drugs, lipid-lowering drugs, and glucose-lowering drugs. Analysis was conducted on 2,084 participants after excluding 23 cases with missing medication data. *OR: odds ratio; CI: confidence interval; GA: glycated albumin;

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## Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Cross-sectional Study

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**Mediation Effect of Body Mass Index on the Association Between Glycated Albumin and 10-Year Atherosclerotic Cardiovascular Disease Risk in Hunan Residents of China: A Retrospective Cross-sectional Study**

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## Abstract

**Objectives:** Glycated albumin (GA) and body mass index (BMI) are associated with the risk of atherosclerotic cardiovascular disease (ASCVD). However, the role of BMI in the association between GA and 10-year ASCVD risk is still not fully understood.

**Design:** A Retrospective Cross-sectional Study

**Setting:** In this retrospective cross-sectional study, 4,646 healthy subjects who received a full health examination at the Health Management Medical Center, Third Xiangya Hospital of Central South University from January 1, 2022 to December 30, 2023, were initially identified. According to the exclusion criteria, 2,107 participants were included in the final analysis.

**Participants:** The inclusion criteria for this study included: a) age is greater than or equal to 18 years old; and b) subjects were long-term residents of Hunan province.

**Primary and secondary outcome measures:** The 10-year ASCVD risk was evaluated via the China-PAR equation. The link between GA and 10-year ASCVD risk was examined through a multivariable logistic regression model and the dose-response relationship was demonstrated using the restricted cubic spline. The potential mediation effect of BMI on this association was explored and the differences in this mediation effect across age and metabolic-associated fatty liver disease (MAFLD) subgroups were analyzed.

**Results:** Elevated GA levels were positively linked to an intensified 10-year ASCVD risk (OR=1.160, 95% CI =1.055–1.276). Additionally, BMI was negatively linked to GA and 10-year ASCVD risk. BMI mediated 13.9% of the connection between GA and 10-year ASCVD risk. Specifically, the mediating effect of BMI remained significant in the 40–60 years age subgroup and non-MAFLD subgroup, with mediation ratios of 43.7% and 8.5%, respectively.

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**Conclusions:** GA is a key predictor of 10-year ASCVD risk, and BMI partially mediates this relationship in healthy populations. Therefore, targeted weight management is recommended to reduce the adverse effect of GA on 10-year ASCVD risk in different populations.

**Strengths and limitations of this study**

1. We used a mediation model to identify BMI as a partial mediator in the association between GA and 10-year ASCVD risk.

2. We applied multivariate logistic regression to explore the link between GA and 10-year ASCVD risk, controlling confounding effects stepwise.

3. Restricted Cubic Spline curves were used to illustrate the dose-response association between GA and 10-year ASCVD risk.

4. The study's retrospective cross-sectional design prevents establishing causality between GA and 10-year ASCVD risk and exploring the GA-BMI bidirectional relationship.

**Keywords:** Glycated Albumin, Body Mass Index, Atherosclerotic Cardiovascular Disease Risk, Mediation, Metabolic Associated Fatty Liver Disease

**1. Introduction**

Cardiovascular diseases (CVDs) represent the primary culprits behind deaths and disability worldwide, presenting a substantial risk to human health. This category of diseases encompasses coronary artery disease (CAD), stroke, and myocardial infarction, which commonly arise due to arteriosclerosis, hypertension, hyperlipidemia, and diabetes[1-5]. Currently, glycated hemoglobin A1c (HbA1c) is recognized as a leading

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indicator in the clinical assessment of long-term blood glucose control. The latest KDIGO guidelines have recommended a target value of 6.5% to 8.0% for HbA1c in those with diabetes and chronic kidney disease (CKD) to reduce CVD risk[6]. Nevertheless, HbA1c can be influenced by hemoglobin levels, red blood cell lifespan, anemia, liver dysfunction, and kidney disease[7]. Concurrently, there is increasing interest in an alternative indicator, glycated albumin (GA), a novel index for glucose monitoring, reflecting average blood glucose levels over the past 2 to 3 weeks[8, 9] GA is calculated as the ratio of GA to total albumin concentration[10]. Unlike HbA1c, GA has the advantage of being independent of hemoglobin and red blood cell turnover and less affected by red blood cell lifespan, thus serving as a favorable marker for assessing blood glucose levels[11].

Numerous guidelines have recommended that the prevention and treatment of atherosclerotic CVD (ASCVD) be based on risk assessment. Traditional risk assessment methods, including the Framingham D'Agostino 2008 model, PCE white model, SCORE model, and QRI SK model, are recognized for their reliability in Western populations. However, the Chinese population presents distinct disease patterns and risk factors, making these models less suitable. Particularly, the Framingham D'Agostino model overestimates the CVD risk in men and underestimates it in women, a trend similarly observed in the PCE model[12]. Since 2003, several large-sample prediction models for CVD risk have been established for Chinese adults. Among them, the 10-year risk prediction models for Chinese CVD[13, 14] and the prediction for ASCVD risk in China (China-PAR) model for ASCVD[15] are notable. China-PAR model, a CVD risk assessment tool developed by Chinese scholars based on large-sample cohort data, is most often externally validated and is considered potentially a better option for predicting CVD risk in China[16]. The China-PAR model

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estimates the 10-year ASCVD risk based on validated demographic, clinical, and lifestyle indicators, including age, sex, family history, blood pressure (systolic blood pressure, diastolic blood pressure), lipid profiles (total cholesterol, high-density lipoprotein cholesterol), smoking, diabetes, abdominal obesity (waist circumference), urban/rural residence, and regional cardiovascular epidemiology.

Obese people have a high prevalence of ASCVD, and body mass index (BMI), as an important indicator for assessing obesity in an individual, has a very close link with ASCVD risk. Similarly, the performance of GA in predicting CVD risk has attracted much attention in recent years. GA, as an early glycated protein, may be more sensitive than other markers to CVD and its complications[5, 17]. Several publications have found a link between GA and cardiovascular mortality in diabetes mellitus (DM) patients[9, 18, 19]. Notably, multiple studies confirm that GA is more strongly than HbA1C associated with CVD in CKD patients requiring dialysis[18, 20, 21]. Several papers have noted a weak negative connection between GA and BMI in DM populations but with a narrow range for BMI or GA values[11, 22-27]. In contrast, an investigation of Caucasian populations with a median BMI of 38.4 (large body weight) similarly unveiled a marked negative link between BMI and GA[7]. Therefore, the association between GA and BMI in a wider population remains to be confirmed by further studies. There are few studies on the association among GA, BMI, and 10-year ASCVD risk in normal populations. Hence, this paper was to illustrate whether BMI mediates the link between GA and 10-year ASCVD risk in the normal population.

