Association between glucose variation and lower extremity amputation incidence in individuals with type 2 diabetes: a nationwide retrospective cohort study

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Abstract

Aims/hypothesis Elevated glucose level is one of the risk factors for lower extremity amputation (LEA), but whether glycaemic variability confers independent risks of LEA remains to be elucidated. This study aimed to investigate the association between visit-to-visit glycaemic variability and minor and major LEA risks during 8 years of follow-up in type 2 diabetic individuals aged 50 years and older.

Methods This retrospective cohort study included 27,574 ethnic Chinese type 2 diabetic individuals aged ≥ 50 years from the National Diabetes Care Management Program in Taiwan. Glycaemic variability measures were presented as the CVs of fasting plasma glucose (FPG-CV) and of HbA_{1c} (A_{1c}-CV). The effect of glycaemic variability on the incidence of LEA events was analysed using Cox proportional hazards models.

Results After a median follow-up of 8.9 years, 541 incident cases of LEA with a crude incidence density rate of 2.4 per 1000 person-years were observed. After multivariate adjustment, FPG-CV and A_{1c}-CV were found to be significantly associated with minor LEA, with corresponding HRs of 1.53 (95% CI 1.15, 2.04) and 1.34 (95% CI 1.02, 1.77) for the third tertiles of FPG-CV and A_{1c}-CV, respectively. In addition, these associations were stronger amongst older adults with longer diabetes duration (≥ 3 years) than amongst those with shorter duration (< 3 years) ($p_{\text{interaction}} < 0.01$).

Conclusions/interpretation Our study suggests that visit-to-visit variations in HbA_{1c} and FPG are important predictors of minor LEA amongst older adults with type 2 diabetes, particularly for those with more than 3 years of diabetes duration.

Keywords Glycaemic variability · Lower extremity amputation · Type 2 diabetes

Abbreviations

A_{1c}-ARV Average real variability in HbA_{1c}
 A_{1c}-CV CV of HbA_{1c}

ARV Average real variability
 CHF Congestive heart failure
 CKD Chronic kidney disease

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Research in context

What is already known about this subject?

- Evidence from meta-analysis research indicates that raised HbA_{1c} is associated with an increased incidence of lower extremity amputation (LEA)
- Limited evidence is available regarding the relationship between glucose variability and the risk of LEA in individuals with diabetes

What is the key question?

- What are the effects of visit-to-visit variation in fasting plasma glucose and HbA_{1c} on minor and major LEA risks in individuals with type 2 diabetes?

What are the new findings?

- The present study indicated that CVs of both fasting plasma glucose (FPG-CV) and HbA_{1c} (A_{1c}-CV) exert independent effects on minor LEA risk
- Amongst individuals with more than 3 years of diabetes duration, those with FPG-CV $\geq$ 34.8% and A_{1c}-CV $\geq$ 16.7% had increased minor LEA risks of 94% and 51%, respectively

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

- Individuals with high glucose variation should be recognised early and monitored closely in order to prevent amputation

COPD	Chronic obstructive pulmonary disease
FPG	Fasting plasma glucose
FPG-ARV	Average real variability in fasting plasma glucose
FPG-CV	CV of fasting plasma glucose
ICD-9-CM	International Classification of Disease, 9th Revision, Clinical Modification
LEA	Lower extremity amputation
NDCMP	National Diabetes Care Management Program
NHIRD	National Health Insurance Research Database
NHI	National Health Insurance
PAD	Peripheral artery disease
PIN	Personal identification number

Introduction

The worldwide prevalence of diabetes has substantially increased and was 8.4% in individuals aged between 18 and 99 years old in 2017, which corresponds to 451 million individuals globally; this number is expected to rise to 693 million by 2045 [1]. In Taiwan, the prevalence was 9.8% in 2013 and is projected to rise to 13.1% in 2035 [1]. This overall increase leads to growth in the number of incident cases of diabetes-related complications, including peripheral neuropathy, peripheral artery disease (PAD) and lower extremity amputation (LEA).

Several risk factors associated with LEA, such as ethnicity, sex, smoking habits, body weight, hypertriglycerolaemia, years of diabetes duration, insulin therapy and elevated glucose level, have

been identified [2–7]. Amongst them, two glucose-related biomarkers, that is, fasting plasma glucose (FPG) and HbA_{1c}, are the primary therapeutic targets of diabetes management to achieve adequate glycaemic control for long periods of time. Individuals with diabetes and high FPG levels are likely to have an amputation [6, 7]. A meta-analysis reported that every 1% increase in HbA_{1c} is associated with an increased LEA incidence [8].

