Hemoglobin glycation index as a screening tool for aging acceleration and cardiovascular disease: a population-based study

Keywords
Diabetes, Cardiovascular disease, Biological Aging, Hemoglobin glycation index, Aging acceleration, Phenotypic age acceleration

Abstract

Introduction
Diabetes promotes aging and contributes to the progression of cardiovascular disease (CVD). Hemoglobin glycation index (HGI) serves as an indicator linking blood glucose to CVD, but the role of aging remains unclear. This study aimed to investigate the association between the HGI and CVD, and to evaluate whether accelerated aging mediates this relationship.

Material and methods
We conducted a cross-sectional analysis from the NHANES 1999-2010. HGI was used to assess interindividual glycemic variability, and phenotypic age acceleration (PAA) was employed to estimate biological aging. Relationships were studied using multivariable linear and logistic regression, LASSO regression, and mediation analysis. Patients with type 2 diabetes admitted to Xijing Hospital were enrolled to validate the efficacy of HGI.

Results
Analysis of 8,449 NHANES adults (median age 45 years; 48% male) revealed that 8.2% had CVD. Individuals with a positive HGI had a significantly higher risk of CVD, independent of age, sex, PAA, lipid levels, and renal function. A positive HGI was also positively associated with PAA (β= 14.034, $P < 0.001$). Mediation analysis showed PAA significantly mediated 23.8% (95% CI: 17.1%–29.0%, $P < 0.001$) of the relationship between HGI and CVD. In the validation cohort, positive HGI inversely correlated with cardiac function parameters, including left atrial strain during contraction phase (LASct) and left ventricular global longitudinal strain (LVGLS).

Conclusions
The HGI is a strong and clinically accessible biomarker for both accelerated biological aging and increased cardiovascular risk, especially in individuals with dysglycemia.

1. Introduction:

Type 2 diabetes (T2D) and prediabetes are highly prevalent among individuals with cardiovascular disease (CVD), and both conditions are strongly associated with elevated risks of adverse cardiovascular outcomes[1]. Glycated hemoglobin A1c (HbA1c), a widely used biomarker of long-term glycemic control, is also an established predictor of CVD in individuals with diabetes[2]. However, accumulating evidence suggests that HbA1c alone may inadequately reflect glycemic status in approximately 40% of patients due to interindividual differences in hemoglobin glycation that are independent of blood glucose levels[3].

To address this variability, the hemoglobin glycation index (HGI), is defined as the difference between measured and predicted HbA1c based on fasting plasma glucose and has emerged as a complementary metric that captures individual glycation propensity and glycemic variability[3]. Recent studies have linked higher HGI values to increased risks of microvascular and macrovascular complications, such as diabetic nephropathy and retinopathy [4, 5]. However, the association between HGI and CVD remains underexplored, particularly in population-based settings. Elucidating this relationship could provide valuable insight into novel risk stratification tools beyond traditional glycemic markers.

CVD is also fundamentally an age-related condition, with aging processes playing a key role in vascular degeneration and disease progression. Biological aging indicators, including leukocyte telomere length, which is significantly influenced by metabolic health status[6] and phenotypic age acceleration (PAA)[7], have shown enhanced predictive value for cardiovascular outcomes compared to chronological age. Importantly, chronic hyperglycemia may accelerate vascular aging and promote cellular senescence, thus mediating the link between glucose dysregulation and CVD[8]. Whether HGI reflects interindividual differences in biological aging and contributes to cardiovascular risk through age-related

pathways remains unclear.

Therefore, the primary objective of this study is to investigate the association between HGI and cardiovascular disease and to determine whether biological aging, as quantified by PAA, mediates this relationship. We hypothesized that HGI, which reflects individual glucose fluctuations, serves as an effective predictor of cardiovascular risk.

2. Methods:

The current study comprises two parts: analysis of U.S. National Health and Nutrition Examination Survey (NHANES) data and validation analysis using data from patients with diabetes at our institution.

Part I. NHANES data

2.1 Study population

The NHANES is a nationally representative, cross-sectional survey conducted by the U.S. Centers for Disease Control and Prevention (CDC), with publicly available data accessible at http://www.cdc.gov/nchs/nhanes.htm. The survey protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board, and written informed consent was obtained from all participants at enrollment.

For this study, we used data from the 1999–2010 NHANES cycles, as these years include measurements of C-reactive protein (CRP), which is required for the calculation of phenotypic age. A total of 59,731 adult participants were initially considered. We excluded 27,267 individuals under 20 years of age and 5,167 participants with missing data required for computing phenotypic age (including serum albumin, creatinine, fasting plasma glucose [FPG], CRP, alkaline phosphatase, and complete blood count with 5-part differential). We further excluded participants with missing HbA1c data (n = 26), as this parameter was necessary for calculating the HGI, and those lacking questionnaire responses related to

cardiovascular disease (n = 18,822). Individuals with severe anemia were also excluded. After these exclusions, the final analytic sample included 8,449 adults aged 20 to 85 years, of whom 985 were identified as having CVD.