## 2. Methods

### 2.1 Participant Selection

This cross-sectional study obtained data from 4646 healthy subjects during health

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checkups at the Health Management Medicine Center, Third Xiangya Hospital of Central South University from January 1, 2022, to June 30, 2023. To control for potential biases, we included all health examination data during this period, rather than relying on random sampling.

The inclusion criteria for this study included: a) age is greater than or equal to 18 years old; and b) subjects were long-term residents of Hunan province. The exclusion criteria of this study were a) subjects did not have complete data required for this study; b) subjects did not provide written informed consent; c) Subjects reported established family history and medical history of CVD and cerebrovascular diseases (including coronary heart disease, stroke, congestive heart failure, myocardial infarction, and angina) in questionnaires; and d) Subjects was discovered severe liver or kidney dysfunction (which may affect GA results) in health checkups. According to the exclusion criteria, 84 participants under the age of 18 were excluded, 1237 participants lacking GA, BMI, and China-PAR equation-related data were excluded, 276 participants with self-reported CVD and cerebrovascular diseases on the questionnaire were excluded, 28 participants with severe liver or kidney dysfunction (defined as a history of severe liver impairment, liver cirrhosis, CKD stage 3 or higher, or end-stage renal disease, which may affect GA results) were excluded, and 914 participants with missing information on cardiovascular risk factors were excluded. Ultimately, 2107 participants were enrolled. The specific selection process is displayed in Figure 1. This retrospective study adhered to the institution and national research committee's ethical standards, and approval was obtained from the institutional ethics committee of the Third Xiangya Hospital of Central South University and the review board (Ethics Approval Number: Quick24559).

**2.2 Measurements**

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### 2.2.1 GA measurement

Compared to HbA1c testing, the detection methods for GA are not yet fully standardized. In this study, enzyme-linked immunosorbent assay (ELISA), a widely adopted and reliable technique[28] was employed to detect GA.

### 2.2.2 Cardiovascular Risk Estimation Based on China-PAR Project

The China-PAR score was employed to appraise ASCVD risk. The China-PAR model encompassed sex, age, systolic blood pressure (SBP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), waist circumference (WC), smoking status, diabetes, region, urbanization, and family history of ASCVD. The participants were allocated into three risk subgroups based on the scores: <5% as low risk, 5–10% as medium risk, and ≥10% as high risk[15].

Health status and lifestyle information of the participants were collected through questionnaires during their health checkups. This questionnaire, designed based on the National Physical Examination Questionnaire[29], included details on the participants' and family history of CVDs and cerebrovascular diseases, and lifestyle information such as history of hypertension, CVDs, DM, smoking, alcohol consumption, and physical activity. Hypertension was diagnosed as SBP ≥140 mmHg and/or diastolic blood pressure (DBP)≥90 mmHg or receiving antihypertensive treatment. Diabetes was diagnosed as fasting plasma glucose (FPG) ≥7.0 mmol/L, HbA1c ≥6.5%, or receiving hypoglycemic treatment. A family history of ASCVD was described as congestive heart failure, stroke, coronary heart disease, myocardial infarction, or angina in first-degree relatives. Based on the Chinese guideline on the primary prevention of cardiovascular diseases (2020) [30], which prioritizes actionable thresholds for public health interventions, participants were allocated into a medium-high risk group and low-risk groups in this study.

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### 2.2.3 Covariate Assessment

Demographic data were obtained from physical examination reports. Age as a continuous variable was categorized into subgroups: 18-39 years, 40-60 years, or >60 years. During the physical examination, weight, height, WC, SBP, and DBP were gauged. BMI was presented as weight (kg) divided by height square (m²). Laboratory tests covered routine blood tests, liver function tests, GA, FBG, HbA1c, fasting insulin, and blood lipids. It should be noted that GA rather than HbA1C was the primary focus of this study, and most research subjects did not have both measurements above, HbA1C was not set as a mandatory enrollment criterion. All participants underwent liver and cervical vascular ultrasonography performed by an ultrasonologist after an overnight fast. These reports were reviewed and confirmed by another senior ultrasonologist. The diagnoses of fatty liver (hepatic steatosis) and cervical vascular plaques were based on liver and cervical vascular ultrasound scans using linear array or convex high-frequency probes with frequencies of 5-10 MHz. These scans evaluated the echo, size, and morphology of the liver, as well as intima-media thickness, plaque size, morphology, and location of cervical vessels. Furthermore, medication history, smoking history, alcohol consumption, and exercise frequency were recorded directly from the physical examination questionnaire. Medication history in this study was defined as medication used for diabetes mellitus, hypertension, and dyslipidemia. Smoking history was defined as smoking continuously or cumulatively for more than six months[29]. Alcohol consumption manifested as consuming over 10 grams of alcohol per day[31]. Physical activity represented moderate- or high-intensity physical activity at least three times per week, with each session lasting at least 30 minutes[32].

### 2.2.4 Diagnosis of MAFLD

According to Eslam et al.[33], metabolic-associated fatty liver disease (MAFLD)

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means steatosis in combination with metabolic dysfunction in the International Expert

Consensus Statement on MAFLD. The diagnostic criteria included hepatic steatosis

through histology (liver biopsy), imaging techniques, or blood-based biomarkers and at

least one of the following conditions: overweight/obesity, T2DM, and metabolic

dysfunction. Metabolic dysfunction refers to at least two of the seven metabolic risk

abnormalities (Figure 2). In this study, all subjects were from a health screening cohort.

Thus, balancing feasibility, cost-effectiveness, and diagnostic accuracy in the screening

population, we uniformly used liver ultrasonography after an overnight fast to assess

hepatic steatosis in all subjects, instead of other imaging techniques (e.g., vibration-

controlled transient elastography or MRI elastography), blood-based biomarkers (e.g.,

FIB-4, APRI), or liver biopsy.

### 2.3 Data Analysis

Continuous variables were denoted as median and interquartile spacing and compared

utilizing the independent samples t-test. Categorical variables were depicted as

unweighted frequencies, weighted percentages, and standard error (SE), and compared

utilizing the chi-square test. Multivariate logistic regression analysis was performed to

assess the association between GA (a continuous exposure variable) and 10-year

ASCVD risk (a binary outcome variable). Variables showing between-group

differences in the baseline information table ($P < 0.05$) were identified and included as

potential confounders, serving as covariates in subsequent analyses. Given the strong

association of fasting glucose and HbA1c with the 10-year ASCVD risk, they were

excluded from the logistic regression model. Three models were constructed to

progressively control for the effects of confounders: Unadjusted crude Model 1; Model

2 adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, and DBP; and

Model 3 adjusted for all covariates, such as albumin, platelet, TC, triglyceride, HDL-

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C, cervical vascular plaques, and MAFLD. Subsequently, restricted cubic spline (RCS) analysis was adopted to demonstrate the dose-response relationship between GA and 10-year ASCVD risk. Finally, a logistic regression model was employed to illustrate the relationships among GA, BMI, and 10-year ASCVD risk. To analyze the mediation effect of BMI [(mediation effect/total effect) × 100%], a simple mediation model was used, with three paths (Figure 3). The total effect represented the impact of GA (exposure) on 10-year ASCVD risk (outcome). Path A assessed the effect of GA on BMI (mediator). Path B evaluated the link between BMI and 10-year ASCVD risk. Path C estimated the direct impact of GA on 10-year ASCVD risk. The mediated effect was calculated as (mediated effect/total effect) × 100%.