HbA_{1c} is the gold standard for blood glucose control monitoring, and it is associated with diabetes complication risk [9, 10]. However, the interpretation is complicated, partly because HbA_{1c} merely reflects the mean blood sugar for the past 3 months; it fails to reflect glycaemic variability and cannot capture the risks associated with extreme glycaemic swings, conferring an additional risk of diabetes-related complications [11, 12]. In the last decade, glycaemic variability has been an emerging glucose control marker and has been treated as a target for diabetes management. Several systematic reviews have reported a positive relationship between diabetes-related complications and glycaemic variability, including variability in HbA_{1c} [13] and short-term blood glucose variation (self-monitoring of blood glucose or continuous glucose monitoring without considering HbA_{1c} variability) [14, 15] in type 1 and type 2 diabetes mellitus. The association between HbA_{1c} variability and wound healing in foot ulcers has been explored in individuals with diabetes [16], but no study has reported the relationship between glucose variability and risk of LEA in individuals with diabetes. Thus, additional data are needed to characterise the effects of glucose variability on incident LEA. This study examined whether visit-to-visit

glucose variation, presented as CVs of FPG (FPG-CV) and of HbA_{1c} (A_{1c}-CV), has an independent association with minor and major LEA incidences in Chinese individuals with type 2 diabetes who participated in the National Diabetes Care Management Program (NDCMP) in Taiwan.

Methods

Study population

The Taiwan Diabetes Cohort Study, a retrospective cohort study, was composed of 50,491 enrollees from the Taiwan NDCMP, who were aged 50 years and over during 2002–2004 and were diagnosed with diabetes on the basis of the ADA criteria (International Classification of Disease, 9th Revision, Clinical Modification [ICD-9-CM] code 250). This study had two sources of datasets: the NDCMP (2002–2004) and the National Health Insurance Research Database (NHIRD) (1996–2011) (electronic supplementary material [ESM] Fig. 1). The NDCMP dataset provided clinical information for individuals at each visit, such as blood glucose tests for deriving 1 year glucose variation. We used the admission and outpatient claim databases of NHIRD from 1996 to 2003 to exclude individuals who had LEA before entry and to ascertain the LEA events and baseline medications and comorbidities. After excluding those who were ineligible (1392 individuals with type 1 diabetes and 296 individuals with LEA), 48,803 individuals remained. A total of 27,574 individuals remained after further excluding those who could not provide at least 1 year of follow-up blood tests for deriving visit-to-visit variation in FPG and HbA_{1c} ($n = 19,561$), because of joining the NDCMP after 1 January 2014, and those who had missing data on baseline characteristics, diabetes-related variables, comorbidities and laboratory urinary and blood tests ($n = 1668$) (ESM Fig. 2). To evaluate the potential bias arising from the exclusion of those who did not have 1 year follow-up measurements for blood glucose tests, we used standardised effect size to evaluate the baseline differences and found no difference (all standardised effect sizes < 0.1) except for FPG and HbA_{1c} (slightly higher than 0.1), indicating that this kind of error might be random, and a low threat to validity (ESM Table 1). This study was approved by the Human Research Committee of China Medical University Hospital. The methods were conducted in accordance with the relevant guidelines and regulations. Informed consent of the study participants was not required because the study was a secondary data analysis using a dataset consisting of de-identified data that were released for research purposes.

Datasets for baseline and follow-up assessments

The National Health Insurance (NHI) programme, a universal insurance system, was launched in 1995. This national insurance programme had covered approximately 99% of the entire population (23.74 million people) in Taiwan since 1999 and had contracts with 100% of hospitals and 92% of clinics nationwide in 2010. The NHI Bureau randomly audits claims data. To enhance the validity of the claims data, expert reviews were conducted quarterly on every 50–100 inpatient and ambulatory claims in each hospital and clinic. Every false diagnostic report incurred a severe penalty, which was administered by the NHI Bureau. We used datasets for inpatient care by admission and outpatient care for visits during 1996–2011 to exclude individuals who had LEA before entry and to ascertain LEA during the follow-up period. The personal identification number (PIN) of all individuals was scrambled cryptographically for security and privacy purposes by the NHIRD and was used for linkage amongst datasets, including the NDCMP registry and all NHI datasets. Information on demographic data, date and source of diagnosis, outpatient care, inpatient admission and outpatient and inpatient treatments was included in the NHIRD. Disease status was ascertained by the ICD-9-CM. The withdrawal rate of enrollees was very low under the comprehensive coverage of the NHI programme. Thus, bias due to loss to follow-up rate was trivial.

