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Association of long-term insulin variability before the onset of diabetes with cardiovascular outcomes in later life: Findings from the coronary artery risk development in young adults (CARDIA) study

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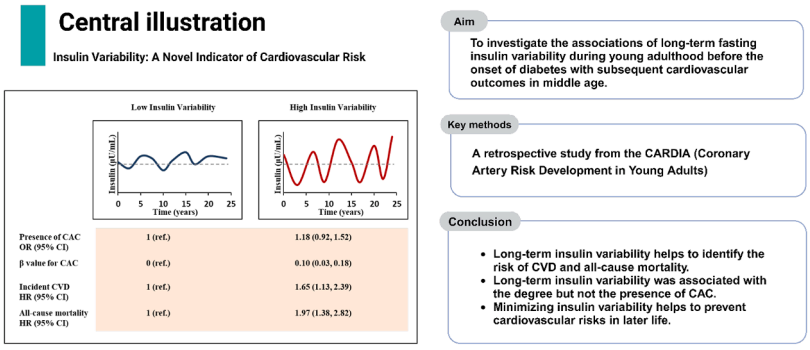
HIGHLIGHTS

- Long-term insulin variability helps to identify the risk of CVD and all-cause mortality.
- Long-term insulin variability was associated with the degree but not the presence of CAC.
- Minimizing insulin variability helps to prevent cardiovascular risks in later life.

GRAPHICAL ABSTRACT



Abbreviation: ARV, average real variability; BMI, body mass index; CAC, coronary artery calcification; CI, confidence interval; CVD, cardiovascular disease; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; SBP, systolic blood pressure; SD, standard deviation.

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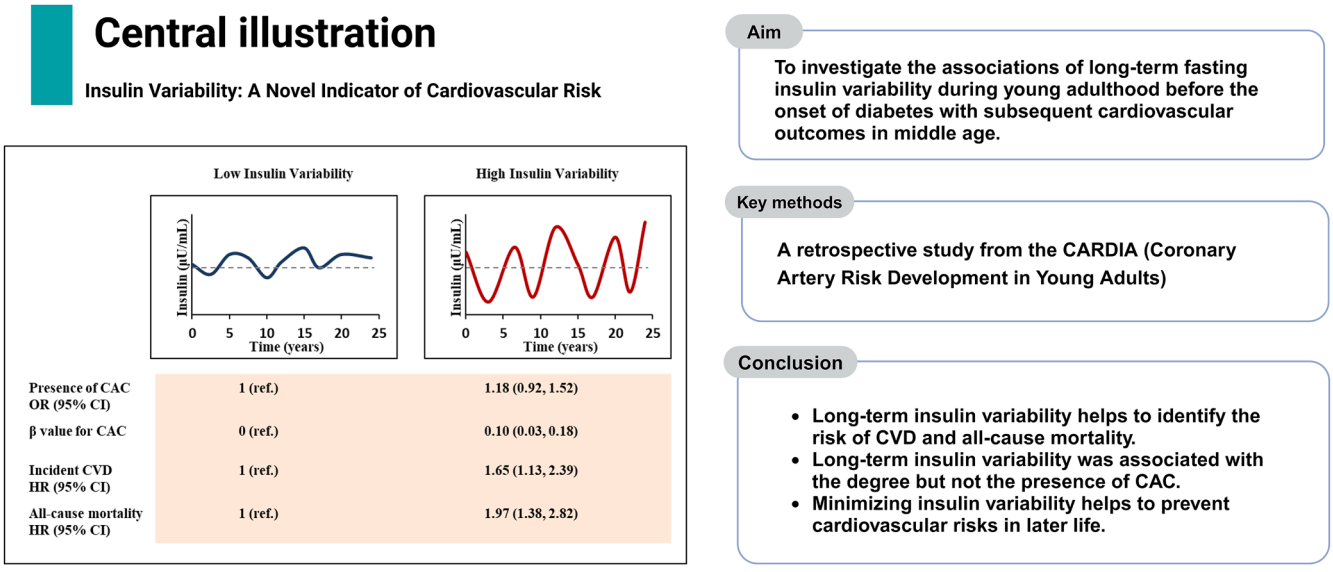
ABSTRACT