All statistical analyses, including mediation analysis, were performed in the R statistical package. Statistical significance was delineated at a two-sided p-value of < 0.05.

## 3. Results

### 3.1. Characteristics of Participants

Table S1 displays the baseline characteristics of study participants. Based on the three risk categories mentioned above, participants were assigned to a medium-high-risk group (n=230) and a low-risk group (n=1877). The differences were statistically notable (p < 0.001) in age, BMI, WC, SBP, DBP, albumin, GA, platelet, FBG, TC, cervical vascular plaques, smoking status, and MAFLD.

Table S2 exhibits the multivariate logistic analysis results. In Model 1, a higher GA was greatly associated with higher odds of 10-year ASCVD risk (OR = 1.138, 95% CI = 1.094, 1.185). After adjusting for covariates (age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD), GA was positively associated with 10-year ASCVD risk

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(OR =1.160, 95% CI =1.055, 1.276) in Model 3.

The RCS curve showed that increased GA levels were significantly linked to an increased 10-year ASCVD risk (P for overall **<** 0.001), with a linear relationship (P for non-linear =0.2616) (Figure 4). The red solid line indicates the point estimate of OR, and the pink shaded area represents the 95% CI. The curve indicates that when GA levels are below 12.95%, the OR remains around 1, suggesting that GA does not significantly affect the risk within this range. However, when GA levels > 12.95%, the OR starts to increase significantly, indicating a marked association between higher GA levels and greater 10-year ASCVD risk.

## 3.2 Mediation Analysis of BMI

We then delved into the mediation effect of BMI on the link between GA and 10-year ASCVD risk. All mediation analyses were carried out after adjustment for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

GA was negatively associated with BMI (p < 0.001) and positively associated with 10-year ASCVD risk (p < 0.001). BMI was positively related to 10-year ASCVD risk (p < 0.001) (Table S3). It is estimated that BMI mediated 13.9% of the total link between GA and 10-year ASCVD risk.

Given the effect of age and MAFLD, subgroup analyses were implemented (Table S4). In the age group, BMI partially mediated the link between GA and 10-year ASCVD risk. In the age subgroup 40–60 years, there was a 43.7% mediating effect of BMI on the link between GA and 10-year ASCVD risk. However, in the age subgroup < 40 years and > 60 years, no mediating effect of BMI was observed on the association between GA and 10-year ASCVD risk. Similarly, BMI mediated 8.5% of the total link

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between GA and 10-year ASCVD risk in the non-MAFLD group. However, the mediating effect was not observed in the MAFLD group.

**3.3 Sensitivity Analysis**

In sensitivity analyses, we adjusted for three medication status variables (antidiabetic, antihypertensive, and lipid-lowering drugs) as potential confounders. After excluding 23 cases with missing data, the analysis of 2,084 eligible participants demonstrated that the positive association between GA and 10-year cardiovascular risk remained highly consistent (OR = 1.153, 95% CI = 1.033–1.277; Table S5). Detailed results of the adjusted model are provided (Table S5). This finding further supported the robustness of our primary analysis, indicating that GA maintained its independent predictive value for 10-year cardiovascular risk even after controlling for the influence of pharmacological interventions.

**4. Discussion**

This study elucidated the association among GA, BMI, and 10-year ASCVD risk in the normal population undergoing physical examination and estimated the mediating role of BMI. This is the first study on their associations, and our results confirm: 1. BMI mediates 13.9% of the association between GA and 10-year ASCVD risk. 2. The mediating role of BMI varies by age and MAFLD subgroups. BMI plays a partially mediating role of 8.5% in the 40-60 age group and no mediating role in the 30-44 and over 60 age groups. Similarly, BMI has an 8.5% partial mediating effect on the non-MAFLD group, but no mediating effect was revealed in the MAFLD group.

The present study examined a real-world normal population undergoing medical examinations and showed a positive association between GA and 10-year CVD risk.

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The association remained after adjustment for established cardiovascular risk factors and socio-behavioral factors. Although GA is widely recognized to reflect blood glucose levels, relevant studies have focused on its application in diverse situations (e.g., anemia, abnormal liver function, and renal disease) where HbA1c accuracy may be impaired. However, there is controversy about the ability of GA to predict long-term ASCVD outcomes. Previous studies have manifested that GA upregulation is positively linked to CAD and its severity in DM patients, whereas HbA1c levels are not as strongly correlated with CAD[34, 35]. Similarly, several publications have highlighted that after adjustment for HbA1c levels, the higher GA in dialysis-requiring patients with CKD and DM, the higher risk of cardiovascular mortality, all-cause mortality, CAD, major adverse cardiovascular events, and stroke; no association was found in non-DM individuals[9, 18, 19]. Zhao et al. found in the meta-analysis that GA had a stronger association with CVD outcomes (including cardiovascular mortality in non-dialysis patients and all-cause mortality in dialysis patients) than HbA1c, and its link with MACCE was independent of traditional risk factors and HbA1c levels[36]. However, Copur et al. revealed a prominent association of GA levels with all-cause mortality in DM patients undergoing dialysis, but not with CVD mortality[37]. Additionally, high GA levels were independently linked to unfavorable intermediate-term efficacy in low-risk populations undergoing percutaneous coronary intervention, but the prognostic role was only present in the DM subgroup, and in the non-DM individuals, this association was not supported by clear evidence[38]. It is well known that atherosclerosis is the most important cause of CVDs[39]. Significant glycemic excursions or fluctuations potentiate oxidative stress and drive atherosclerosis, which can lead to ASCVD[40, 41]. We hypothesized that relative to HbA1c, GA may better indicate glycemic fluctuations (eg, acute hyperglycemia or significant postprandial glycemic excursion) before the

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onset of acute coronary syndrome (within a short period[40, 42], thereby promoting oxidative stress and accelerating atherosclerosis in both DM and non-DM subjects[43, 44]. In non-DM subjects, short-term glycemic fluctuations, especially significant postprandial glycemic excursion, may be manifested as small but notable differences in GA. The effect of HbA1c on non-DM participants may be confounded by non-glycemic factors, like abnormalities in liver and kidney function and differences in red blood cell lifespan. In summary, studies comparing GA and HbA1c for long-term ASCVD prediction had mainly focused on diabetic patients and those with renal dysfunction, and there had been a lack of research on the longitudinal comparison of GA and HbA1c changes in the normal population. Therefore, further investigations are warranted to elucidate the complex pathophysiologic mechanisms that link GA to ASCVD risk.