All enrollees underwent assessment upon entry to the NDCMP, which included a series of urinary and blood tests, body measurements and ascertainment of disease status and complication history. A case management nurse administered a standardised, computerised questionnaire to record disease and medication histories and lifestyle behaviours. After a 12 h overnight fast, blood was drawn in the morning and sent for analysis within 4 h post collection.

Measurements

Sociodemographic factors, biomarkers and comorbidities

Sociodemographic variables, lifestyle habits and anthropometric measurements were retrieved from the NDCMP database in 2002–2004. The information included age, sex, diabetes duration, smoking status, alcohol consumption status and BMI. Smoking and alcohol consumption statuses were categorised into two: yes and no. Diabetes duration was derived by calculating the number of days between the dates of entry to NDCMP and diabetes onset and then dividing by 365 days. BMI, systolic blood pressure, diastolic blood pressure, serum creatinine, total cholesterol, high-density lipoprotein, low-density lipoprotein, HbA_{1c}, FPG and triacylglycerols at the entry date to NDCMP were also retrieved. Measurements of FPG and HbA_{1c} within the 1 year period after entry date for each individual were analysed to calculate CV and average real variability (ARV) in FPG and HbA_{1c}. CV

and ARV were considered because these two measures reflect long-term visit-to-visit glycaemic variation rather than within-day or within-multiple-day glycaemic fluctuation. CV was chosen because it measures the relative variability by weighting the mean. FPG-CV, ARV in FPG (FPG-ARV), A_{1c} -CV or ARV in HbA_{1c} (A_{1c} -ARV) was calculated only when an individual had two or more FPG or HbA_{1c} measurements in the first year after enrolment. To adjust for the impact of visit number on variation, the CV value was divided by the square root of the ratio of visit number divided by visit number minus 1 [17]. The ARV values for FPG and HbA_{1c} were calculated by the summation of absolute differences in two consecutive measurements divided by total visits minus 1 [18]. Individuals were classified into subgroups according to tertiles of FPG-CV, A_{1c} -CV, FPG-ARV and A_{1c} -ARV. Serum creatinine was used to derive the eGFR by using the Chronic Kidney Disease Epidemiology Collaboration equation that had been recalibrated for individuals of Chinese ancestry [19].

The exposure periods for comorbidities and hypoglycaemic drugs were defined as the 1 year period preceding entry to the cohort using ambulatory and inpatient claims data. The comorbidities included PAD (ICD-9-CM codes 443.9 and 440.21), hypertension (401–405), hyperlipidaemia (272), coronary artery disease (410–413, 414.01–414.05, 414.8 and 414.9), cancer (140–149, 150–159, 160–165, 170–175, 179–189, 190–199, 200, 202, 203, 210–213, 215–229, 235–239, 654.1, 654.10, 654.11, 654.12, 654.13 and 654.14), atrial fibrillation (427.31), congestive heart failure (CHF) (428, 398.91 and 402.x1), stroke (430–438), chronic obstructive pulmonary disease (COPD) (490–496), chronic hepatitis (571, 572.2, 572.3, 572.8, 573.1, 573.2, 573.3, 573.8 and 573.9), diabetes-related retinopathy (362.0 and 250.5), hypoglycaemia (2510–2512), peripheral neuropathy (356), foot ulcer (707.1 and 440.23) and alcohol dependence (303 and 305).

Outcome ascertainment By using the unique PIN, we linked the study participants to inpatient claims records (2002–2011) to identify the first episodes of primary or secondary procedure codes for minor amputation, referring to any LEA below the ankle joint (ICD-9-CM codes 84.11–84.12), and/or major amputation, referring to any LEA above the ankle joint (ICD-9-CM codes 84.13–84.19) [20]. The study period was between 1 January 2002 and 31 December 2011. The cohort was followed up over 8 years from the index date to 31 December 2011 or until LEA events, death or withdrawal from the insurance programme occurred.