Background: The important effects of variability of some physiological/biological characteristics (such as LDL cholesterol, blood pressure) on cardiovascular outcomes have been elucidated, while the role of insulin variability is undefined.
Objectives: To investigate the associations of long-term fasting insulin variability during young adulthood before the onset of diabetes with subsequent cardiovascular outcomes in middle age.
Methods: We included 3,983 CARDIA (Coronary Artery Risk Development Study in Young Adults) participants aged 18 to 30 years with at least three fasting insulin measurements. Intra-individual fasting insulin variability was defined by the average real variability (ARV) of insulin and standard deviation (SD) of insulin during 30-year follow-up. The presence and the degree of coronary artery calcification (CAC) were assessed by computed tomography at year 25. Incident cardiovascular disease (CVD) and all-cause mortality were adjudicated.
Results: After multivariable adjustment, comparing high versus low tertile of insulin ARV, the hazard of CVD increased by 65 % (HR, 1.65; 95 % CI, 1.13–2.39) and all-cause mortality by 97 % (HR, 1.97; 95 % CI, 1.38–2.82). Higher tertile of insulin ARV was associated with significantly worse degree of CAC (β =0.1; 95 % CI, 0.03–0.18) but not with the presence of CAC ($P = 0.197$). Similar results were also observed in insulin SD.
Conclusion: High long-term insulin variability in young adulthood before the onset of diabetes was associated with an increased risk of CVD and all-cause mortality in later life, independent of average FG, HOMA-IR and other established cardiovascular risk factors. Long-term insulin variability was associated with the degree but not the presence of CAC.

1. Introduction

The incidence of type 2 diabetes is increasing. Individuals with diabetes are at more than twice risk of cardiovascular disease (CVD) compared with those without diabetes [1,2]. CVD is the main cause of death in patients with diabetes [3]. Identifying people at high risk of diabetes and intervening before the onset of diabetes may reduce the burden of adverse cardiovascular outcomes. Dysfunction in glucose homeostasis is regarded as one of the important mechanisms linking diabetes to CVD [4,5]. As it is well known, insulin contributes significantly to glucose homeostasis. Abnormal insulin levels, such as hyperinsulinemia and insulin resistance, are also an critical process involved in diabetes-related CVD [6]. Prior studies have shown that high insulin level is associated with increased CVD in both individuals with or without diabetes [7]. However, most observational studies on the association between insulin level and CVD risk have focused on insulin level measured at a single time point [8,9]. High fasting insulin level measured at a single time point might be attributed to a rapid increase caused by short-term stimulates or a persistent high level. Therefore, it does not adequately reflect long-term insulin level and its fluctuation. To date, the potential association between long-term insulin variability, particularly the natural physiological levels of insulin during the pre-diabetic phase, and its independent predictive value for major adverse cardiovascular events remains incompletely characterized.

Coronary Artery Risk Development in Young Adults (CARDIA) is a prospective cohort study with repeated insulin measurements during 25-year follow-up [10]. It is well-suited to use CARDIA dataset to capture the long-term insulin variability before the onset of diabetes and further analyze its association with subsequent cardiovascular outcomes. In the present study, we aimed to explore the associations of long-term fasting insulin variability during young adulthood before the onset of diabetes with subsequent risks of cardiovascular outcomes including coronary artery calcification (CAC), CVD, and all-cause mortality in later life.



Central Illustration. Long-term fasting insulin variability during young adulthood before the onset of diabetes was associated with cardiovascular outcomes in later life.

2. Methods

2.1. Study population

CARDIA is a multicenter longitudinal cohort study of 5115 healthy young adults aged 18 to 30 years recruited from four US metropolitan communities (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California) in 1985–1986. A detailed design of CARDIA has been published previously [10]. Participants were contacted via telephone annually and invited to participate in serial follow-up examinations at years 2, 5, 7, 10, 15, 20, 25, and 30 following baseline. The retention rate was high, with 71 % of surviving participants completing the year 30 (2015–2016) examination. All participants provided written informed consent at each examination, and the institutional review board at each study site and coordinating center approved the study procedure for all examinations.

From the CARDIA cohort enrolled at baseline, we included participants who had at least three valid insulin measurements before the onset of diabetes and had endpoint events assessment over 30-year follow-up. We excluded participants who withdrew study consent ($n = 1$), had a myocardial infarction at baseline examination ($n = 1$), and had fewer than three valid insulin values from baseline to year 30 examination. A total of 3983 participants were included for analyzing the association between insulin variability and cardiovascular outcomes. In addition, we further excluded participants without CAC assessment at year 25 examination ($n = 1005$), and the final sample size for the analysis of insulin variability and CAC was 2978 participants (Supplementary Fig. 1).