Our experimental results showed that GA was negatively associated with BMI. Research in normoglycemic populations, pre-DM patients, and T2DM patients has reported a link between BMI and HbA1c or GA/HbA1c[27, 45]. However, studies on the link between GA and BMI are very limited in normal populations in China. As mentioned earlier, several papers have demonstrated a weak negative connection between GA and BMI among DM populations, but mainly in normal-weight subjects with little fluctuation in BM[11, 22-27], or Caucasian populations with large body weights (mean BMI of 38.4). In contrast, the two papers that did not discover a negative association between GA and BMI were conducted in the Japanese population with type 1 DM with a mean BMI of 20.1[46] or without DM with a nearly constant GA[47]. Thereby, the negative link between BMI and GA may not be exclusively attributable to glycemic exposure, but there are other factors. An increase in BMI increases renal blood flow, glomerular filtration rate, and tubular reabsorption, resulting in glomerular

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enlargement and obesity-associated glomerular disease[48, 49]. There is a difference in the rate of GA synthesis in participants with higher BMI and normal BMI[22]. BMI may affect GA clearance. A mild increment in BMI is related to an increased prevalence of microalbuminuria, which is a marker of early renal damage and is also closely related to cardiovascular prognosis[50]. Elevated GA levels may reflect the presence of microalbuminuria and cardiovascular events, but the exact mechanism is currently unknown. Therefore, future studies should refine the proteinuria and renal clearance of GA, which may influence the relationship between GA and BMI. Additional research utilizing labeled albumin in animal models of obesity and DM may offer valuable insight into this process.

Interestingly, the mediation analysis unraveled that BMI mediated 13.9% of the link between GA and 10-year ASCVD risk. This result emphasizes the importance of reducing BMI in lowering 10-year ASCVD risk, especially in high-GA populations, regardless of DM. Overweight and obesity are drivers of insulin resistance[51, 52]. Insulin resistance is the core connecting several events of cardiometabolic disorders[51-56], molecularly due to impaired insulin signaling transduction via the PI3K pathway and intact signaling transduction via the MAPK pathway, leading to altered glucose-insulin homeostasis and increased glucose fluctuations. Significant shifts in blood glucose values facilitate oxidative stress and atherosclerosis[40, 41]. Our study results supported this hypothesis. Although antihypertensive, lipid-lowering, and antidiabetic drugs might influence ASCVD risk through different pathways, sensitivity analysis showed that GA's independent predictive value remained unweakened after adjusting for these clinical interventions. Similarly, elevated serum GA levels are notably related to augmented carotid intima-media thickness and vascular endothelial dysfunction, a sign of early atherosclerosis[57]. Vascular smooth muscle cell proliferation, vascular

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endothelial dysfunction, and overproduction of collagen and inflammatory cytokines contribute to atherosclerosis and adverse cardiovascular events. Patients in the high GA group were more susceptible to metabolic disorders and severe CAD than those in the low and mediate GA groups and therefore had an intensified risk of CAD. When GA levels are substantially elevated (in the higher range, e.g., more than 17.1%), the CAD risk augments by approximately two folds[38]. Akane Mihara et al. disclosed findings from a 10-year follow-up of the Hisayama study, suggesting that the highest quartile of GA levels (≥15.7%) was connected with a 2.2-fold increase in CAD incidence and a 2.5-fold increase in stroke risk[58]. The exact mechanism of the mediating effect of BMI is currently unclear and may be different in different populations. Additional research is warranted to illustrate the mechanisms.

Interestingly, we found age and MAFLD were involved in the mediating role of BMI in the association between GA and 10-year ASCVD risk. Age-stratified subgroup analyses found that BMI did not mediate the association in the <40 and >60 groups. This finding suggests that BMI reduction in the <40 and >60 groups with increased GA may not be the cause for reducing 10-year CVD risk compared with the partial mediation effect in the 40-60 group. We suggest that in the 40-60 group, BMI reduction will be the focus of reducing the 10-year CVD risk induced by increased GA. In addition, subgroup analyses by MAFLD discovered that the link between GA and 10-year CVD risk was partially mediated by BMI in the non-MAFLD group, whereas BMI had no mediating effect in the MAFLD group. This finding suggests that BMI reduction in the MAFLD group with increased GA may not be the cause for reducing 10-year CVD risk. In the non-MAFLD group, BMI reduction will be the focus of preventing 10-year CVD risk. Since GA is an indicator of short-term glucose fluctuations and insulin resistance is the main mechanism of MAFLD, it is primarily a long-term effect.

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Therefore, long-term monitoring of GA is of limited benefit in assessing 10-year CVD risk in MAFLD populations. This may provide new insights into assessing and reducing 10-year CVD risk in these two groups.

Our study first investigates the mediating role of BMI in the link between GA and 10-year ASCVD risk. Nevertheless, we must acknowledge certain inherent limitations of our study. Firstly, multiple multivariate models were adopted to unveil the independent link between GA and 10-year ASCVD risk. Essential confounding factors like alcohol consumption and smoking were obtained through self-reported data, which are potentially influenced by recall bias and misinterpretations and potentially affect the accuracy of our findings. Secondly, it is crucial to analyze the association between GA and proteinuria. However, since this study involved non-hospitalized populations and the incidence of proteinuria among participants was exceedingly low, statistical analysis was not possible. This limitation underscores further investigations into the renal clearance of proteinuria and GA, which is pivotal in elucidating the link between BMI and GA. Lastly, given the cross-sectional nature, it is not feasible to establish the exact causality as well as the bidirectional association between GA and 10-year ASCVD risk. Therefore, prospective cohort studies are imperative to further unveil this relationship. Despite these limitations, our study, based on a real-world context and substantial sample size, has fully adjusted for confounders, so the conclusions drawn are relatively reliable.

In conclusion, our cross-sectional study of 2,107 participants demonstrates for the first time that BMI effectively mediates the impact of GA on the 10-year ASCVD risk. Consequently, healthy populations, especially those aged 40-60 without MAFLD, should realize the beneficial effects of GA in reducing their 10-year ASCVD risk through lowering BMI, rather than just preventing diabetes. Prospective studies are

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crucial to validate these findings and elucidate intrinsic mechanisms, thus paving the way for targeted therapeutic strategies.

## List of abbreviations

Glycated albumin (GA); atherosclerotic cardiovascular disease (ASCVD); cardiovascular diseases (CVDs); coronary artery disease (CAD); hemoglobin A1c (HbA1c); chronic kidney disease (CKD); systolic blood pressure (SBP); waist circumference (WC); total cholesterol (TC); metabolic-associated fatty liver disease (MAFLD); standard error (SE)

## Declarations

### Ethics approval and consent to participate

This retrospective study adhered to the institution and national research committee's ethical standards, and approval was obtained from the institutional ethics committee of the Third Xiangya Hospital of Central South University and review board (Ethics Approval Number: Quick24559).