Statistical analysis

We assessed the normality of variables by visually assessing the histogram curves and conducting the Kolmogorov–Smirnov test. If the variables were normally distributed, continuous variables were presented as the mean (standard

deviation); otherwise, they were presented as the median (interquartile range). Number and percentage were presented for categorical variables. Baseline characteristics were compared between LEA status by the χ^2 test or Fisher's exact test for categorical variables and by one-way ANOVA for continuous variables. The relationships of FPG-CV and A_{1c} -CV categories with minor or major LEA risks were assessed by Cox proportional hazards models. HRs and their 95% CIs were presented by adjusting for age, sex and multiple variables. Three multivariate models were conducted, and the first one adjusted for age at entry (continuous) and sex (male, female). The second model additionally adjusted for lifestyle behaviours and diabetes-related factors of alcohol consumption (yes, no), smoking (yes, no), diabetes duration (continuous), types of hypoglycaemic drugs (no medication, sulfonylurea monotherapy, metformin monotherapy, oral glucose-lowering drug monotherapy other than sulfonylurea or metformin [glucose-lowering-drug-other], sulfonylurea plus metformin combination, sulfonylurea plus glucose-lowering-drug-other combination, metformin plus glucose-lowering-drug-other combination, two glucose-lowering-drug-others, three glucose-lowering drugs, more than three glucose-lowering drugs, insulin monotherapy, insulin plus one glucose-lowering drug, insulin plus two or more glucose-lowering drugs), antihypertensive medication (yes, no), obesity (yes, no) and baseline FPG (continuous). The third model further adjusted for comorbidities at baseline, such as CHF (yes, no), coronary artery disease (yes, no), chronic kidney disease (CKD) (eGFR <60 ml min⁻¹ 1.73 m⁻²), cancer (yes, no), hyperlipidaemia (yes, no), PAD (yes, no), atrial fibrillation (yes, no), hypertension (yes, no), stroke (yes, no), COPD (yes, no), chronic hepatitis (yes, no), hypoglycaemia (yes, no), diabetes-related retinopathy (yes, no), foot ulcer (yes, no), peripheral neuropathy (yes, no) and alcohol dependence (yes, no). The linear trend was examined across the FPG-CV and A_{1c} -CV categories. We also tested the proportionality assumption by entering an interaction term for the quartiles of FPG-CV and A_{1c} -CV with person-time in the multivariate Cox models. Likelihood ratio tests were applied to explore the interactions of FPG-CV and A_{1c} -CV with diabetes duration by adding their product terms into the full model for statistical significance. In addition, a dose–response (log-linearity) or non-linear association between each glucose variation variable and LEA outcome was checked by restricted cubic splines in Cox models, and the Wald test was used to test its statistical significance. The multiple imputation method was used to handle missing data in A_{1c} -CV and FPG-CV based on information on baseline variables. SAS version 9.4 (SAS, Cary, NC, USA) was used for all analyses. Two-tailed *p* values were considered, and statistical significance was considered at *p* < 0.05.

Results

A total of 541 incident LEA cases (310 minor, 195 major and 36 both minor and major LEA cases) were ascertained in our

cohort during a median 8.9 years of follow-up, resulting in a crude incidence density rate of 2.4 per 1000 person-years. In the subgroups of the first, second and third tertiles, the crude minor LEA incidence density rates per 1000 person-years of FPG-CV were 0.9, 1.5 and 2.3 for minor LEA and 0.7, 1.0 and 1.3 for major LEA; those of A_{1c}-CV were 1.1, 1.6 and 1.9 for minor LEA and 0.9, 1.0 and 1.2 for major LEA, respectively. The crude incidence density rates for minor and major LEA were 1.3 and 0.9 per 1000 person-years in women and 1.8 and 1.2 per 1000 person-years in men, respectively.

Table 1 shows the baseline characteristics according to the LEA status. Individuals with LEA were more likely to be male and smokers and have high mean age, diabetes duration, and prevalence of CHF, hypertension, stroke, COPD, hypoglycaemia and diabetes-related retinopathy. Figure 1 shows the Kaplan–Meier cumulative incidence curves for LEA within subgroups of 1 year A_{1c}-CV and FPG-CV. Individuals with A_{1c}-CV $\geq 16.7\%$ and FPG-CV $\geq 34.8\%$ were at increased risk for LEA (both logrank $p < 0.001$, Fig. 1).