2.2 Disease Assessment

Cardiovascular disease status was determined using the NHANES Medical Conditions Questionnaire items 160B through 160E, which asked participants whether a healthcare professional had ever informed them of having congestive heart failure, coronary heart disease, angina pectoris, or myocardial infarction. Participants who answered "yes" to any of these items were classified as having CVD. Responses such as "don't know" or "refused" were treated as missing data.

Diabetes and prediabetes were classified based on the diagnostic criteria established by the American Diabetes Association[9]. Participants were considered to have diabetes or prediabetes if they self-reported a diagnosis by a healthcare professional and met at least one of the following laboratory criteria: fasting plasma glucose (FPG) ≥ 5.6 mmol/L or HbA1c ≥ 5.7%.

2.3 Key variables

2.3.1 Biological aging markers

Phenotypic age was calculated using a validated algorithm based on nine aging-related biomarkers [10], including chronological age, serum albumin, creatinine, fasting glucose, CRP, lymphocyte percentage, mean cell volume, red cell distribution width, alkaline phosphatase, and white blood cell count. The formula used is as follows:

$$Phenotypic\ \ age = 141.50 + \frac{\ln\left[-0.00553 \times \ln\left(exp\left(\frac{-1.51714 \times exp(xb)}{0.076927}\right)\right)\right]}{0.09165}$$

where

$$xb = -19.907 - 0.0336 \times \text{Albumin} + 0.0095 \times \text{Creatinine} + 0.1953 \times \text{Glucose} + 0.0954 \times \text{Ln CRP} - 0.0120 \times \text{Lymphocyte Percent} + 0.0268 \times \text{Mean Cell Volume} + 0.3306 \times \text{Red Cell Distribution Width} + 0.00188 \times \text{Alkaline Phosphatase} + 0.0554 \times \text{White Blood Cell Count} + 0.0804 \times \text{Chronological Age}.$$

PAA was calculated as the residual from a linear regression model of phenotypic age regressed on chronological age. A PAA value < 0 indicates decelerated aging, while PAA ≥ 0 reflects accelerated biological aging.

2.3.2 HGI

The HGI quantifies the discrepancy between observed and predicted HbA1c values, offering insight into individual glycation tendencies beyond blood glucose levels. Predicted HbA1c was calculated from a linear regression model of HbA1c on fasting plasma glucose (FPG): Predicted HbA1c = (0.45 × FPG) + 2.99. HGI = Observed HbA1c − Predicted HbA1c. Participants were categorized into two groups: Positive HGI: Observed HbA1c > predicted HbA1c; Negative HGI: Observed HbA1c < predicted HbA1c.

2.3.3 Covariates

To account for potential multicollinearity among clinical covariates (e.g., lipid profiles, blood pressure), we employed the adaptive least absolute shrinkage and selection operator (LASSO) logistic regression for variable selection. The outcome variable was CVD status (0 = no CVD, 1 = CVD), and 15 candidate predictors were included: age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), and uric acid

(UA). Based on prior literature and the LASSO results (Supplementary Figure 1), the final models adjusted for the following covariates: age, sex, SBP, TC, BUN, Cr, UA, and PAA.

2.4 Statistical analysis

All statistical analyses were conducted using R version 4.3.2. The "survey" package was used to account for the NHANES complex survey design, incorporating stratification (SDMVSTRA) and clustering (SDMVPSU). Missing data were imputed using the multiple imputation by chained equations (MICE) method.

Descriptive statistics are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) for continuous variables, and as frequencies and percentages for categorical variables. Group differences between participants with and without CVD were assessed using independent t-tests for continuous variables and chi-square tests for categorical variables.

LASSO regression was applied to minimize multicollinearity and identify key predictors of CVD. The penalty parameter (λ) was optimized via 10-fold cross-validation over 1,000 iterations to minimize the mean squared error.

To evaluate the association between HGI and CVD risk, we performed multivariable logistic regression adjusting for selected covariates. Linear regression models were used to assess the relationship between HGI and PAA, while logistic regression assessed the association between HGI and CVD incidence.

Mediation analysis was conducted to explore the potential mediating effect of PAA in the relationship between HGI and CVD. Stratified subgroup analyses were performed among participants with a positive HGI, with effect modification evaluated by sex (male/female), age (< 65/≥ 65 years), TC (< 6.19/≥ 6.19 mmol/L), Cr (< 133/≥ 133 μmol/L), UA (< 420/≥ 420 mmol/L), and PAA (< 0/≥ 0).

A two-sided *P* value < 0.05 was considered statistically significant.

Part II. Validation Analysis:

To examine the performance of HGI in predicting CVD risk across different population, we analyzed data from our institutiony. A total of 200 individuals with T2D (age 40–80 years) was consecutively recruited from the department of endocrinology between February and December 2023. We collected data on age, sex, and diabetes duration, height, weight, fasting glucose and HbA1c. All participants underwent carotid artery ultrasound to assess carotid intima-media thickness (CIMT), conventional echocardiography and two-dimensional speckle-tracking echocardiography (2D-STI) to measure left ventricular global longitudinal strain (LVGLS), left atrial storage strain during reservoir phase (LASr), left atrial strain during conduit phase (LAScd), left atrial strain during contraction phase (LASct). After excluding those did not complete both the carotid artery ultrasound and 2D-STI, a total of 151 participants with T2D was included in the final analysis.