### 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.

### Consent for publication

Not applicable

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**Author contributions**

All authors contributed to the study conception and design. Writing - original draft

preparation: XZ, YH, SX, NC, YZ, ML; Writing - review and editing: XZ;

Conceptualization: YH, NC; Methodology: SX, YZ; Formal analysis and investigation:

XZ, YH; Funding acquisition: ML, XZ; Resources: XZ; the guarantor: ML;

Supervision: ML, and all authors commented on previous versions of the manuscript.

All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial

or financial relationships that could be construed as a potential conflict of interest.

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**Patient and Public Involvement**

Not applicable

**References**

1.  *1. Improving Care and Promoting Health in Populations: Standards of Medical Care in Diabetes-2020.* Diabetes Care, 2020. **43**(Suppl 1): p. S7-s13.

2.  Health, T.W.C.o.t.A.R.o.C. and D.i. China, *Interpretation of the Annual Report on Cardiovascular Health and Diseases in China 2020.* Cardiology Discovery, 2022. **2**(4): p. 269-285.

3.  Zhao, D., et al., *Epidemiology of cardiovascular disease in China: current features and implications.* Nat Rev Cardiol, 2019. **16**(4): p. 203-212.

19

4.  Roth, G.A., et al., *Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study.* J Am Coll Cardiol, 2020. **76**(25): p. 2982-3021.

5.  Schram, M.T., et al., *Advanced glycation end products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study.* Hypertension, 2005. **46**(1): p. 232-7.

6.  *KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease.* Kidney Int, 2022. **102**(5s): p. S1-s127.

7.  Selvin, E., et al., *Fructosamine and Glycated Albumin and the Risk of Cardiovascular Outcomes and Death.* Circulation, 2015. **132**(4): p. 269-77.

8.  Takahashi, S., et al., *Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control.* Endocr J, 2007. **54**(1): p. 139-44.

9.  Rooney, M.R., et al., *Glycated Albumin and Risk of Mortality in the US Adult Population.* Clin Chem, 2022. **68**(3): p. 422-430.

10. Kouzuma, T., et al., *Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method.* Clin Chim Acta, 2004. **346**(2): p. 135-43.

11. Koga, M., et al., *Body mass index negatively influences glycated albumin, but not glycated hemoglobin, in diabetic patients.* Endocr J, 2006. **53**(3): p. 387-91.

12. Damen, J.A., et al., *Performance of the Framingham risk models and pooled cohort equations for predicting 10-year risk of cardiovascular disease: a systematic review and meta-analysis.* BMC Med, 2019. **17**(1): p. 109.

13. Wu, Y., et al., *Estimation of 10-year risk of fatal and nonfatal ischemic cardiovascular diseases in Chinese adults.* Circulation, 2006. **114**(21): p. 2217-

20

25.

14. Liu, J., et al., *Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study.* Jama, 2004. **291**(21): p. 2591-9.

15. Yang, X., et al., *Predicting the 10-Year Risks of Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China).* Circulation, 2016. **134**(19): p. 1430-1440.

16. Zhiting, G., et al., *Cardiovascular disease risk prediction models in the Chinese population- a systematic review and meta-analysis.* BMC Public Health, 2022. **22**(1): p. 1608.

17. Bunn, H.F., K.H. Gabbay, and P.M. Gallop, *The glycosylation of hemoglobin: relevance to diabetes mellitus.* Science, 1978. **200**(4337): p. 21-7.

18. Hoshino, J., et al., *Glycated albumin and hemoglobin A1c levels and cause-specific mortality by patients' conditions among hemodialysis patients with diabetes: a 3-year nationwide cohort study.* BMJ Open Diabetes Res Care, 2020. **8**(1).

19. Ciardullo, S., F. Rea, and G. Perseghin, *Glycated albumin is associated with all-cause and cardiovascular mortality among U.S. adults with and without diabetes: A retrospective cohort study.* Nutr Metab Cardiovasc Dis, 2022. **32**(10): p. 2375-2382.

20. Shafi, T., et al., *Serum fructosamine and glycated albumin and risk of mortality and clinical outcomes in hemodialysis patients.* Diabetes Care, 2013. **36**(6): p. 1522-33.

21. Fukuoka, K., et al., *Glycated albumin levels predict long-term survival in diabetic patients undergoing haemodialysis.* Nephrology (Carlton), 2008. **13**(4): p. 278-83.

21

22. Sullivan, V.K., et al., *Inverse Associations between Measures of Adiposity and Glycated Albumin in US Adults, NHANES 1999-2004.* J Appl Lab Med, 2023. **8**(4): p. 751-762.

23. Sumner, A.E., et al., *Glycated Albumin Identifies Prediabetes Not Detected by Hemoglobin A1c: The Africans in America Study.* Clin Chem, 2016. **62**(11): p. 1524-1532.

24. Miyashita, Y., et al., *Glycated albumin is low in obese, type 2 diabetic patients.* Diabetes Res Clin Pract, 2007. **78**(1): p. 51-5.

25. Koga, M., et al., *Negative association of obesity and its related chronic inflammation with serum glycated albumin but not glycated hemoglobin levels.* Clin Chim Acta, 2007. **378**(1-2): p. 48-52.

26. He, X., et al., *Associations of body mass index with glycated albumin and glycated albumin/glycated hemoglobin A(1c) ratio in Chinese diabetic and non-diabetic populations.* Clin Chim Acta, 2018. **484**: p. 117-121.

27. Reynolds, A.N., et al., *Glycated albumin is associated with body mass index in euglycemic adults but is not predictive of postprandial blood glucose response.* J Clin Lab Anal, 2017. **31**(5).

28. Danese, E., et al., *Advantages and pitfalls of fructosamine and glycated albumin in the diagnosis and treatment of diabetes.* J Diabetes Sci Technol, 2015. **9**(2): p. 169-76.

29. Vejbjerg, P., et al., *The impact of smoking on thyroid volume and function in relation to a shift towards iodine sufficiency.* Eur J Epidemiol, 2008. **23**(6): p. 423-9.

30. *[Chinese guideline on the primary prevention of cardiovascular diseases].* Zhonghua Xin Xue Guan Bing Za Zhi, 2020. **48**(12): p. 1000-1038.

22

31. Wood, A.M., et al., *Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies.* Lancet, 2018. **391**(10129): p. 1513-1523.

32. van der Kolk, N.M., et al., *Effectiveness of home-based and remotely supervised aerobic exercise in Parkinson's disease: a double-blind, randomised controlled trial.* Lancet Neurol, 2019. **18**(11): p. 998-1008.