2.2. Insulin variability measurements

Fasting insulin was measured by radioimmunoassay (Linco Research, St. Charles, Missouri) in nonpregnant participants who reported fasting ≥8 h, at baseline (year 0) and years 7, 10, 15, and 20, as well as by an Elecsys sandwich immunoassay (Roche Diagnostics, Rotkreuz, Switzerland) at year 25. The main parameters of intra-individual fasting insulin variability were calculated for each participant with at least three successive insulin measurements: the average real variability (ARV) of insulin and standard deviation (SD) of insulin (insulin-SD). The concept of ARV inspired by the total variability concept of real analysis in mathematics was widely applied to the study of blood pressure variability [11–13]. ARV reflects within-subject variability and the overall variability of differences between successive measurements of fasting insulin over different time points, thus largely unaffected by trends. The proposed ARV index is a more reliable representation of time series variability than SD and may be less sensitive to the relative low sampling frequency of the data. Insulin variability was defined similar to suggested literature using by following parameters: (i) SD of insulin calculated as: $SD = \sqrt{\frac{1}{n-1}\sum_{k=1}^{n}(X_k - \overline{X})^2}$ $(1 \le k \le n)$ (ii) ARV of insulin calculated as: $\mathrm{ARV} = \frac{1}{\sum w}\sum_{k-1}^{n-1} w \times |X_{k+1} - X_k|$ $(1 \le k \le n-1)$ (where "n" means the number of measurements, X_k=the insulin level at baseline or follow-up year and $\overline{X}$=their mean, "w" is the time interval between the consecutive measurements.)

2.3. Covariates ascertainment

Standardized questionnaires and protocols were used to collect data on participant demographic characteristics and smoking status [10]. Height and weight were measured without shoes and in light clothing and were used to calculate body mass index (BMI, weight in kilograms divided by the square of height in meters). Blood pressure was measured in triplicate after a 5-min rest using either a random-zero mercury sphygmomanometer (model HEM907XL; OMRON) [14]. Glucose was assayed at baseline with the hexokinase UV method by American BioScience Laboratories (Van Nuys, California) and by hexokinase coupled to glucose-6-phosphate dehydrogenase (Merck Millipore, Billerica, Massachusetts) at years 7, 10, 15, 20, and 25. Other laboratory measures, such as serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were collected for analysis.

2.4. CAC assessment

CAC, a noninvasive marker of subclinical atherosclerosis, was assessed using multidetector computed tomography scanners (Chicago and Oakland centers, Birmingham and Minneapolis centers) at year 25. A standardized protocol for the CAC assessment process has previously been described in detail [15]. Contiguous 3-mm-thick slices from the root of the aorta to the apex of the heart were obtained. Image data were transmitted electronically to the CARDIA Reading Center, and a trained technician blinded to participant characteristics identified a region of interest for each potential foci of CAC. A calcium score in Agatston units was calculated for each calcified lesion, and scores from the four major coronary arteries (left main, left circumflex, left anterior descending, and right coronary) were summed to compute a total calcium score [16]. All image data were analyzed with high between reader and within-reader reproducibility [15].

2.5. Cardiovascular outcomes

Participants were contacted annually to identify the possible outcomes through December 2016. The primary outcome was fatal and nonfatal cardiovascular events, including coronary heart disease, cerebrovascular disease, and other heart or vascular diseases, specifically fatal and nonfatal myocardial infarction, acute coronary syndrome, hospitalization for heart failure, intervention for peripheral arterial disease, stroke, transient ischemic attack, or death from cardiovascular causes [17].

All-cause mortality (all cases of death) was examined as a secondary outcome. For each outcome, medical records, death certificates, informant interviews (for outpatient deaths), and autopsy reports, when available, were adjudicated independently by two reviewers of the Endpoint Committee. Definitions of each outcome have been previously described [17,18].

2.6. Diabetes incidence

Diabetes was assessed at each examination according to American Diabetes Association diagnostic criteria for laboratory measures and a history of diabetes medication use [19]. In this study, insulin resistance was assessed by calculating homeostatic model assessment of insulin resistance (HOMA-IR). The Homeostasis Model Assessment (HOMA) is a computational model widely recognized for its utility in predicting homeostatic concentrations associated with varying degrees of β-cell dysfunction and insulin resistance. This model facilitates the assessment of insulin resistance and β-cell function by analyzing a patient's fasting plasma insulin and glucose levels [20]. In this study, insulin resistance was assessed by calculating homeostatic model assessment of insulin resistance (HOMA-IR) as described in prior study.