The association between HGI and cardiovascular structure and function parameters were analyzed using multivariable linear regression with restricted cubic splines (RCS) to allow for nonlinear relationships. Three knots were placed at the 10th, 50th, and 90th percentiles of the HGI distribution. The model adjusted for sex, age, BMI, waist-to-height ratio (WHtR), C-peptide level, and diabetes duration. The overall and nonlinear effects of HGI were tested using analysis of variance from the fitted model. Results were visualized by plotting the adjusted association (with 95% confidence intervals) between HGI and cardiovascular structure and function parameters. The overall and nonlinear p-values were annotated on the plot.

The study was approved by the Ethical Committee of our institution (Ethics Board Approval Number: KY20242029-C-1). The requirement for informed consent was waived because the

study involved analysis of anonymized pre-existing data, and the research posed no more than minimal risk to participants. All procedures followed the ethical standards of the Declaration of Helsinki.

3. Results:

3.1 General characteristics in participants with and without self-reported CVD.

A total of 8,449 participants were included in the analysis, with a median age of 45.0 years (interquartile range [IQR]: 13.0) and 48% being male. Among them, 4,027 participants (42%) had prediabetes or diabetes, and 867 (10.3%) self-reported a history of cardiovascular disease (CVD). Compared with those without CVD, individuals with CVD were significantly older, more likely to be male, and exhibited higher levels of systolic and diastolic blood pressure, BMI, HbA1c, and less favorable lipid profiles (i.e., higher triglycerides, lower HDL-C). They also showed poorer renal function as indicated by elevated levels of creatinine, blood urea nitrogen (BUN), and uric acid.

Importantly, participants with CVD had significantly higher phenotypic age acceleration (PAA) (median [IQR]: 1.53 [3.80] vs. –1.75 [3.07]) and hemoglobin glycation index (HGI) (0.05 [0.25] vs. –0.01 [0.25]) than those without CVD. All between-group differences were statistically significant except for alanine aminotransferase (ALT) **(Table 1)**.

3.2 Associations between HGI and total CVD incidence.

Multivariable logistic regression analyses **(Table 2)** revealed that among participants with a positive HGI, the odds of having CVD were significantly higher (adjusted odds ratio [aOR] = 1.545, 95% CI: 1.183–2.019; Model 2), even after controlling for major confounders. When PAA was added to the model (Model 3), the association between HGI and CVD remained robust (aOR = 1.469, 95% CI: 1.094–1.971), suggesting that accelerated aging may partly mediate this relationship. Given that positive HGI demonstrated a more pronounced

association with CVD than negative HGI, subsequent analyses were focused on the positive HGI subgroup. Consistent associations were observed across different types of CVD. Interestingly, positive HGI conferred an increased risk for CVD in individuals with dysglycemia (prediabetes and diabetes), whereas a protective association was noted in normoglycemic individuals.

3.3 Relationship between HGI and accelerated aging.

While HbA1c primarily reflects glycemic exposure, it is also influenced by erythrocyte lifespan. To explore whether aging plays a role in the association between HGI and CVD, we examined the relationship between HGI and PAA. Linear regression analyses demonstrated a significant positive association between HGI and PAA in participants with prediabetes or diabetes ($\beta = 14.034$, SE = 6.350, $P < 0.001$), indicating that individuals with higher HGI values exhibited greater biological age acceleration. In contrast, a consistent inverse relationship was found in participants with normal glucose metabolism (NGM) **(Supplementary Table 1)**. These findings suggest that positive HGI is associated with accelerated aging, independent of common metabolic confounders.

3.4 The modifying effect of aging acceleration on the association between HGI and CVD risk.

To further elucidate the underlying mechanism, we performed mediation analysis to determine whether PAA mediates the effect of HGI on CVD risk. Results indicated a significant mediating effect of PAA ($\beta = 2.64$, SE = 0.30, $P < 0.001$), accounting for 23.8% (95% CI: 17.1%–29.0%) of the total association between positive HGI and CVD **(Supplementary Figure 2)**. This supports the hypothesis that accelerated biological aging partially explains the link between elevated HGI and increased cardiovascular risk.

3.5 Subgroup analysis.

Subgroup analyses revealed consistent associations between positive HGI and increased CVD risk across strata defined by sex, age, BMI, uric acid, PAA, and glucose metabolism status. However, when stratified by total cholesterol and creatinine levels, significant associations were observed only in participants with total cholesterol < 6.19 mmol/L and creatinine < 133 μmol/L. Interaction tests showed no statistically significant effect modification across subgroups (*P* for interaction > 0.05 for all), suggesting that the relationship between positive HGI and CVD risk is stable regardless of sex, age, renal function, lipid profile, or aging status (**Figure 1**).

