Fasting glucose variability in young adulthood and incident diabetes, cardiovascular disease and all-cause mortality

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Abstract

Aims/hypothesis The aim of this study was to determine whether long-term intra-individual variability in fasting glucose (FG) during young adulthood is associated with incident diabetes, cardiovascular disease (CVD) and mortality.

Methods We included participants from the Coronary Artery Risk Development in Young Adults (CARDIA) study, ages 18–30 years at baseline (1985–1986) and followed with eight examinations for up to 30 years. Long-term glucose variability was assessed using the CV (CV-FG) and the absolute difference between successive FG measurements (average real variability; ARV-FG). For participants who developed any event (diabetes, CVD or mortality), FG variability measurement was censored at the examination prior to event ascertainment. We estimated HRs for incident diabetes, CVD and mortality with adjustment for demographics, baseline FG, change in FG (censor – baseline) and time-varying education, smoking, alcohol consumption, BMI, physical activity, systolic BP, BP medications, LDL-cholesterol and cholesterol medications (and incident diabetes and diabetes medications for CVD and mortality outcomes).

Results Among 3769 black and white participants, there were 317 incident diabetes cases (102,677 person-years), 159 incident CVD events (110,314 person-years) and 174 deaths (111,390 person-years). After adjustment, HRs per 1 SD higher ARV-FG were 1.64 (95% CI 1.52, 1.78) for diabetes, 1.15 (95% CI 1.01, 1.31) for CVD and 1.25 (95% CI 1.11, 1.40) for mortality. The HRs per 1 SD higher CV-FG were 1.39 (95% CI 1.21, 1.58) for diabetes, 1.32 (95% CI 1.13, 1.54) for CVD and 1.08 (95% CI 0.92, 1.27) for mortality, after adjustment. The cause-specific HRs per 1 SD higher ARV-FG were 1.29 (95% CI 1.14, 1.47) for non-CVD death and 1.05 (95% CI 0.76, 1.45) for CVD death. We did not observe evidence for effect modification of any association by sex or race.

Conclusions/interpretation Our results suggest that higher intra-individual FG variability during young adulthood before the onset of diabetes is associated with incident diabetes, CVD and mortality.

Keywords Cardiovascular disease · Epidemiology · Fasting blood glucose · Glucose variability · Mortality · Type 2 diabetes · Young adulthood

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Research in context

What is already known about this subject?

- Blood glucose variability is present during middle and older age, particularly among individuals with diabetes
- Greater glucose variability is associated with higher risk for incident cardiovascular disease and mortality

What is the key question?

- Is variability in blood glucose level present during young adulthood before the onset of diabetes and, if so, associated with risk for diabetes, cardiovascular disease and death?

What are the new findings?

- Greater fasting glucose variability during young adulthood before the onset of diabetes was associated with greater risk for diabetes, cardiovascular disease and death during middle age

How might this impact on clinical practice in the foreseeable future?

- If further work proves variable glucose levels are a marker of future risk, healthcare providers should consider monitoring of glucose levels for individuals who experience variable glucose levels over time

Abbreviations

ARV	Average real variability
ARV-FG	Average real variability of fasting glucose
CARDIA	Coronary Artery Risk Development in Young Adults
CVD	Cardiovascular disease
CV-FG	CV of fasting glucose
EU	Exercise unit
FG	Fasting glucose

Introduction

For individuals with diabetes, prevention of complications requires maintenance of blood glucose levels within a commonly accepted range and limiting the hypo- and hyperglycaemic excursions from this range [1, 2]. Recent epidemiological research suggests that greater long-term variability in traditional measures of blood or plasma glucose during middle and older age is associated with higher risk for cardiovascular disease (CVD) events and mortality among adults with diabetes [3–14]. However, among adults without diabetes at study initiation, studies that have reported an association between glucose variability and outcomes have not taken into account subsequent development of diabetes and diabetes medication use. Furthermore, blood glucose tends to increase over the lifespan and it is important to distinguish long-term variability in glucose from increases in glucose that occur with aging and in association with aging-related comorbidities.