2.7. Statistical analysis

Continuous variables are described as mean (standard deviation, SD) for normally distributed data or median (range) for non-normally distributed data. Categorical variables are reported as frequency (percentage). The incidence rate of CVD and all-cause mortality was calculated by dividing the number of incident cases by the total follow-up duration (person-years). The survival probability of primary outcomes according to the tertile of insulin variability was showed using unadjusted Kaplan–Meier curves. Cox proportional hazards regression was

used to estimate hazard ratios (HRs) for CVD and all-cause mortality. Prevalent CAC, a binary variable (Yes/No CAC), was assessed in logistic regression models with odds ratios (ORs), whereas linear regression models were used to calculate beta coefficients for log (CAC + 1). Model 1 was adjusted for age, race, sex, BMI, systolic blood pressure (SBP), smoking status, HDL-C and total cholesterol levels. Model 2 was adjusted for model 1 covariates and incidence of diabetes. Model 3 was adjusted for model 1 covariates and average fasting glucose (FG) levels. To account for the possible influence of insulin resistance on this association, the model further adjusted for HOMA-IR level. The potential effect modification by sex, race and diabetes status was estimated using stratified analysis (with an accompanying test for statistical interaction). A two-sided P value <0.05 was considered statistically significant. All analyses were conducted using SPSS version 24 (SPSS, Inc, Chicago, IL).

3. Results

A total of 3983 participants who satisfied the study inclusion criteria were available for analysis. The baseline characteristics are presented in Table 1. Participants were divided into three groups according to tertile of insulin ARV. Fasting insulin, insulin-SD, HOMA-IR, FG, average FG, and incident diabetes gradually increased with an increasing tertile of insulin ARV (all $P < 0.01$, Table 1). Participants with higher tertile of insulin ARV were younger, more likely black people, had higher levels of triglycerides, LDL-C, SBP, and BMI while a lower HDL-C level (all $P < 0.01$, Table 1).

The incidence and degree of CAC at year 25 are shown in Fig. 1 and Table 2. Participants with high tertile of insulin ARV had a higher incidence of CAC [32.9 % vs. 26.0 %; OR, 1.40; (95 % CI, 1.14–1.72), $P = 0.002$, Fig. 1A, Table 2]. However, after adjusting for age, race, sex, BMI, SBP, smoking status, HDL-C, and total cholesterol levels, there were no significant differences in the presence of CAC between high tertile of insulin ARV group and low tertile group (OR, 1.22; 95 % CI, 0.95–1.57, $P = 0.111$, Table 2). Our investigation extended beyond documenting CAC incidence to include quantitative analysis of calcification burden using established Agatston scoring protocols, enabling comprehensive characterization of atheroma progression dynamics. It was found that participants with high tertile of insulin ARV had more severe CAC (Fig. 1B) even if demographic characteristics and other risk factors were adjusted [Model 3, β=0.10; (95 % CI, 0.03–0.18), $P = 0.008$, Table 2]. Interestingly, higher tertile of insulin ARV was associated with significantly worse degree of CAC (β =0.1; 95 % CI, 0.03–0.18, $P = 0.008$) but not with the presence of CAC (OR, 1.18; 95 % CI, 0.92–1.52, $P = 0.197$). Similar trends were also observed in insulin-SD (Supplementary Table 1).

During a median 30.9-year follow-up, a total of 210 incident CVD events (5.27 %) and 216 deaths (5.42 %) occurred overall. CVD and death incidence rate were 1.74 and 1.77 per 1000 person-years at risk, respectively. The incidence rates of both CVD (from 1.22 to 2.92 per 1000 person-years, P value for trend < 0.001) and all-cause mortality (from 1.40 to 2.95 per 1000 person-years, P value for trend < 0.001) gradually increased with an increasing tertile of insulin ARV (Fig. 2, Supplementary Table 2). After adjusting for demographic characteristics and traditional cardiovascular risk factors, the associations between insulin ARV and CVD, and all-cause mortality remained significant [CVD, HR (95 %CI): High tertile,1.65 (1.13–2.39), $P = 0.009$; all-cause mortality, HR (95 % CI): High tertile, 1.97 (1.38–2.82), $P < 0.001$] (Table 2).

To account for a potential influence of insulin resistance, we performed another analysis adjusting by demographic characteristics, clinical covariates, and HOMA-IR level (Table 3). Association of the tertile of insulin ARV with incident CVD and all-cause mortality remained significant after adjustment for HOMA-IR level. Additionally, adjusting for HOMA-IR level also yielded similar results in the presence or the degree of CAC.

We performed a stratified analysis according to age, sex, race and diabetes status (Supplementary Table 3–6). The associations between insulin ARV and CAC progression, CVD and all-cause mortality were similar in various subgroups (all P interaction>0.05). Furthermore, we found that white participants with high insulin ARV had a significantly higher incidence rate of CAC [OR,1.45; 95 % CI, (1.01–2.10)] and CVD [HR,2.02; 95 % CI, (1.10–3.72)] compared with low insulin ARV group (Supplementary Table 5). In participants without diabetes, we also found that incidence rate of CAC [OR,1.33; 95 % CI, (1.01–1.78)], degree of CAC [β=0.13; 95 % CI, 0.04–0.21)], CVD [HR,1.57; 95 % CI, (1.01–2.43)] and all-cause mortality [HR,2.04; 95 % CI, (1.4–2.98)] significantly increased in participants with high insulin ARV (Supplementary Table 6). To assess the potential influence of fasting insulin measurement number, sensitivity analysis restricting individuals with 6 to 7 insulin measurements also produced similar results (Supplementary Table 7).