3.6 Associations of HGI with Cardiovascular Structure and Function Parameters

Within the 151 patients with T2D from our institution, mean (SD) age was 58.0 (6.0) years, 63% were male, median (IQR) duration of T2D was 11.15 (6.77) years, mean (SD) BMI was 24.06 (2.72), mean (SD) WHtR was 40.10 (5.19), mean C peptide was 1.82 (0.37), mean (SD) fasting glucose was 7.69 (2.53) mmol/L, mean (SD) HbA1c (%) was 8.23 (1.4). The three-node restricted cubic spline analysis was performed to assess the strength of the association between HGI and cardiovascular structure and function parameters. We observed a near-linear inverse association between HGI and both LASct (Overall $P = 0.0448$; Non-linear $P = 0.4647$) and LVGLS (Overall $P = 0.007$; Non-linear $P = 0.4502$), after adjustment for sex, age, BMI, WHtR, duration of T2D, and C peptide **(Figure 2)**, consistent with our findings in NHANES data.

4. Discussion:

This study yielded two principal findings. First, we found that a positive hemoglobin glycation index (HGI), but not a negative one, was significantly associated with an increased risk of cardiovascular disease (CVD), particularly among individuals with diabetes. Second, a

positive HGI appeared to be a strong and previously underappreciated indicator of biological aging acceleration. These findings suggest that identifying individuals with elevated HGI levels may be critical for the early prevention and intervention of age-related diseases, especially CVD.

HbA1c is a widely recognized biomarker of long-term glycemic control and a key predictor of cardiovascular risk. However, HbA1c is influenced not only by blood glucose levels but also by erythrocyte lifespan [11] and glucose permeability across red cell membranes [12]. Although some studies suggest that erythrocyte lifespan does not differ significantly between individuals with and without diabetes [11]. The discrepancy between predicted and observed HbA1c may reflect interindividual glycemic variability and differences in glycation propensity. Increasing evidence indicates that a positive HGI is linked to higher degrees of protein glycation, endothelial dysfunction, and vascular injury [4, 5] which may contribute to the development of CVD and other diabetic vascular complications.

Our analysis of 8,449 adults from the NHANES cohort confirmed that individuals with self-reported CVD exhibited significantly higher HGI, phenotypic age acceleration (PAA), and a greater prevalence of dysglycemia. Importantly, the association between a positive HGI and CVD persisted across different types of cardiovascular events. These findings highlight the potential of HGI as a clinically accessible biomarker for CVD risk stratification, particularly in populations with impaired glucose metabolism.

CVD has long been conceptualized as a disease of accelerated aging. Individual differences in biological aging rates can result in varying susceptibilities to cardiovascular complications, even among people of the same chronological age[8]. Hyperglycemia not only contributes to metabolic stress but also exacerbates cellular senescence and tissue aging, thereby increasing CVD risk [13]. Although the precise biological mechanisms linking HGI and CVD remain to be fully elucidated, our results suggest that accelerated aging may be a critical intermediary

in this pathway.

Biological aging can be measured through various means, including epigenetic clocks, leukocyte telomere length (LTL)[14], and more recently, clinically derived metrics such as PAA. PAA has demonstrated strong predictive value for age-related diseases, including CVD, and reflects multisystem physiological dysregulation [15]. In our study, HGI was significantly correlated with PAA among individuals with diabetes and prediabetes, but not among those with normoglycemia. Mediation analysis further revealed that accelerated aging (as measured by PAA) accounted for approximately 24% of the association between a positive HGI and CVD risk. These results suggest that HGI may partly reflect underlying aging-related physiological changes that contribute to cardiovascular vulnerability.

Several plausible biological mechanisms may explain the relationship between HGI and accelerated aging. First, elevated blood glucose can reduce erythrocyte Na^+/K^+-ATPase activity, increasing intracellular glucose concentrations and thereby enhancing glycation reactions[16] Second, aging itself is associated with diminished erythrocyte ATP content [17], which may impair red blood cell function and further modify HbA1c levels. Collectively, these changes may contribute to higher HGI and, in turn, to increased CVD risk through the aging pathway.

To overcome the limitation of the part 1 NHANES data in only one ethnic population, we examined whether our study findings can be extrapolated to our Chinese groups by assess the strength of the association between HGI and cardiovascular structure and function parameters in individuals with T2D using data derived from our institution. 2D-STI uses myocardial strain to objectively assess cardiac function, identifying subtle changes and improving prognosis over standard echo. Our findings indicate that, divergent relationships occurred between HGI and cardiovascular parameters in negative versus positive HGI. A negative relationship between HGI and cardiovascular parameters (LASct, and LVGLS).

CIMT, a marker of cardiovascular disease risk, showed a positive association trend with HGI. Notably, the inverse relationship between HGI and cardiovascular parameters was particularly noticeable in the positive HGI value, which supports our conclusion that a positive HGI can serve as a reliable indicator of increased CVD risk.