The Coronary Artery Risk Development in Young Adults (CARDIA) study is an ideal setting to determine the natural variability of glucose over 30 years beginning during young

adulthood in a population followed into middle age for incident diabetes, CVD events and mortality. Our objective was to assess whether variability in fasting glucose (FG) during young adulthood is associated with incident diabetes, CVD and mortality. We hypothesised that higher variability of FG during young adulthood would be associated with higher risk for incident diabetes, CVD and mortality as compared with lower FG variability, independent of increases in glucose over time.

Methods

Study population CARDIA is a longitudinal observational study of individuals recruited from four US metropolitan communities. Details regarding the CARDIA study design have been published previously [15]. Briefly, 5115 black and white men and women aged 18–30 years who were free from CVD were enrolled in 1985–1986 [15]. Participants were invited to participate in follow-up examinations 2, 5, 7, 10, 15, 20, 25 and 30 (2015–2016) years after baseline. Participation rates at these examinations were high: 91%, 86%, 81%, 79%, 74%, 72%, 72% and 71%, respectively, of the surviving cohort. Written informed consent was obtained at enrolment and each subsequent examination. Institutional review boards at each study site and coordinating centre have granted approval for all examinations and contacts.

Structured questionnaires were used at each clinic visit to collect information on participant demographics, medical history and use of medications, and health behaviours, such as past and current use of tobacco, regular alcohol consumption and leisure-time physical activity [15, 16]. CARDIA researchers derived an overall physical activity score in

‘exercise units’ (EU), with a score of 300 EU corresponding to 30 min of moderate physical activity, five times per week. [17] Prior to examination year 20, seated BP was measured in triplicate after 5 min of rest using a random-zero sphygmomanometer. At year 20 and later, BP was measured using an automated oscillometric BP monitor (Omron HEM-907XL; Online Fitness, Santa Monica, CA, USA) with values standardised across examinations to the sphygmomanometric measures; systolic and diastolic BP were determined as the average of the last two measurements [18]. Body weight was measured with a calibrated balance beam scale and height was measured with a vertical ruler. BMI was calculated as weight in kilograms divided by height in meters squared. At each examination, blood was drawn by venipuncture and serum separation was performed before aliquots were stored at -70°C and shipped on dry ice to central laboratories. Total cholesterol and triacylglycerols were measured enzymatically within 6 weeks of collection, HDL-cholesterol was determined by precipitation with dextran sulphate-magnesium chloride and LDL-cholesterol was determined by the Friedewald equation [19–21].

FG and incident diabetes FG was determined in non-pregnant individuals who reported fasting ≥ 8 h at baseline and 7, 10, 15, 20, 25 and 30 years after baseline. At baseline, serum glucose was measured using the hexokinase ultraviolet method by American Bio-Science Laboratories (Van Nuys, CA, USA). At examination years 7–20, serum glucose was measured using hexokinase coupled to glucose 6-phosphate dehydrogenase by Linco Research (St Louis, MO, USA). Quality control was performed using a commercially purchased pool of controls; within-run precision was below 1% CV and between-run precision was below 2%. Serum glucose was measured at years 25 and 30 at the Collaborative Studies Clinical Laboratory at the University of Minnesota. Test–retest correlation between the year 25 and 30 assays was 0.988. The data used herein employ findings from a recalibration study, performed to harmonise glucose values across CARDIA examinations [22]. Diabetes was determined at each examination using a combination of available information on $\text{FG} \geq 7.0$ mmol/l (examination years 0 and 7–30), 2 h 75 g OGTT ≥ 11.1 mmol/l (examination years 10, 20 and 25), non-fasting glucose ≥ 11.1 mmol/l (examination years 0 and 7–30), $\text{HbA}_{1c} \geq 48$ mmol/mol ($\geq 6.5\%$) (examination years 20 and 25) and use of diabetes medications (all examinations), previously assessed in CARDIA [23].