Table 1
Baseline characteristics for 3983 participants by tertile of insulin ARV.

Characteristic	Insulin ARV			P value
	Low tertile	Middle tertile	High tertile	
N	1748	1465	770	
Age, years	25.3 (3.5)	24.9 (3.6)	24.6 (3.7)	<0.001
Woman, n (%)	993 (56.8)	808 (55.2)	421 (54.7)	0.506
Black, n (%)	661 (37.8)	738 (50.4)	511 (66.4)	<0.001
BMI, kg/m^2	23.1 (3.7)	24.4 (4.5)	26.6 (5.6)	<0.001
SBP, (mmHg)	108.9 (10.5)	110.2 (10.7)	112.6 (11.4)	<0.001
Current smoker, n (%)	506 (28.9)	398 (27.2)	221 (28.7)	0.413
Total cholesterol, mg/dL	176 (32.7)	176.9 (33.1)	178.8 (33.7)	0.148
Triglycerides, mg/dL	65 (35.8)	73.2 (44.2)	80.7 (55.3)	<0.001
LDL-C, mg/dL	107.4 (30.7)	109.6 (30.7)	112.2 (31.9)	0.002
HDL-C, mg/dL	55.6 (13.3)	52.7 (12.6)	50.4 (12.1)	<0.001
Incident diabetes, n (%)	137 (7.8)	184 (12.6)	186 (24.2)	<0.001
FG, mg/dL	81 (7.7)	81.7 (8.1)	82.2 (8.7)	0.002
Average FG, mg/dL	86.3 (7.7)	88.7 (9.7)	92.3 (12.4)	<0.001
Fasting insulin, μU/mL	9.5 (2.9)	11.5 (4.7)	14.9 (8.1)	<0.001
HOMA-IR	1.9 (0.7)	2.3 (1.0)	3.1 (1.7)	<0.001
Insulin variability				
ARV, μU/mL	2.3 (0.7)	4.8 (1.0)	13.8 (15.1)	<0.001
SD, μU/mL	2.4 (0.9)	4.5 (1.6)	12.2 (11.4)	<0.001

Data were expressed as mean (SD) or n (%) as appropriate.
*P value for global test: ANOVA for continuous variables and chi-square tests for categorical variables.
ARV, average real variability; BMI, body mass index; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SD, standard deviation.

4. Discussion

In the present study, we found that young adults with high insulin variability, as determined by insulin ARV and insulin SD, have higher risk of CAC progression and increased risk of CVD and all-cause mortality, even after adjusting for average FG, incident diabetes, HOMA-IR and other traditional risk factors. In all, high insulin variability (both insulin ARV and SD) during young adulthood before the onset of diabetes well predicts increased risk of CAC progression, CVD and all-cause mortality in midlife.

Insulin is the only hormone to lower blood glucose and plays important roles in physiological and pathological functions. There is an imbalance between insulin level and glucose level in patients with type 2 diabetes, thus leading to cardio-metabolic diseases [6]. The effects of hyperinsulinemia and insulin resistance on CAC, CVD and all-cause mortality have been extensively explored. Most studies [21–23], but not all [24], found that fasting insulin and insulin resistance were independent predictors of CAC, a surrogate marker of subclinical coronary