To our knowledge, this is the first study to demonstrate that interindividual variation in HGI is closely tied to biological aging and may serve as a proxy for the rate of physiological aging. Given its ease of calculation from routinely collected clinical parameters, HGI holds promise as a practical, cost-effective biomarker for identifying individuals at elevated cardiovascular risk, particularly those with dysglycemia who may be overlooked by conventional metrics. The use of such accessible biomarkers supports recent expert recommendations for a more comprehensive approach to cardiovascular prevention[18].

Nevertheless, this study has several limitations. First, the absence of oral glucose tolerance test data may have led to underdiagnosis of glucose abnormalities. Second, CVD status was assessed through self-report, which introduces potential recall bias. Specifically, self-reported angina may include non-cardiac chest pain, leading to potential misclassification. Nevertheless, the observed associations remained consistent across more definitive cardiovascular events, such as myocardial infarction and congestive heart failure, providing support for the overall reliability of the main findings. Third, due to the cross-sectional nature of NHANES, causal inferences cannot be made. Cardiovascular risk extends beyond glycemic control. Although we addressed this complexity by adjusting for phenotypic age acceleration, reflecting systemic inflammation, renal function, and metabolic status, along with variables like insulin resistance and lipid profiles, unmeasured confounders may still have influenced the results. Longitudinal studies and mechanistic research are needed to validate these findings and determine whether interventions targeting cellular aging pathways could mitigate CVD risk in individuals with elevated HGI.

5. Conclusion

This study demonstrates that a positive HGI is significantly associated with increased CVD risk and accelerated biological aging, particularly among individuals with type 2 diabetes. HGI may serve as a practical biomarker for identifying those at heightened risk of age-related cardiovascular complications, thereby facilitating earlier and more targeted preventive interventions.

6. List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BUN	Blood urea nitrogen
CIs	Confidence intervals
Cr	Creatinine
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
FPG	Fasting plasma glucose
HbA1c	Glycated hemoglobin a1c
HDL	High-density lipoprotein
HGI	Hemoglobin glycation index
HOMA-IR	Homeostasis model assessment-insulin resistance
IQR	Interquartile range
LDL	Low-density lipoprotein
NHANES	National Health and Nutrition Examination Survey
NGM	Normal glucose metabolism
ORs	Odds ratios
PAA	Phenotypic age acceleration
SBP	Systolic blood pressure
T2D	Type 2 diabetes
TC	Total cholesterol
TG	Triglycerides
UA	Uric acid
WHtR	Waist-to-height ratio

7. Declarations

Ethics approval and consent to participate: This study comprises two parts. The NHANES is a nationally representative survey conducted by the U.S. Centers for Disease Control and Prevention (CDC). The study protocol was reviewed and approved by the National Center

for Health Statistics (NCHS) Research Ethics Review Board. Written informed consent was obtained from all NHANES participants prior to their inclusion. Validation was performed using anonymized clinical data from our institution. The study was approved by the Ethics Committee of our institution (Approval No.: KY20242029-C-1). All procedures complied with the ethical principles of the Declaration of Helsinki. The committee granted a waiver of written informed consent for this research.

Consent for publication: Not applicable.

Availability of data and materials: The analyses utilized existing data, and we were not involved in participant recruitment. The NHANES data that support the findings of this study are available on the NHANES database website at https://www.cdc.gov/nchs/nhanes/index.htm. The datasets were derived from the Department of Endocrinology at our institution. Due to data security and privacy regulations, these datasets are not publicly available. However, they may be obtained from the corresponding author upon reasonable request and with permission from the originating institution.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

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Figure captions:

Figure 1. Subgroup analysis of the association between positive HGI and CVD incidence.

Figure 2. Association Between HGI and Cardiovascular Parameters Using a Restricted Cubic Spline Regression Model.

Supplementary materials

Supplementary Figure 1. Feature selection using least absolute shrinkage and selection operator (LASSO) logistic regression.

Supplementary Figure 2. The relationship among positive HGI, PAA and CVD risk according to mediation analysis adjusted for age, sex, TC, BUN, Cr and UA.

Table 1. Weighted baseline characteristics by CVD incidence among participants in NHANES 1999-2010