CVD events and mortality During study examinations and annual contacts completed via mail, phone or email using standardised questionnaires, participants or designated proxies were queried about overnight hospital admissions, outpatient procedures and deaths that occurred since the last study contact. Additional semi-annual contacts were conducted, also

via mail, phone or email, and were designed to provide research staff with the opportunity to update participants’ vital status and contact information. Medical records were requested for potential cardiovascular hospitalisations and outpatient procedures. Vital status was also obtained through periodic surveillance of the National Death Index. Trained physicians using detailed event definitions reviewed medical records, autopsy reports, death certificates, informant interviews and other evidence, and adjudicated cases independently in pairs. In the instance of disagreement in initial event adjudication, the case was reviewed by the full CARDIA Endpoints Committee. The primary CVD outcome was the first occurrence of any fatal or non-fatal CVD event, including myocardial infarction, non-myocardial infarction acute coronary syndrome, coronary revascularisation, stroke, transient ischaemic attack, hospitalisation for acute decompensated heart failure, intervention for peripheral arterial disease or underlying cause of death due to CVD. We recorded incident CVD events and mortality that occurred between the baseline examination and 31 August 2016.

Statistical analysis We excluded one person who withdrew consent during follow-up and 1345 individuals who had fewer than three FG measures. We calculated two measures of intra-individual FG variability for each participant: CV (CV-FG) and average real variability (ARV; ARV-FG). CV-FG (%) was calculated for each individual as the SD of FG divided by mean FG, and then divided by the square root of the ratio of FG measurements (n) to $n - 1$, $\sqrt{n/(n-1)}$, to account for the possible influence on CV of the FG measurement number [24]. ARV has been previously modelled for BP in CARDIA [25]. Here, ARV-FG (mmol/l per year) was calculated as the absolute difference between successive measurements of FG, divided by the duration between FG measurements, to create annualised ARV-FG between examinations. We summed over examinations and divided by the number of years contributing to ARV-FG determination, to account for variable duration of ARV-FG. To minimise the potential for diabetes medications or clinical CVD events to influence glucose variability determination, FG values measured at or after examinations where identification of diabetes or a CVD event occurred did not contribute to CV-FG or ARV-FG. This novel methodological approach is in contrast to the prior research on this topic, where glucose measures with concomitant medication use contributed to variability [26, 27]. Change in FG was determined as the difference in FG at the last examination for FG variability measurement before censoring and baseline FG (i.e., last FG value minus baseline FG).

Participants who missed an examination were encouraged to participate in subsequent examination years, but missing data are mostly due to participants not returning and are not missing at random. Missing covariate data were generated

using two-step fully conditional specification imputation methods [28, 29]. The proportion of observations missing data before imputation was largest for LDL-cholesterol, ranging from 8% to 17% over all examinations, and less than 10% for all other covariates at each examination.

We used extended Cox proportional hazards regression to estimate the association of a 1 SD increment for continuous ARV-FG and CV-FG with incident diabetes, CVD and all-cause mortality. Our modelling approach included adjustment for age, sex, race, field centre, baseline FG value, change in FG and time-varying data for educational attainment, smoking status, regular alcohol consumption, BMI, physical activity, systolic BP, use of BP-lowering medication, LDL-cholesterol and use of cholesterol-lowering medication. For the outcomes of CVD and mortality, we included time-varying incident diabetes and use of diabetes medication in the adjustment scheme. Person-time at risk for each respective

outcome started at baseline and ended at the examination at which the respective event was ascertained, date of respective event from medical/death records or date of last contact. We assessed for collinearity between adjustment variables by calculating variance inflation factors and did not find evidence for collinearity; all variance inflation factors were <1.6. We assessed the sensitivity of our estimates (1) when excluding individuals with missing baseline covariate data, without imputing missing data and with carrying forward prior values; (2) when censoring individuals who developed diabetes during follow-up and treating this as a competing risk for CVD and mortality outcomes; and (3) when restricting the sample to individuals who had FG values at each of the first four glucose assessments. To better understand the association between FG variability and cause-specific death, we assessed the HRs for CVD-related deaths and non-CVD deaths taking into account competing risk of the alternative cause of death [30]. We

Table 1 Characteristics at baseline for 3769 participants by quartile of ARV in FG from 1985–1986 to 2010–2011