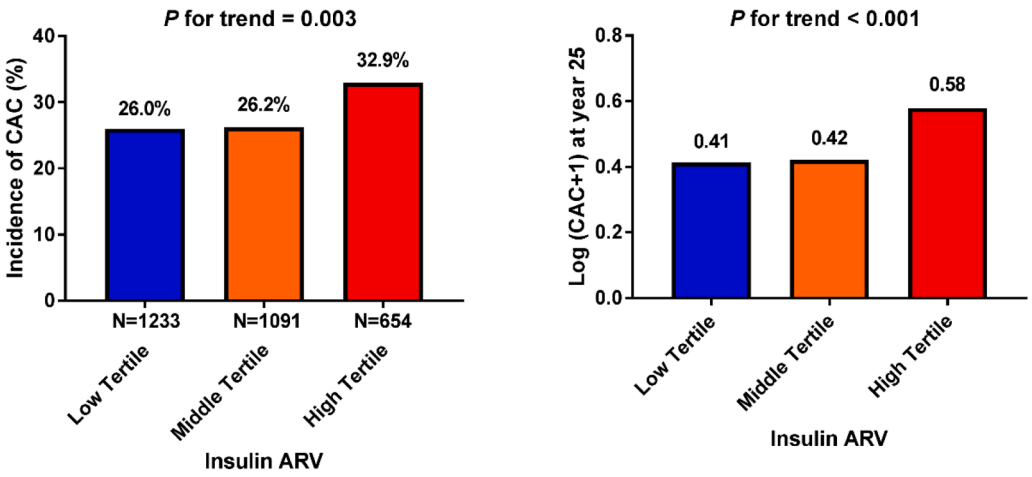


Fig. 1. The incidence (*A*) and severity (*B*) of coronary artery calcification (CAC) at year 25 in participants according to the tertile of insulin average real variability (ARV).

Table 2
Unadjusted and Multivariable-Adjusted Associations of the Tertile of Insulin ARV With Cardiovascular Outcomes.

Event	Unadjusted		Multivariable-Adjusted					
			Model 1[a]		Model 2[b]		Model 3[c]	
	Effect Size (95 % CI)	*P* value	Effect Size (95 % CI)	*P* value	Effect Size (95 % CI)	*P* value	Effect Size (95 % CI)	*P* value
Presence of CAC, OR (N = 2978)[d]								
Low tertile	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Middle tertile	1.01 (0.84, 1.22)	0.886	0.97 (0.79, 1.20)	0.791	0.97 (0.79, 1.20)	0.794	0.96 (0.78, 1.18)	0.688
High tertile	1.40 (1.14, 1.72)	0.002	1.22 (0.95, 1.57)	0.111	1.20 (0.93, 1.54)	0.155	1.18 (0.92, 1.52)	0.197
P for trend		0.003		0.156		0.222		0.239
β value for CAC (logarithm-transformed) (N = 2978)[e]								
Low tertile	0 (ref.)		0 (ref.)		0 (ref.)		0 (ref.)	
Middle tertile	0.01 (−0.06, 0.08)	0.817	0.002 (−0.06, 0.06)	0.948	0.002 (−0.06, 0.06)	0.952	−0.002 (−0.06, 0.06)	0.939
High tertile	0.17 (0.09, 0.24)	<0.001	0.12 (0.04, 0.19)	0.003	0.11 (0.03, 0.18)	0.005	0.10 (0.03, 0.18)	0.008
P for trend		<0.001		0.008		0.010		0.019
Incident CVD, HR (N = 3983)[f]								
Low tertile	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Middle tertile	1.45 (1.04, 2.01)	0.027	1.31 (0.94, 1.83)	0.117	1.30 (0.93, 1.81)	0.128	1.28 (0.91, 1.79)	0.151
High tertile	2.44 (1.74, 3.43)	<0.001	1.75 (1.21, 2.53)	0.003	1.69 (1.17, 2.45)	0.006	1.65 (1.13, 2.39)	0.009
P for trend		<0.001		0.013		0.022		0.032
All-cause mortality, HR (N = 3983)[f]								
Low tertile	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
Middle tertile	1.14 (0.82, 1.57)	0.435	1.10 (0.79, 1.53)	0.572	1.12 (0.80, 1.55)	0.519	1.12 (0.81, 1.56)	0.486
High tertile	2.11 (1.52, 2.92)	<0.001	1.85 (1.30, 2.63)	0.001	1.97 (1.38, 2.80)	<0.001	1.97 (1.38, 2.82)	<0.001
P for trend		<0.001		0.001		<0.001		<0.001

ARV, average real variability; BMI, body mass index; CAC, coronary artery calcification; CI, confidence interval; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; OR, odds ratio; SBP, systolic blood pressure.

[a] Adjusted for age, race, sex, BMI, SBP, smoking status, HDL-C and total cholesterol levels.

[b] Adjusted for model 1 covariates and incidence of diabetes.

[c] Adjusted for model 1 covariates and average fasting glucose levels.

[d] Modeling framework was logistic regression.

[e] Modeling framework was general linear models.

[f] Modeling framework was Cox proportional hazards regression.

artery disease. The associations of fasting insulin level and insulin resistance with CVD are also inconsistent in prior studies [7,25]. A meta-analysis including 11 prospective studies showed that high insulin level was related to increased cardiovascular mortality in Europeans without diabetes after adjusting for traditional risk factors [7]. However, in an 11-year follow-up longitudinal study recruiting 604 Caucasians aged 50–75 years with or without diabetes, fasting insulin level and insulin resistance were not significantly associated with CVD mortality after adjusting for age and sex [25]. A prospective study, recruiting 13,446 middle-aged participants without coronary heart disease from four U.S. communities, found that fasting insulin was independently associated with coronary heart disease only in women without diabetes, but not in men without diabetes [26]. Most studies on the association of insulin with CAC or CVD only measure insulin at a single time point [8,9,22,23]. However, insulin level at a single time point can be regulated by short-term factors, including diet, physical activity and stress [27–29]. Insulin level at a single time point may not well reflect long-term insulin level and its variability. In the present study, we firstly defined long-term insulin variability by calculating ARV and SD of fasting insulin, which may well reflect the natural level and changes of insulin over time. High long-term insulin variability during young adulthood was found to be an independent predictor of CAC progression and CVD in middle age, especially in individuals without diabetes.