Table 1 Comparisons of baseline sociodemographic factors, lifestyle behaviours, diabetes-related variables, drug-related variables, comorbidities and blood biochemical measurements according to LEA incidence in individuals with type 2 diabetes enrolled in the NDCMP, Taiwan

Variable	LEA				<i>p</i> value
	Minor (<i>n</i> = 310)	Major (<i>n</i> = 195)	Both (<i>n</i> = 36)	No (<i>n</i> = 27,033)	
Sociodemographic factors					
Male, <i>n</i> (%)	155 (50.00)	97 (49.74)	22 (61.11)	12,112 (44.8)	0.03
Age (years), mean (SD)	65.48 (8.20)	67.48 (8.28)	68.53 (9.12)	64.54 (8.47)	<0.001
Lifestyle behaviours, <i>n</i> (%)					
Smoking	53 (17.10)	37 (18.97)	2 (5.56)	3515 (13.00)	0.006
Alcohol drinking	17 (5.48)	13 (6.67)	2 (5.56)	1954 (7.23)	0.65
Diabetes-related variables					
Diabetes duration (years), mean (SD)	10.53 (7.92)	10.57 (8.16)	10.69 (6.75)	7.29 (6.87)	<0.001
Type of glucose-lowering drug use, <i>n</i> (%)					<0.001
No medication	1 (0.32)	0 (0.00)	0 (0.00)	469 (1.73)	
One glucose-lowering drug	18 (5.81)	16 (8.21)	1 (2.78)	4684 (17.33)	
Two glucose-lowering drugs	86 (27.74)	56 (28.72)	9 (25.00)	11,226 (41.53)	
Three glucose-lowering drugs	53 (17.1)	35 (17.95)	7 (19.44)	4873 (18.03)	
>3 glucose-lowering drugs	16 (5.16)	8 (4.1)	3 (8.33)	1378 (5.1)	
Insulin only	24 (7.74)	15 (7.69)	2 (5.56)	755 (2.79)	
Insulin + glucose-lowering drug	112 (36.13)	65 (33.33)	14 (38.89)	3648 (13.49)	
Drug-related variables, <i>n</i> (%)					
Hypertension drug treatment	133 (42.9)	87 (44.62)	21 (58.33)	11,275 (41.71)	0.18
Comorbidities, <i>n</i> (%)					
Obesity (BMI ≥ 27 kg/m ²)	106 (34.19)	56 (28.72)	11 (30.56)	9489 (35.10)	0.27
CAD	34 (10.97)	18 (9.23)	6 (16.67)	2642 (9.77)	0.48
CHF	12 (3.87)	13 (6.67)	2 (5.56)	777 (2.87)	0.008
Cancer	8 (2.58)	8 (4.1)	1 (2.78)	603 (2.23)	0.24 ^a
Hyperlipidaemia	75 (24.19)	55 (28.21)	6 (16.67)	6774 (25.06)	0.48
Hypertension	174 (56.13)	117 (60.00)	26 (72.22)	13,402 (49.58)	<0.001
Atrial fibrillation	2 (0.65)	2 (1.03)	0 (0)	166 (0.61)	0.61 ^a
Stroke	29 (9.35)	28 (14.36)	6 (16.67)	1574 (5.82)	<0.001
COPD	17 (5.48)	19 (9.74)	5 (13.89)	1364 (5.05)	0.002
Hypoglycaemia	0 (0.00)	7 (3.59)	0 (0)	117 (0.43)	<0.001 ^a
CKD (eGFR < 60 ml min ^{−1} 1.73 m ^{−2})	140 (45.16)	111 (56.92)	15 (41.67)	8352 (30.9)	<0.001
Diabetes-related retinopathy	22 (7.1)	16 (8.21)	3 (8.33)	538 (1.99)	<0.001 ^a
Peripheral neuropathy	9 (2.9)	0 (0)	1 (2.78)	244 (0.9)	0.004 ^a
PAD	11 (3.55)	10 (5.13)	1 (2.78)	670 (2.48)	0.07 ^a
Foot ulcer	8 (2.58)	3 (1.54)	2 (5.56)	71 (0.26)	<0.001 ^a
Blood biochemical measurements, mean (SD)					
Mean FPG (mmol/l)	10.77 (3.29)	10.37 (3.21)	10.93 (3.93)	9.24 (2.67)	<0.001
Mean HbA _{1c} (mmol/mol) [%]	75.60 (18.56)	70.83 (19.00)	74.63 (22.67)	63.18 (16.84)	<0.001
	[9.07 (1.70)]	[8.63 (1.74)]	[8.98 (2.07)]	[7.93 (1.54)]	
eGFR (ml min ^{−1} 1.73 m ^{−2})	63.24 (24.20)	55.02 (24.16)	59.32 (23.84)	70.37 (20.85)	<0.001
Number of FPG	2.91 (0.88)	3.11 (0.93)	3.11 (0.98)	3.07 (0.95)	0.03
Number of HbA _{1c}	3.24 (1.30)	3.47 (1.30)	3.25 (1.30)	3.45 (1.39)	0.05