Characteristic	ARV in FG				<i>p</i> value ^a
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
ARV glucose, mmol/l per year, range	0.0001, 0.008	0.009, 0.012	0.013, 0.018	0.019, 0.08	
<i>n</i>	942	942	943	942	
Age, years	24.7 ± 3.7	25.3 ± 3.5	24.9 ± 3.6	25.1 ± 3.6	<0.005
Women	560 (59)	542 (58)	542 (57)	463 (49)	<0.001
Black	470 (50)	398 (42)	409 (43)	499 (53)	<0.001
Education, >4 years college	91 (10)	133 (14)	105 (11)	70 (7)	<0.001
Current smoker	247 (26)	240 (25)	250 (27)	301 (32)	<0.05
No daily alcohol	389 (41)	354 (38)	371 (39)	352 (37)	0.53
BMI, kg/m ²	24.1 ± 5.0	23.9 ± 4.3	24.1 ± 4.3	24.8 ± 5.2	<0.001
Systolic BP, mmHg	109.6 ± 10.9	109.7 ± 10.8	109.5 ± 10.3	111.1 ± 10.8	<0.005
Diastolic BP, mmHg	68.4 ± 9.2	68.5 ± 9.0	68.0 ± 9.7	68.8 ± 9.9	0.34
BP-lowering medication use	29 (3)	9 (1)	20 (2)	18 (2)	<0.05
Physical activity, EU	429 ± 305	408 ± 298	424 ± 289	419 ± 296	0.48
LDL-cholesterol, mmol/l	2.81 ± 0.8	2.86 ± 0.8	2.83 ± 0.76	2.81 ± 0.82	0.50
FG at baseline, mmol/l	4.57 ± 0.38	4.54 ± 0.43	4.51 ± 0.44	4.50 ± 0.52	<0.05
FG at variability censoring examination, mmol/l	5.13 ± 0.43	5.22 ± 0.51	5.27 ± 0.57	5.40 ± 0.66	<0.001
Weighted average FG, mmol/l	4.93 ± 0.32	4.98 ± 0.33	5 ± 0.34	5.08 ± 0.38	<0.001
Incident diabetes before event of administrative censoring	39 (4)	46 (5)	67 (7)	165 (18)	<0.001
Number of glucose measurements ^b	4.7 ± 1.3	5.6 ± 1.3	5.9 ± 1.3	5.5 ± 1.5	<0.001
CV glucose, %	5.7 ± 2.6	7.0 ± 2.6	7.7 ± 2.4	10.0 ± 3.2	<0.001
ARV glucose, mmol/l per year	0.006 ± 0.002	0.01 ± 0.001	0.015 ± 0.002	0.025 ± 0.008	<0.001
Proportion of ARV in a positive direction, %	0.75 ± 0.24	0.70 ± 0.20	0.66 ± 0.18	0.63 ± 0.19	<0.001
Have the majority of ARV in a positive direction	799 (85)	785 (83)	793 (84)	727 (77)	<0.001

Data are mean ± SD or *n* (%) unless stated otherwise

ARV in FG was calculated as the absolute difference between successive measurements of FG, divided by the duration between FG measurements, to create annualised ARV-FG between examinations. Annualised ARV-FG was then summed over examinations and divided by the number of years contributing to ARV-FG determination

^a *p* value for global test: ANOVA for continuous variables and Pearson χ^2 tests for categorical variables

^b Range *n*: 3–7

assessed effect measure modification of each association by race and sex. SAS software version 9.4 (SAS Institute, Cary NC) was used for all analyses.

Results

Among 3769 CARDIA participants initially free from CVD, the mean (SD) age at enrolment was 25.0 (3.6) years, 47% were African-American and 56% were women. Table 1 presents participant characteristics according to quartile of ARV-FG only, as characteristics were generally similar for CV-FG quartiles (electronic supplementary material [ESM] Table 1). Current smoking at enrolment was associated with greater subsequent variability for both ARV-FG and CV-FG. For both ARV-FG and CV-FG, the incidence of diabetes over follow-up was highest among the top quartile of variability. Older age, male sex and greater BMI, SBP and number of glucose measurements were all associated with greater ARV-FG over follow-up, but not with CV-FG. Lower percentage of variability in the positive direction was associated with greater ARV-FG over follow-up.