Characteristic	Overall	Without CVD*	With CVD* (N=867, 8.2%)	*P*†
Age (Years)	45 (32, 58)	43.00 (31.00, 55.00)	65.00 (54.00, 75.00)	<0.001
Sex (male,	4,038 (48%)	3,531 (48%)	507 (55%)	0.001
SBP (mmHg)	118.00 (109.33, 130.00)	117.33 (108.67, 128.67)	126.00 (114.67, 141.33)	<0.001
DBP (mmHg)	70.00 (63.33, 77.33)	70.00 (63.33, 77.33)	68.67 (60.00, 76.00)	<0.001
BMI (kg/m^2)	27.36 (23.80, 31.80)	27.23 (23.72, 31.65)	28.99 (25.26, 33.01)	<0.001
Glucose	5.37 (5.00, 5.83)	5.33 (4.98, 5.77)	5.83 (5.33, 6.62)	<0.001
HbA1c (%)	5.30 (5.10, 5.60)	5.30 (5.10, 5.60)	5.60 (5.30, 6.10)	<0.001
HOMA-IR	2.17 (1.34, 3.71)	2.11 (1.31, 3.59)	2.95 (1.87, 5.39)	<0.001
TC (mmol/L)	5.04 (4.40, 5.79)	5.07 (4.42, 5.79)	4.86 (4.19, 5.66)	0.001
TG (mmol/L)	1.26 (0.89, 1.85)	1.25 (0.87, 1.82)	1.49 (1.07, 2.22)	<0.001
LDL (mmol/L)	3.00 (2.41, 3.60)	3.03 (2.43, 3.62)	2.72 (2.17, 3.44)	<0.001
HDL (mmol/L)	1.32 (1.09, 1.60)	1.32 (1.09, 1.63)	1.22 (1.03, 1.53)	<0.001
ALT (U/L)	21 (17, 29)	21 (17, 29)	22 (17, 29)	0.8
AST (U/L)	23 (19, 27)	23 (19, 27)	23 (20, 29)	0.002
BUN (mmol/L)	4.30 (3.57, 5.40)	4.28 (3.57, 5.36)	5.36 (3.93, 6.80)	<0.001
Cr (μmol/L)	70.72 (61.88, 88.40)	70.72 (61.88, 86.63)	79.60 (70.70, 97.24)	<0.001
UA (mmol/L)	315.20 (261.70, 374.70)	315.20 (261.70, 374.70)	350.90 (291.50, 416.40)	<0.001
HGI	-0.01 (-0.25, 0.23)	-0.01 (-0.26, 0.22)	0.05 (-0.20, 0.31)	<0.001
PAA	-1.54 (-4.66, 2.14)	-1.75 (-4.82, 1.80)	1.53 (-2.27, 6.32)	<0.001
Prediabetes and	4,027 (42%)	3,397 (39%)	630 (71%)	<0.001

All results were survey-weighted except for sample counts.

* Median (IQR); n (unweighted) (%)

† Wilcoxon rank-sum test for complex survey samples; chi-squared test with Rao & Scott's second-order correction.

Table 2. Logistic regression analysis for the association between HGI and cardiovascular disease

	Crude	Model 1	Model 2	Model 3
Total, N=8449	1.535(1.249-1.887)***	1.425(1.152-1.761)***	1.319(1.074-1.621)**	1.286(1.048-1.577)*
Stratified by HGI				
Negative HGI	0.594(0.376-0.938)*	0.643(0.392-1.055)	0.822(0.489-1.381)	0.898(0.536-1.503)
Positive HGI	1.764(1.317-2.363)***	1.713(1.305-2.248)***	1.545(1.183-2.019)***	1.469(1.094-1.971)**
Stratified by glucose status†				
NGM	0.187(0.033-1.060)	0.172(0.032-0.918)*	0.118(0.020-0.703)*	0.106(0.017-0.650)*
Prediabetes and diabetes	1.367(1.062-1.759)**	1.469(1.144-1.886)**	1.406(1.092-1.811)**	1.383(1.113-2.456)*
Stratified by CVD*				
Congestive heart failure	1.870(1.257-2.782)**	1.880(1.316-2.688)***	1.656(1.199-2.288)**	1.586(1.134-2.216)**
Coronary heart disease	1.757(1.199-2.574)**	1.747(1.218-2.505)**	1.675(1.147-2.445)**	1.632(1.093-2.437)*
Angina/angina pectoris	1.674(1.132-2.476)*	1.659(1.125-2.445)*	1.571(1.042-2.370)*	1.531(0.994-2.357)
Heart attack	1.769(1.255-2.493)**	1.734(1.269-2.369)***	1.595(1.135-2.242)**	1.522(1.052-2.204)*

HGI, hemoglobin glycation index; OR, odds ratio.

*P<0.05, **P<0.01, *** P<0.001.

†Subgroups in positive HGI group

Model 1, adjusted for sex and age; Model 2, adjusted for TC, BUN, Cr, and UA based on Model 1. Model 3, further adjusted for PAA based on Model 2.

Variable	OR (95% CI)	P value	P for interaction
Sex			0.446
Male	1.85 (1.44 to 2.38)	< 0.001	
Female	1.57 (1.07 to 2.30)	0.025	
Age (Years)			0.771
< 65	1.72 (1.16 to 2.57)	0.009	
>= 65	1.86 (1.36 to 2.54)		
TC (mmol/L)			0.064
< 6.19	1.95 (1.26 to 3.02)	0.004	
>= 6.19	1.21 (0.86 to 1.72)	0.281	
Cr (μmol/L)			0.06
< 133	1.76 (1.31 to 2.35)	< 0.001	
>= 133	1.11 (0.68 to 1.81)	0.675	
UA (mmol/L)			0.394
< 420	1.80 (1.30 to 2.51)	0.001	
>=420	1.51 (1.06 to 2.15)	0.025	
PAA			0.054
< 0	2.28 (1.54 to 3.39)	< 0.001	
>= 0	1.39 (1.03 to 1.86)	0.032	
Glucose metabolism			0.033
NGM	0.22 (0.04 to 1.17)	0.081	
Prediabetes and Diabetes	1.37 (1.07 to 1.75)	0.016	

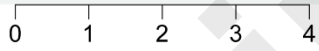


Figure 1. Subgroup analysis of the association between positive HGI and CVD incidence.