33. Eslam, M., et al., *A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement.* J Hepatol, 2020. **73**(1): p. 202-209.

34. Shen, Y., et al., *Glycated albumin is superior to hemoglobin A1c for evaluating the presence and severity of coronary artery disease in type 2 diabetic patients.* Cardiology, 2012. **123**(2): p. 84-90.

35. Pu, L.J., et al., *Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients.* Circ J, 2007. **71**(7): p. 1067-73.

36. Zhao, H., et al., *Glycated albumin and risk of cardiovascular diseases and mortality in patients with and without dialysis: A meta-analysis.* Diabetes Obes Metab, 2023. **25**(8): p. 2203-2217.

37. Copur, S., et al., *Serum glycated albumin predicts all-cause mortality in dialysis patients with diabetes mellitus: meta-analysis and systematic review of a predictive biomarker.* Acta Diabetol, 2021. **58**(1): p. 81-91.

38. Zhang, J., et al., *Elevated Glycated Albumin in Serum Is Associated with Adverse Cardiac Outcomes in Patients with Acute Coronary Syndrome Who Underwent Revascularization Therapy.* J Atheroscler Thromb, 2022. **29**(4): p. 482-491.

39. Doménech, M., et al., *Mediterranean diet reduces 24-hour ambulatory blood*

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*pressure, blood glucose, and lipids: one-year randomized, clinical trial.* Hypertension, 2014. **64**(1): p. 69-76.

40. Oka, S., et al., *Glycemic variability is associated with myocardial damage in nondiabetic patients with ST-elevation myocardial infarction.* Cardiovasc Endocrinol Metab, 2018. **7**(2): p. 47-53.

41. Newsholme, P., et al., *Molecular mechanisms of ROS production and oxidative stress in diabetes.* Biochem J, 2016. **473**(24): p. 4527-4550.

42. Hashimoto, K., et al., *Association of variation range in glycated albumin (GA) with increase but not decrease in plasma glucose: implication for the mechanism by which GA reflects glycemic excursion.* Clin Biochem, 2015. **48**(6): p. 397-400.

43. Brownlee, M. and I.B. Hirsch, *Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications.* Jama, 2006. **295**(14): p. 1707-8.

44. Ceriello, A., et al., *Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients.* Diabetes, 2008. **57**(5): p. 1349-54.

45. Selvin, E., et al., *Establishment of Community-Based Reference Intervals for Fructosamine, Glycated Albumin, and 1,5-Anhydroglucitol.* Clin Chem, 2018. **64**(5): p. 843-850.

46. Hirata, T., et al., *Glycated albumin is not significantly correlated with body mass index in patients with acute-onset type 1 diabetes.* Clin Chim Acta, 2015. **438**: p. 248-51.

47. Furusyo, N., et al., *Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS).* Diabetologia, 2011. **54**(12): p. 3028-36.

24

48. D'Agati, V.D., et al., *Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis.* Nat Rev Nephrol, 2016. **12**(8): p. 453-71.

49. Levitt, D.G. and M.D. Levitt, *Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements.* Int J Gen Med, 2016. **9**: p. 229-55.

50. Ren, M., et al., *Association between obesity measures and albuminuria: A population-based study.* J Diabetes Complications, 2016. **30**(3): p. 451-6.

51. Johnson, A.M. and J.M. Olefsky, *The origins and drivers of insulin resistance.* Cell, 2013. **152**(4): p. 673-84.

52. DeFronzo, R.A., *Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009.* Diabetologia, 2010. **53**(7): p. 1270-87.

53. Pedersen, D.J., et al., *A major role of insulin in promoting obesity-associated adipose tissue inflammation.* Mol Metab, 2015. **4**(7): p. 507-18.

54. Samuel, V.T. and G.I. Shulman, *The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux.* J Clin Invest, 2016. **126**(1): p. 12-22.

55. Fryk, E., et al., *Hyperinsulinemia and insulin resistance in the obese may develop as part of a homeostatic response to elevated free fatty acids: A mechanistic case-control and a population-based cohort study.* EBioMedicine, 2021. **65**: p. 103264.

56. Czech, M.P., *Insulin action and resistance in obesity and type 2 diabetes.* Nat Med, 2017. **23**(7): p. 804-814.

57. Mukai, N., et al., *Association of hemoglobin A1c and glycated albumin with carotid atherosclerosis in community-dwelling Japanese subjects: the Hisayama Study.* Cardiovasc Diabetol, 2015. **14**: p. 84.

25

58. Mihara, A., et al., *Association between serum glycated albumin and risk of cardiovascular disease in a Japanese community: The Hisayama Study.* Atherosclerosis, 2020. **311**: p. 52-59.