Over a median follow-up of 29.8 years, there were 317 incident diabetes cases (102,677 person-years), 159 incident CVD events (110,314 person-years) and 174 deaths from all causes (111,390 person-years). In an unadjusted model, the HR for incident diabetes per 1 SD higher ARV-FG was 1.78 (95% CI 1.66, 1.91) (Fig. 1a). After adjustment for age, sex, race, field centre, baseline FG, and change in FG and time-varying risk factors, the diabetes HR per 1 SD higher ARV-FG was 1.64 (1.52, 1.78). The association between 1 SD higher CV-FG and incident diabetes was similar in direction and lower in magnitude for each model (unadjusted HR 1.50; 95% CI 1.35, 1.66 and adjusted HR 1.39; 95% CI 1.21, 1.58) (Fig. 1b).

Before adjustment, greater ARV-FG was associated with greater HRs for incident CVD (Fig. 1a). The HR for incident CVD per 1 SD higher ARV-FG was 1.34 (95% CI 1.17, 1.52). The HR for CVD after adjustment for age, sex, race, field centre, baseline FG and change in FG per 1 SD higher ARV-FG was 1.25 (95% CI 1.10, 1.42) and this association remained after adjustment for time-varying CVD risk factors. The association between CV-FG and incident CVD was similar in magnitude and direction for each model (Fig. 1b).

In an unadjusted model, 1 SD higher ARV-FG was associated with 37% higher HR (95% CI 1.22, 1.55) for all-cause mortality (Fig. 1a). These HRs were slightly attenuated after adjustment for basic demographics, baseline FG and change in glucose and after further adjustment for time-varying CVD risk factors, but remained significantly associated with all-cause mortality (HR 1.25; 95% CI 1.11, 1.40). The association between 1 SD higher CV-FG and mortality was similar in direction, but not strong in magnitude (Fig. 1b). Of the 174

Fig. 1 Multivariable adjusted HRs (95% CIs) for incident diabetes (blue marker), CVD (red marker) and all-cause mortality (grey marker) according to 1 SD higher (a) ARV (1 SD: 0.009 mmol/l per year) in FG and (b) CV (1 SD: 3.4%) in FG. Model 1 is adjusted for age, sex, race, field centre, baseline FG and change in FG from baseline to the examination of last glucose variability measurement. Model 2 includes the variables in model 1 and further adjustment for time-varying educational attainment, smoking status, alcohol consumption, physical activity, BMI, systolic BP, BP-lowering medication use, LDL-cholesterol and cholesterol-lowering medication use. For CVD and mortality outcomes, model 2 also includes adjustment for time-varying incidence of diabetes and diabetes medication use

deaths, 29 were classified as CVD mortality. Deaths due to any cancer ($n = 34$) were the most frequent non-CVD causes. The association between ARV-FG and overall mortality was mainly due to non-CVD deaths. The cause-specific HRs per 1 SD higher ARV-FG were 1.29 (95% CI 1.14, 1.47) for non-CVD death and 1.05 (95% CI 0.76, 1.45) for CVD death. HRs from the subdistribution model taking into account competing risk of death were similar in magnitude and direction to the cause-specific HRs.

When assessing each association according to quartile of ARV-FG, we observed a potential dose-response for ARV-FG quartile with hazards for diabetes (Fig. 2). We observed a possible threshold effect wherein HRs were substantively higher for the highest quartile, but not the interquartile range, compared with the lowest quartile for both incident CVD and mortality. We did not observe evidence for effect modification on the multiplicative scale of any association by sex or race

Fig. 2 Multivariable adjusted HRs (95% CIs) for incident diabetes (blue marker), CVD (red marker) and all-cause mortality (grey marker) according to quartile of ARV in FG. HRs are adjusted for age, sex, race, field centre, baseline FG, change in FG from baseline to the examination of last glucose variability measurement and time-varying educational attainment, smoking status, alcohol consumption, physical activity, BMI, systolic BP, BP-lowering medication use, LDL-cholesterol and cholesterol-lowering medication use. For CVD and mortality outcomes, model 2 also includes adjustment for time-varying incidence of diabetes and diabetes medication use

(all p for interaction >0.05). Estimates from non-imputed data and when censoring individuals at the time of diabetes development during follow-up were similar in strength and direction of association to the primary findings (ESM Tables 2 and 3). When the sample was restricted to individuals who had FG values at each of the first four glucose assessments, we observed negligible attenuation in the HRs for each association, with the exception of the association between CV-FG and all-cause mortality where we observed a strengthening of the association (ESM Table 4).