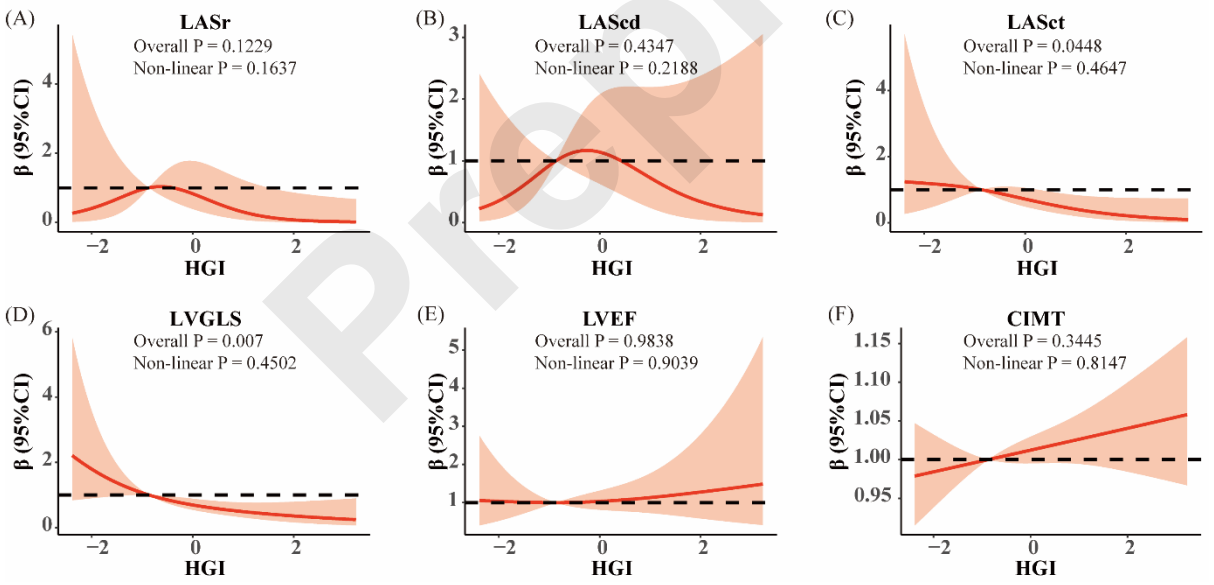
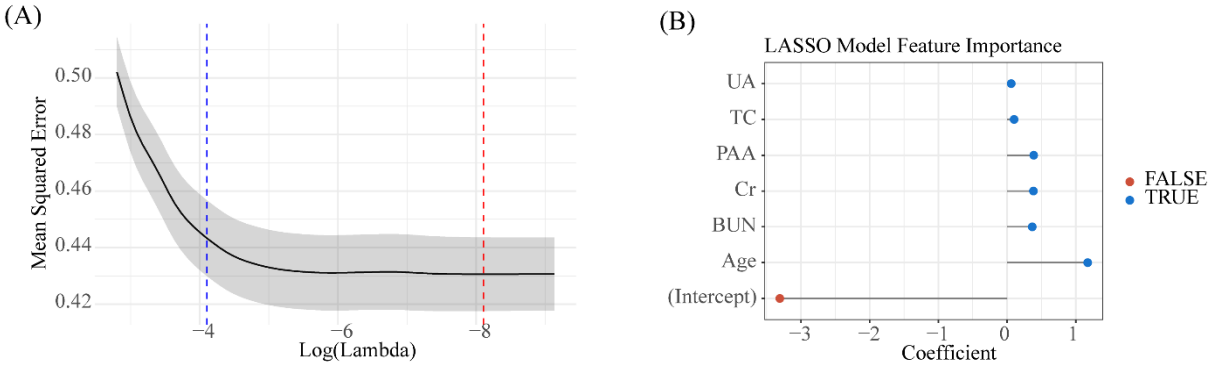


Figure 2. Association Between HGI and Cardiovascular Parameters Using a Restricted Cubic Spline Regression Model.