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**Figure legends**

**Figure 1:** Flowchart for study population selection.

**Figure 2:** Diagnostic standard flowchart of MAFLD.

**Figure 3:** Path diagram of the mediation analysis models

**Figure 4:** Restricted Cubic Spline Curve for association between odds ratio of GA and 10-year ASCVD risk

**Supplementary Materials**

**Table S1** Baseline characteristics of enrolled participants

**Table S2**. Logistic regression models of the link between GA and 10-year ASCVD risk

**Table S3.** Mediation effect of BMI on the association between GA and 10-year ASCVD risk

**Table S4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk by age and MAFLD

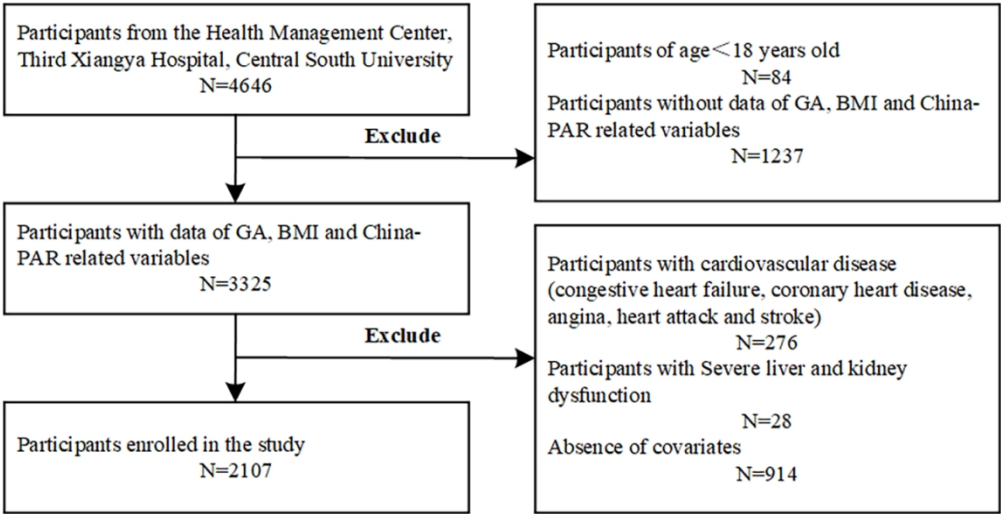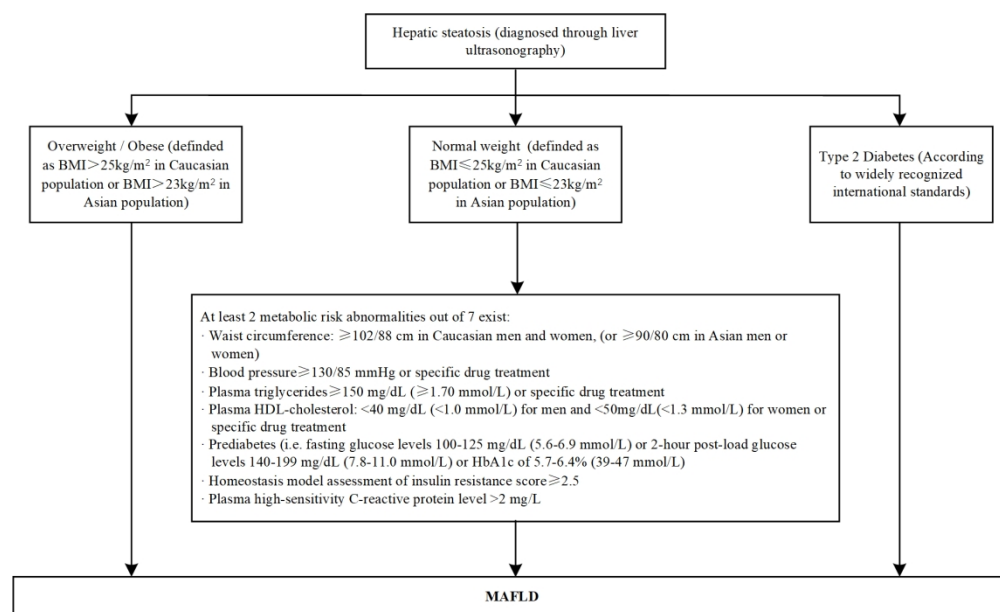**Table S5.** Sensitivity Analysis Adjusted for Pharmacotherapy (Model 4)

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Figure 1: Flowchart for study population selection.

112x57mm (300 x 300 DPI)

Diagnostic standard flowchart of MAFLD.
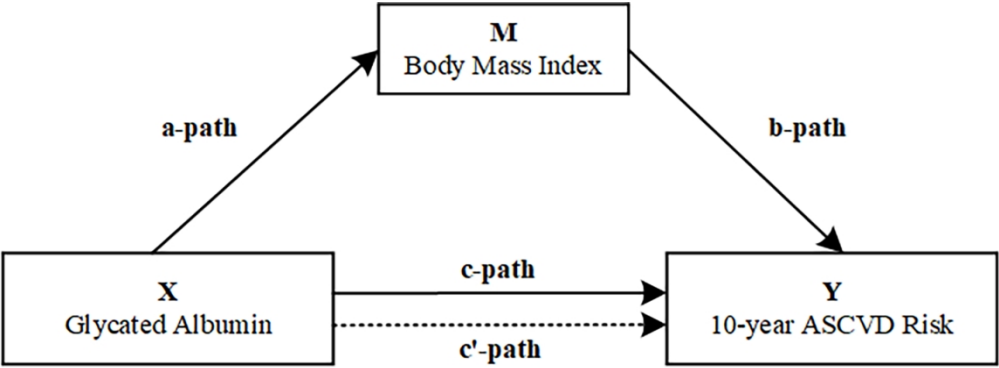
169x102mm (300 x 300 DPI)

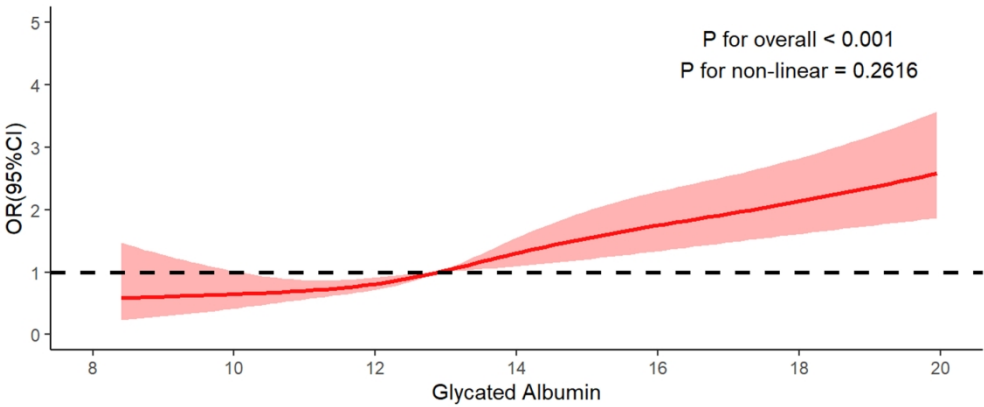Figure 3: Path diagram of the mediation analysis models

169x63mm (300 x 300 DPI)

Figure 4: Restricted Cubic Spline Curve for association between odds ratio of GA and 10-year ASCVD risk

169x75mm (300 x 300 DPI)