Discussion

In this prospective cohort of adults followed for 30 years, greater intra-individual variability in FG during young adulthood was associated with higher risk for incident diabetes, CVD and all-cause mortality by middle age. Importantly, glucose variability was assessed before the onset of diabetes, measured by both absolute average difference between successive measurements and as a percentage of mean exposure, and was consistently associated with incident diabetes, CVD and mortality. Both measures of glucose variability were associated with incident diabetes, CVD and mortality after adjustment for baseline glucose level, change in glucose over follow-up, and time-varying changes in traditional diabetes and CVD risk factors, including the development of diabetes and diabetes medication use after the assessment of glucose variability for CVD and mortality outcomes. Furthermore, the findings for CVD and all-cause mortality were consistently observed with multiple approaches for sensitivity analyses to

address the development of diabetes over follow-up. Together, these results suggest that higher glucose variability before the onset of diabetes may be a harbinger of serious health outcomes.

Our findings of an association of long-term glycaemic variability with CVD and mortality align with other studies on this topic [3–12, 31, 32]. A preponderance of this previous research has been focused on glycaemic variability among individuals with frank type 2 diabetes [3–7, 9, 10]. Findings from interventions among diabetes populations suggest that greater visit-to-visit variability over 2–7 years is associated with greater risk for incident micro- and macrovascular events [3, 7] and mortality [3, 4]. Our multivariable adjusted incident CVD HR estimates per 1 SD in ARV-FG are similar to those reported from the Veteran's Affairs Diabetes Trial [7]. Our estimates per 1 SD in ARV-FG, but not CV-FG, with incident CVD and mortality were similar to those observed in the Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation Trial [3]. The unadjusted and adjusted estimates for the association per 1 SD CV-FG with incident diabetes observed here in CARDIA are higher compared with those reported from a study population drawn from the Korean National Health Insurance System [31]. This study included an older population at baseline, shorter follow-up, annual FG assessment and a different diabetes case definition of $\text{FG} \geq 7.0$ mmol/l or an insurance claim for diabetes medication use, compared with our current work. In contrast to our findings, Ghose et al did not observe an association between glucose variability and incident diabetes using a cohort drawn from the Copenhagen (Denmark) General Practitioner's Laboratory [32]. This work by Ghose et al differed methodologically from our work and included an older study population, shorter follow-up, three annual HbA_{1c} measurements and a diabetes case definition of discharge diagnosis of type 2 diabetes or treatment with oral glucose-lowering drugs. Investigators of the Kailuan study, a large population-based cohort in China, reported an association of CV-FG with incident CVD and mortality [8]. They observed a potential threshold effect for higher risk limited to the top quartile of variability and most pronounced among individuals who did not have diabetes [8]. In our current study, we focused on assessing glucose variation before the onset of diabetes in relation to incident CVD and mortality and we adjusted for the subsequent development of diabetes. Our approach is a methodological strength because we only included FG measurements prior to diabetes onset in our glucose variability measures to reduce the potential for residual confounding due to the initiation of glucose-lowering medications. Diabetes medication use disrupts the natural course of glucose variability and may alter any association of glucose variability with incident CVD and mortality.

We were able to extend prior work in this area on the causes of death associated with higher glucose variability. In our

study, over 80% of deaths were due to non-CVD causes; glucose variability was associated with higher risk for incident CVD events and for non-CVD mortality, but not with CVD mortality. One potential explanation is that a non-fatal CVD event may result in clinical intervention of CVD risk factors and secondary prevention of CVD mortality. Glucose variability was not associated with CVD mortality, but we had a small number of events. The association we observed with non-CVD mortality of various origins suggests that glucose variability may be marker for underlying poor health.