Fasting insulin regulation is a heterogeneous process involving FG,

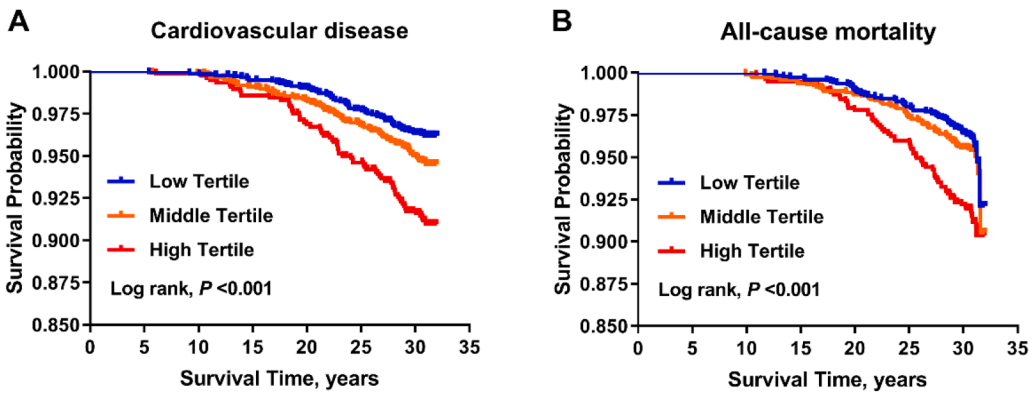


Fig. 2. Unadjusted Kaplan–Meier curves for the survival probability of cardiovascular disease (*A*) and all-cause mortality (*B*) in participants according to the tertile of insulin average real variability (ARV).

Table 3
Associations of the Tertile of Insulin ARV With Cardiovascular Outcomes Adjusting for Clinical Variables and Insulin Resistance[a].

	Effect Size (95 % CI)	*P* value
Presence of CAC, OR (N = 2978)[b]		
Low tertile	1 (ref.)	
Middle tertile	0.98 (0.79, 1.21)	0.841
High tertile	1.25 (0.97, 1.62)	0.088
P for trend		0.129
β value for CAC (logarithm-transformed) (N = 2978)[c]		
Low tertile	0 (ref.)	
Middle tertile	0.02 (−0.06, 0.06)	0.953
High tertile	0.11 (0.04, 0.19)	0.004
P for trend		0.011
Incident CVD, HR (N = 3983)[d]		
Low tertile	1 (ref.)	
Middle tertile	1.30 (0.93, 1.82)	0.127
High tertile	1.71 (1.17, 2.51)	0.006
P for trend		0.021
All-cause mortality, HR (N = 3983)[d]		
Low tertile	1 (ref.)	
Middle tertile	1.08 (0.78, 1.51)	0.631
High tertile	1.78 (1.24, 2.55)	0.002
P for trend		0.004

ARV, average real variability; BMI, body mass index; CAC, coronary artery calcification; CI, confidence interval; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; OR, odds ratio; SBP, systolic blood pressure.
HOMA-IR, homeostatic model assessment of insulin resistance.
[a] Adjusted for age, race, sex, BMI, SBP, smoking status, HDL-C, total cholesterol levels and HOMA-IR.
[b] Modeling framework was logistic regression.
[c] Modeling framework was general linear models.
[d] Modeling framework was Cox proportional hazards regression.