Differences in continuous variables were tested using one-way ANOVA. Differences in categorical variables were tested using the χ^2 test

^a Fisher's exact test

CAD, coronary artery disease

Fig. 1 Risks of minor LEA over time for (a) FPG-CV and (b) A_{1c}-CV

Table 2 shows the crude incidence density rates and HRs of minor and major LEA for the tertile subgroups of FPG-CV, A_{1c}-CV, FPG-ARV and A_{1c}-ARV. The minor LEA risk of individuals in the third tertile subgroup of FPG-CV was significant (adjusted HR 1.53 [1.15, 2.04], $p = 0.004$) after adjusting for all covariates. Similarly, individuals in the third tertile of A_{1c}-CV manifested a significant increase in minor LEA risk (adjusted HR 1.34 [1.02, 1.77], $p = 0.04$). The minor LEA risk of individuals in the third tertile subgroup of FPG-ARV was significant (adjusted HR 1.78 [1.33, 2.38], $p < 0.001$) after adjusting for all covariates. By contrast, A_{1c}-ARV was not significantly associated with minor LEA. After using restricted cubic splines, a non-linear association was found for FPG-CV and A_{1c}-CV with the risk of LEA (Fig.

2). All four glycaemic variability measures were not associated with major LEA. The HRs of minor LEA for all factors considered in the final models are presented in ESM Table 2.

Considering that FPG-CV and A_{1c}-CV were more sensitive in capturing the associations with minor LEA, further analyses focused on the associations of these two glucose variation measures with minor LEA. We excluded individuals with existing comorbidities from sensitivity analyses to rule out the potential bias, including hyperglycaemic hyperosmolar nonketotic coma, diabetes-related ketoacidosis ($n = 653$), hypoglycaemia ($n = 124$), peripheral neuropathy ($n = 254$), foot ulcer ($n = 84$) and all comorbidities ($n = 1140$). Individuals with FPG-CV of $\geq 34.8\%$ exhibited a similar significant HR for minor LEA of 1.49 (1.10, 2.01; $p = 0.01$), and the effect of A_{1c}-CV was also significant (1.36 [1.02, 1.81], $p = 0.04$) (Table 3). When no adjustment was made for foot ulcers, our study results remained similar. We also evaluated whether the cut-off point values affected our findings by categorising FPG-CV and A_{1c}-CV at 20% and 35% and found similar results (ESM Table 3). A total of 46,628 people were included after multiple imputation for missing data. The adjusted HRs estimated were similar to those that used the complete dataset (ESM Table 4).

The interactions of FPG-CV and A_{1c}-CV with diabetes duration and CKD status were examined to determine whether the magnitudes of the associations of FPG-CV and A_{1c}-CV with minor LEA risk differ according to diabetes duration and renal function. We found significant interaction effects ($p < 0.01$ for FPG-CV with diabetes duration and borderline significance for A_{1c}-CV with renal function, $p = 0.13$). Thus, we further explored the effects of FPG-CV and A_{1c}-CV stratified by diabetes duration (Table 4) and renal function (ESM Fig. 3). We found that the multivariate-adjusted HRs for the second and third FPG-CV tertiles were significantly linked to minor LEA in individuals with more than 3 years of diabetes duration (1.53 [1.07, 2.18], $p = 0.02$; and 1.94 [1.38, 2.72], $p < 0.001$, respectively) but not in those with diabetes duration of less than 3 years. The third A_{1c}-CV tertile was also significantly correlated with minor LEA risk only in individuals with more than 3 years of diabetes duration (1.51 [1.12, 2.05], $p = 0.008$). For the stratification analysis of renal function status, we observed that multivariate-adjusted HRs in the third FPG-CV and A_{1c}-CV tertiles were significantly linked to minor LEA in individuals with eGFR ≥ 60 ml min⁻¹ 1.73 m⁻² (1.52 [1.04, 2.22], $p = 0.03$; and 1.47 [1.02, 2.11], $p = 0.04$, respectively).