There are multiple potential pathophysiological mechanisms that link long-term glucose variability with incident diabetes, CVD and mortality. Firstly, acute glucose fluctuations are shown to trigger production and release of oxidative stress isomers to a greater extent than sustained episodes of hyperglycaemia [33]. Secondly, transient hyperglycaemia may induce lasting epigenetic changes that activate inflammatory pathways [34]. Lastly, higher long-term variability may be a corollary of underlying poor health and other comorbidities. While CARDIA participants were generally healthy at enrolment, participants who had the highest variability over follow-up were more likely to smoke, and had higher BMI and BP at baseline. We observed slightly stronger associations of ARV-FG with CVD and mortality than for CV-FG, though associations were consistently in the same direction. Different metrics of variability rely on unique statistical assumptions which may lend to stronger correlation with other statistical estimates (e.g., mean and change) and may also reflect distinct physiological conditions (e.g., direction of variability and absolute variability rather than percentage) [13]. Future research should assess the utility of multiple measures of blood or plasma glucose and variability with various morbidities and mortality.

Strengths of our study include generalisability of our findings to populations with similar demographic, health behaviour and cardiovascular risk profiles. Unlike prior studies, we characterised long-term glucose variability during young adulthood before the onset of diabetes in a biracial cohort. CARDIA employs thorough CVD event adjudication and we used rigorous statistical methods to disentangle glucose variability from bulk increases and to update time-varying CVD risk factors. Some limitations of our study merit consideration. The number of FG measurements per individual was not equal across the spectrum of glucose variability; however, the pattern of association was similar when accounting for the variable number of measurements and duration between measurements. The duration between FG measurements was 3–7 years and may not reflect short-term variability in glucose traditionally assessed among individuals with diabetes. HbA_{1c} and 2 h glucose were measured less frequently and at later points during CARDIA follow-up, preventing the assessment of variability for these measures and their association with incident CVD and mortality. It is possible that the assessment of glucose variability in this study is less sensitive than variability that incorporates

information from fasting and 2 h glucose and HbA_{1c}. We are unable to distinguish whether the lowering of glucose is intentional or unintentional. Among individuals with high normal glucose levels, there is unequivocal benefit from lowering glucose with intentional lifestyle and pharmacologic therapies [35, 36]. We caution not to discourage lowering high glucose with established therapies and we encourage achieving normal consistent levels of glucose. Lastly, we did not observe similar strengths in magnitude of associations between each variability measure and higher risk for mortality. It is possible that CV-FG is a less precise measurement of variability than ARV-FG, particularly at lower glucose levels where a given SD would have a greater influence on the CV-FG than a similar SD at higher glucose levels. With a less precise exposure and a non-specific outcome such as all-cause mortality, we would expect to observe attenuation toward the null and wider confidence limits. To that point, while our estimates per 1 SD increment in CV-FG with risk for all-cause mortality are comparable to those of another recent study, our precision around these HR estimates is lower [37].

In this longitudinal study of black and white adults, we observed that greater glucose variability during young adulthood was associated with higher risk for incident diabetes, CVD and mortality. Associations with mortality were predominantly due to deaths from non-CVD origin. Future research should focus on understanding whether young adults experience variability in other measures of glucose such as HbA_{1c} and 2 h glucose and whether glucose variability is a corollary of other morbidity or a potential modifiable risk factor for fatal events.

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Data availability The datasets analysed during the current study are not publicly available due to the possibility that some information in these data might compromise research participants' privacy or consent. However, data are available from the corresponding author on request and de-identified data to the end of CARDIA examination year 25 are publicly available at: <https://biolincc.nhlbi.nih.gov/studies/cardia/>.

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Contribution statement MPB is the guarantor of this work, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. MPB and MRC conceptualised the study and designed the analysis plan. MPB performed all statistical analyses and drafted the manuscript. MRC provided supervision to MPB. All coauthors, MPB, APC, CEL, EPG, JPR, PJS, YY and MRC, contributed to the acquisition, analysis or interpretation of data; provided critical revision of the manuscript for important intellectual content and contributed to the discussion; and approve the final version of this manuscript.

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