insulin secretion, insulin clearance, insulin resistance, and insulin sensitivity [30]. It is known that hyperinsulinemia and insulin resistance can directly disrupt insulin signaling in endothelial cells, vascular smooth muscle cells and macrophages, and thus increase the progression of atherosclerosis [6]. Besides, hyperinsulinemia and insulin resistance contribute to systemic risk factors, including obesity, hypertension, dyslipidemia and hyperglycemia, all of which promote the development of CVD [6]. However, our study found that the associations of insulin variability (both insulin ARV and SD) with CAC progression and CVD were still significant after adjustment for insulin resistance indicator HOMA-IR and related systemic factors, including BMI, SBP, cholesterol levels and average FG levels. Therefore, insulin variability may be an important characteristic of insulin dysregulation independent of insulin resistance and other traditional risk factors. In addition, advanced glycation end products (AGEs) play a prominent role in atherosclerosis and CAC progression [31,32]. Anand et al. emphasized that hyperglycemia's major role in advancing CAC progression in diabetes patients and the crucial need for maintaining optimal blood sugar levels to curb this process [33]. Hereon, we explained that elevated insulin variability indirectly reflects to blood glucose fluctuations, which subsequently promote calcified plaque development through AGEs-mediated pathways. Moreover, coexisting insulin resistance exacerbates vascular endothelial dysfunction and creates a permissive microenvironment for accelerated calcium deposition through multifactorial mechanisms [34]. In the present study, the associations of insulin variability with CAC and CVD were more significant in participants without diabetes than in those with diabetes. The possible reason is that diabetes medications and interventions have an impact on insulin level or variability and further alleviate the development of CAC and CVD [3,35].

The association of insulin with all-cause mortality in individuals without diabetes or general population in previous studies is also conflicting [36–38]. A meta-analysis including 26,976 participants without diabetes from seven studies found that HOMA-IR, but not fasting insulin, had independent association with elevated risk of all-cause mortality [36]. A large perspective study including 22,837 participants from the Women's Health Initiative (WHI) found that high insulin resistance, as measured by HOMA-IR, predicted higher all-cause mortality in postmenopausal women [39]. However, in a prospective study recruiting 8533 individuals aged over 35 years from the general population in Australian showed that HOMA-IR was not significantly associated with all-cause mortality [38]. In our study, we found that higher insulin variability during young adulthood independently predicted all-cause mortality, even after adjusting for HOMA-IR. Insulin dysregulation is also associated with non-cardiovascular morbidity.

The contemporary understanding of cardiovascular risk continuum establishes that cardiovascular disease progression constitutes a spectrum rather than a binary classification (e.g., high/low risk). This continuum encompasses sequential phases from initial risk factors through subclinical manifestations to overt cardiovascular events [40]. Our research demonstrates that elevated insulin variability may promotes atherogenesis via oxidative stress and inflammatory activation mechanisms. These findings position insulin variability as a potential youth-specific predictive marker within this continuum. In clinical practice, strategies such as dipeptidyl peptidse 4 (DPP-4) inhibitor administration, closed-loop insulin pump therapy, resistance training protocols, and mediterranean dietary adherence are recommended to reduce insulin variability [41]. The investigation of cardiovascular risk continuum is transitioning from unidimensional stratification towards dynamic multidimensional appraisal, with strategic integration of technological innovations and evidence-based interventions serving as the pivotal driver.

The current study has several strengths. First, an advantage of the present study is its status as a prospective study with a relatively large sample size and a long follow-up period in young adults. Its data

collection protocols and quality control were well supervised. The long-term insulin variability was mainly assessed in participants without diabetes, avoiding the disturbance of medications and short-time stimulates and showing the nature level of insulin before the onset of diabetes. The associations of insulin variability with cardiovascular outcomes were highlighted after adjusting for multiple variables including average FG levels, HOMA-IR and other traditional risk factors. However, there are also several limitations in this study. First, this study specifically recruited black and white participants. Therefore, the conclusion may not be generalizable to other racial and ethnic groups. Second, we did not assess CAC at baseline, so we do not know with certainty that CAC developed over the years. However, epidemiological evidence showed the incidence of CAC is very rare in young adults [36, 42]. Lastly, as an observational study, there may be other residual and unknown confounding factors that involve in the associations between insulin variability and cardiovascular outcomes despite multiple adjustments.

5. Conclusion

In total, this prospective study found that high long-term insulin variability (both insulin ARV and SD) during young adulthood before the onset of diabetes helps to predict CAC progression, CVD and all-cause mortality in middle age, independently of average FG levels, HOMA-IR and other traditional risk factors. Further studies are needed to explore the characteristic of insulin variability and its relation to other indicators of insulin dysregulation.

Author agreement

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

CRediT authorship contribution statement

Kun Zhang: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Chunlan Huang:** Writing – review & editing, Writing – original draft, Funding acquisition. **Junping Li:** Writing – review & editing, Writing – original draft, Software. **Peibiao Mai:** Writing – review & editing, Writing – original draft, Formal analysis. **Shuwan Xu:** Visualization, Data curation. **Feifei Huang:** Methodology, Data curation. **Wanbing He:** Methodology, Data curation. **Huanji Zhang:** Validation, Supervision, Resources. **Yang Liu:** Funding acquisition, Conceptualization. **Weijing Feng:** Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajpc.2025.100952.

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