Discussion

On the basis of the analysis of a large nationwide cohort of 27,574 Taiwan Diabetes Study enrollees with type 2 diabetes mellitus, this observational study first showed that high levels

Table 2 HRs of LEA according to the tertiles of FPG-CV, A_{1c}-CV, FPG-ARV and A_{1c}-ARV in individuals with type 2 diabetes

Variable	n	Cases	Person-years	IR (95% CI) ^a	LEA					
					Model I		Model II		Model III	
					HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Minor LEA										
FPG-CV (%)										
<17.5	9099	69	76,157	0.91 (0.69, 1.12)	1.00		1.00		1.00	
17.5–34.7	9099	112	75,133	1.49 (1.21, 1.77)	1.65 (1.22, 2.23)	0.001	1.38 (1.02, 1.86)	0.04	1.30 (0.96, 1.76)	0.09
≥34.8	9376	165	73,377	2.25 (1.91, 2.59)	2.53 (1.91, 3.35)	<0.001	1.71 (1.28, 2.28)	<0.001	1.53 (1.15, 2.04)	0.004
<i>P</i> _{trend}					<0.001		<0.001		0.004	
A _{1c} -CV (%)										
<8.4	9095	84	75,487	1.11 (0.87, 1.35)	1.00		1.00		1.00	
8.4–16.6	9103	120	74,911	1.60 (1.32, 1.89)	1.45 (1.09, 1.91)	0.01	1.26 (0.95, 1.67)	0.10	1.16 (0.87, 1.53)	0.31
≥16.7	9376	142	74,269	1.91 (1.60, 2.23)	1.73 (1.32, 2.27)	<0.001	1.53 (1.16, 2.01)	0.003	1.34 (1.02, 1.77)	0.04
<i>P</i> _{trend}					<0.001		0.002		0.04	
FPG-ARV (%)										
<17.3	9099	65	76,162	0.85 (0.65, 1.06)	1.00		1.00		1.00	
17.3–35.8	9098	104	74,897	1.39 (1.12, 1.66)	1.63 (1.20, 2.23)	0.002	1.38 (1.01, 1.88)	0.05	1.31 (0.96, 1.79)	0.09
≥35.9	9377	177	73,608	2.40 (2.05, 2.76)	2.87 (2.16, 3.82)	<0.001	1.96 (1.47, 2.63)	<0.001	1.78 (1.33, 2.38)	<0.001
<i>P</i> _{trend}					<0.001		<0.001		<0.001	
A _{1c} -ARV (%)										
<7.8	9099	91	75,629	1.20 (0.96, 1.45)	1.00		1.00		1.00	
7.8–16.1	9100	121	74,660	1.62 (1.33, 1.91)	1.35 (1.03, 1.78)	0.03	1.21 (0.92, 1.59)	0.17	1.14 (0.86, 1.49)	0.36
≥16.2	9375	134	74,379	1.80 (1.50, 2.11)	1.51 (1.15, 1.97)	0.003	1.34 (1.02, 1.76)	0.04	1.20 (0.91, 1.57)	0.20
<i>P</i> _{trend}					0.003		0.03		0.21	
Major LEA										
FPG-CV (%)										
<17.5	9099	55	76,196	0.72 (0.53, 0.91)	1.00		1.00		1.00	
17.5–34.7	9099	77	75,282	1.02 (0.79, 1.25)	1.45 (1.02, 2.05)	0.04	1.21 (0.85, 1.71)	0.29	1.13 (0.79, 1.60)	0.51
≥34.8	9376	99	73,730	1.34 (1.08, 1.61)	1.92 (1.38, 2.67)	<0.001	1.31 (0.93, 1.83)	0.12	1.13 (0.80, 1.59)	0.48
<i>P</i> _{trend}					<0.001		0.13		0.51	
A _{1c} -CV (%)										
<8.4	9095	67	75,605	0.89 (0.67, 1.10)	1.00		1.00		1.00	
8.4–16.6	9103	73	75,143	0.97 (0.75, 1.19)	1.11 (0.80, 1.55)	0.54	0.98 (0.70, 1.37)	0.91	0.87 (0.62, 1.21)	0.40
≥16.7	9376	91	74,460	1.22 (0.97, 1.47)	1.42 (1.04, 1.95)	0.03	1.26 (0.92, 1.74)	0.16	1.05 (0.76, 1.45)	0.78
<i>P</i> _{trend}					0.03		0.14		0.71	
FPG-ARV (%)										
<17.3	9099	51	76,217	0.67 (0.49, 0.85)	1.00		1.00		1.00	
17.3–35.8	9098	73	75,033	0.97 (0.75, 1.20)	1.49 (1.04, 2.13)	0.03	1.26 (0.88, 1.80)	0.21	1.19 (0.83, 1.70)	0.35
≥35.9	9377	107	73,958	1.45 (1.17, 1.72)	2.23 (1.60, 3.12)	<0.001	1.54 (1.09, 2.17)	0.01	1.33 (0.94, 1.88)	0.10
<i>P</i> _{trend}					<0.001		0.01		0.10	
A _{1c} -ARV (%)										
<7.8	9099	68	75,741	0.90 (0.68, 1.11)	1.00		1.00		1.00	
7.8–16.1	9100	75	74,902	1.00 (0.77, 1.23)	1.13 (0.81, 1.57)	0.47	1.02 (0.73, 1.41)	0.92	0.91 (0.65, 1.27)	0.58
≥16.2	9375	88	74,564	1.18 (0.93, 1.43)	1.36 (0.99, 1.86)	0.06	1.21 (0.88, 1.67)	0.25	1.02 (0.74, 1.41)	0.91
<i>P</i> _{trend}					0.06		0.23		0.87	