**Table S1** Baseline characteristics of enrolled participants

| Characteristics [median (25th, 75th)] | Total (n=2107) | China-PAR Medium-high Risk Group (n=230) | China-PAR Low Risk Group (n=1877) | p-Value |
|---|---|---|---|---|
| Age (year) | 45.00 [36.00, 52.00] | 59.00 [56.00, 66.00] | 43.00 [35.00, 50.00] | <0.001 |
| BMI*(kg/m²) | 24.20 [21.85, 26.50] | 25.20 [23.63, 27.90] | 24.00 [21.70, 26.20] | <0.001 |
| Waist circumference(cm) | 83.00 [74.00, 91.00] | 89.00 [83.00, 96.00] | 82.00 [73.00, 90.00] | <0.001 |
| Systolic Pressure(mmHg) | 119.00 [109.00, 130.00] | 138.00 [130.00, 147.75] | 118.00 [108.00, 127.00] | <0.001 |
| Diastolic Pressure(mmHg) | 73.00 [65.00, 81.00] | 83.00 [77.00, 89.00] | 71.00 [65.00, 80.00] | <0.001 |
| albumin(g/L) | 45.50 [43.80, 47.60] | 44.60 [42.90, 46.10] | 45.70 [43.90, 47.70] | <0.001 |
| GA*(%) | 12.90 [11.60, 14.20] | 13.60 [12.20, 15.40] | 12.80 [11.50, 14.10] | <0.001 |
| platelet(10⁹/L) | 227.00 [192.00, 261.00] | 208.00 [176.25, 245.75] | 229.00 [194.00, 262.00] | <0.001 |
| ALT*(U/L) | 20.00 [14.00, 31.50] | 20.00 [15.00, 28.00] | 21.00 [14.00, 32.00] | 0.391 |
| AST*(U/L) | 22.00 [18.00, 27.00] | 23.00 [20.00, 26.00] | 22.00 [18.00, 27.00] | 0.043 |
| Fasting blood glucose(mmol/L) | 5.23 [4.90, 5.67] | 5.74 [5.20, 6.51] | 5.20 [4.87, 5.59] | <0.001 |
| Total cholesterol(mmol/L) | 4.97 [4.42, 5.68] | 5.18 [4.59, 5.92] | 4.95 [4.41, 5.64] | 0.006 |
| triglyceride(mmol/L) | 1.42 [0.92, 2.26] | 1.72 [1.05, 2.66] | 1.38 [0.90, 2.22] | <0.001 |
| HDL-C*(mmol/L) | 1.25 [1.07, 1.47] | 1.21 [1.04, 1.40] | 1.25 [1.07, 1.48] | 0.006 |
| LDL-C*(mmol/L) | 2.90 [2.39, 3.45] | 2.94 [2.41, 3.55] | 2.90 [2.39, 3.44] | 0.408 |
| **Characteristics [n (%)]** | | | | |
| Sex | | | | 0.001 |
| Female | 1131 (53.7) | 148 (64.3) | 983 (52.4) | |
| Male | 976 (46.3) | 82 (35.7) | 894 (47.6) | |
| Cervical vascular plaques | | | | <0.001 |
| No | 1632 (77.5) | 101 (43.9) | 1531 (81.6) | |
| Yes | 475 (22.5) | 129 (56.1) | 346 (18.4) | |
| Smoking status | | | | <0.001 |
| No | 1538 (73.0) | 131 (57.0) | 1407 (75.0) | |
| Yes | 569 (27.0) | 99 (43.0) | 470 (25.0) | |
| Drinking status | | | | 0.449 |
| No | 1344 (63.8) | 141 (61.3) | 1203 (64.1) | |
| Yes | 763 (36.2) | 89 (38.7) | 674 (35.9) | |
| Sport status | | | | 0.012 |
| No | 854 (40.5) | 75 (32.6) | 779 (41.5) | |
| Yes | 1253 (59.5) | 155 (67.4) | 1098 (58.5) | |
| MAFLD* | | | | <0.001 |
| No | 1116 (53.0) | 62 (27.0) | 1054 (56.2) | |
| Yes | 991 (47.0) | 168 (73.0) | 823 (43.8) | |

**Notes**: Values are median (25th, 75th), or n (%). P values were computed utilizing Student's t-test or the Mann-Whitney U test for continuous variables, and the chi-squared test was adopted for categorical variables. *BMI: body mass index; GA: glycated albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MAFLD: metabolic associated fatty liver disease.

**Table S2**. Logistic regression models of the link between GA and 10-year ASCVD risk

|  | Model 1 | | | Model 2 | | | Model 3 | | |
|---|---|---|---|---|---|---|---|---|---|
|  | OR | 95%CI | p | OR | 95%CI | p | OR | 95%CI | p |
| GA | 1.138 | (1.094, 1.185) | < 0.001 | 1.142 | (1.049, 1.240) | 0.002 | 1.160 | (1.055, 1.276) | 0.002 |

**Notes:** Model 1 = GA; Model 2 = Model 1 + Age + Sex + Smoking status + Sport status + BMI + WC + SBP +DBP; Model 3 = Model 2 + Albumin + Platelet + Total Cholesterol + Triglyceride + HDL-C + Cervical vascular plaques + MAFLD.

**Table S3.** Mediation effect of BMI on the association between GA and 10-year ASCVD risk

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| BMI | 2107 | -0.146 (0.026) p<0.001 | 0.190 (0.015) p<0.001 | 0.229 (0.040) p<0.001 | -0.028 (0.006) p<0.001 | 0.201 (0.040) p<0.001 | 13.9% |

**Notes:** Exposure: GA; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, TC, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

**Table S4.** Mediation effect of BMI on the link between GA and 10-year ASCVD risk by age and MAFLD

| Mediator | Sample | Exposure to Mediator | Mediator to Outcome | Direct Effect | Mediated (Indirect Effect) | Total Effect (Exposure to Outcome) | Proportion Mediated (%) |
|---|---|---|---|---|---|---|---|
| **Stratification by Age** | | | | | | | |
| <40 | 743 | -0.388 (0.064) p < .001 | 0.056 (0.005) p < .001 | 0.030 (0.022) p =.177 | -0.022(0.007) 95%CI (-0.037, -0.011) p = .002 | 0.008 (0.023) p = .714 | - |
| 40-60 | 1214 | -0.147 (0.030) p < .001 | 0.210 (0.016) p < .001 | 0.102 (0.029) p < .001 | -0.031(0.007) 95%CI (-0.046, -0.020) p < .001 | 0.071 (0.030) p = .017 | **43.7** |
| >60 | 150 | 0.136 (0.075) p =.270 | 0.185 (0.093) p < .05 | 0.415 (0.121) p < .001 | 0.025(0.022) 95%CI (-0.016, 0.069) p = .245 | 0.440 (0.117) p < .001 | - |
| **Stratification by MAFLD** | | | | | | | |
| MALFD | 991 | -0.040 (0.029) p =.092 | 0.081 (0.029) p < .01 | 0.275 (0.043) p < .001 | -0.003(0.003) 95%CI (-0.010, 0.002) p = .310 | 0.272 (0.043) p < .001 | - |
| Non-MAFLD | 1116 | -0.107*** (0.029) p < .001 | 0.127*** (0.026) p < .001 | 0.178 (0.068) p = .009 | -0.014(0.005) 95%CI (-0.025, -0.006) p = .004 | 0.164 (0.067) p = .014 | **8.5** |

**Notes:** Exposure: GA; Mediator: BMI; Outcome: 10-year ASCVD risk; Model adjusted for age, sex, smoking status, sport status, BMI, WC, SBP, DBP, albumin, platelet, total cholesterol, triglyceride, HDL-C, cervical vascular plaques, and MAFLD.

**Table S5**. Sensitivity Analysis Adjusted for Pharmacotherapy (Model 4)

| | Model 4 | | |
| --- | --- | --- | --- |
| | OR* | 95%CI* | p |
| GA* | 1.153 | (1.033, 1.277) | 0.008 |

**Notes:** Model 4 adjusted for antihypertensive drugs, lipid-lowering drugs, and glucose-lowering drugs. Analysis was conducted on 2,084 participants after excluding 23 cases with missing medication data. *OR: odds ratio; CI: confidence interval; GA: glycated albumin;

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