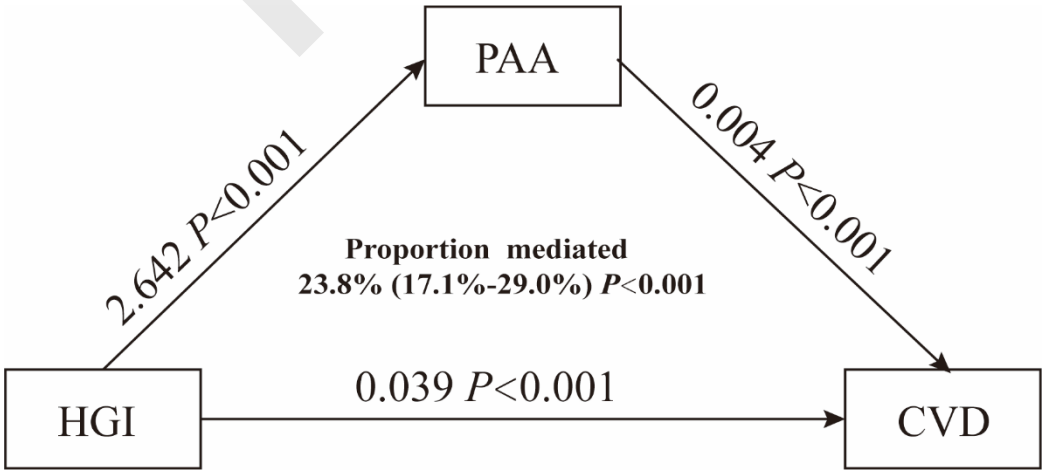
Graphs show β for cardiovascular parameters according to HGI adjusted for age, sex, BMI, WHtR, C peptide, and duration of diabetes. (A) left atrial storage strain during reservoir phase, LASr; (B) left atrial strain during conduit phase, LAScd; (C)left atrial strain during

contraction phase, LASct; (D) left ventricular global longitudinal strain, LVGLS; (E) left ventricular ejection fraction, LVEF; (F) carotid intima media thickness, CIMT.



Supplementary Figure 1. Feature selection using LASSO regression.

(A) Ten-fold cross-validation plot for selecting the optimal penalty parameter (lambda) in the LASSO model. The x-axis shows the log-transformed lambda values, while the y-axis represents the mean squared error. The left blue dashed line indicates the lambda with minimum mean squared error, and the right red dashed line shows the most regularized model within one standard error of the minimum (lambda.1se). (B) Feature importance plot of variables selected by the optimal LASSO model. Variables retained in the model (blue dots) include uric acid (UA), total cholesterol (TC), phenotypic age acceleration (PAA), creatinine (Cr), blood urea nitrogen (BUN), and age. The intercept term (red dot) was excluded.



Supplementary Figure 2. Mediation analysis illustrating the indirect effect of phenotypic age acceleration (PAA) on the association between hemoglobin glycation index (HGI) and

cardiovascular disease (CVD).

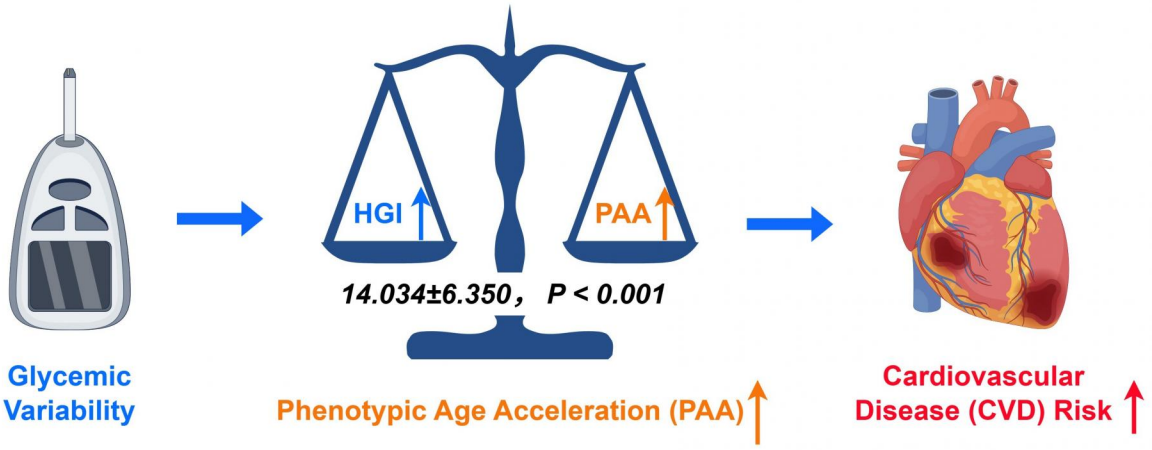
The total effect of HGI on CVD is partially mediated by PAA (indirect effect: 2.642, P < 0.001; path from HGI to PAA: 2.642, P < 0.001; path from PAA to CVD: 0.004, P < 0.001). The direct effect of HGI on CVD remains significant (0.039, P < 0.001). The proportion of the effect mediated by PAA was estimated to be 23.8% (95% CI: 17.1%–29.0%), P < 0.001.

Supplementary Table 1. Linear regression analysis for the association between positive HGI and PAA

	Crude	Model 1	Model 2
Total, N=4671	41.701 (19.198-90.584) *	39.007 (17.932-84.851) ***	14.034 (7.684-25.633) ***
NGM	3.834 (0.622-23.645)	4.142 (0.632-27.125)	2.389 (0.361-15.802)
Prediabetes and diabetes	10.229 (4.994-20.952) ***	10.022 (4.843-20.740) ***	6.213 (3.080-12.53) ***

†Subgroups in positive HGI group

*P<0.05, **P<0.01, *** P<0.001.



HGI: A Biomarker for Accelerated Aging and CVD Risk

NHANES 1999-2010 (n=8449)
Independent T2D Cohort (n=151)