Model I adjusted for age and sex

Model II adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of glucose-lowering drugs and hypertension drug treatment

Model III adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of hypoglycaemic drug, hypertension drug treatment, mean level of FPG, mean level of HbA_{1c}, obesity, coronary artery disease, CHF, cancer, hyperlipidaemia, hypertension, atrial fibrillation, stroke, COPD, hypoglycaemia, CKD, diabetes-related retinopathy, peripheral neuropathy, PAD and foot ulcer^a Exact CIs for Poisson distribution

IR, incidence density rate (number of incident cases/person-years × 1000)

of visit-to-visit FPG and HbA_{1c} variabilities defined by CVs were linked to increased minor LEA risk. These associations remained significant after adjustments were made for baseline FPG and HbA_{1c} and traditional risk factors. Both FPG-CV and A_{1c}-CV exerted independent effects on minor LEA risk. After excluding individuals with comorbidities and acute

complications, our findings demonstrated similar associations of FPG-CV and A_{1c}-CV with minor LEA risks. In addition, diabetes duration modified the associations of FPG-CV and A_{1c}-CV with minor LEA risk. Amongst individuals with more than 3 years of diabetes duration, those with FPG-CV ≥34.8% and A_{1c}-CV ≥16.7% had increased minor LEA risks of 95%

Fig. 2 Restricted multivariable cubic spline plots (with 95% CI) for (a) FPG-CV and (b) A_{1c}-CV. The reference value (i.e. the cut-off point for the first tertile) for FPG-CV was set at 17.5% and for A_{1c}-CV at 8.4%. The HRs were adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of hypoglycaemic drug, hypertension drug treatment, FPG, HbA_{1c}, obesity, coronary artery disease, CHF, cancer, hyperlipidaemia, hypertension, atrial fibrillation, stroke, chronic hepatitis, COPD, hypoglycaemia, CKD, diabetes-related retinopathy, peripheral neuropathy, PAD and foot ulcer

and 52%, respectively. We observed significant FPG-CV and A_{1c}-CV effects on LEA only in individuals with eGFR ≥ 60 ml min⁻¹ 1.73 m⁻².

Oscillatory plasma glucose is more detrimental than stable high glucose in inflammation, insulin resistance, generation of oxidative stress, vascular endothelial dysfunction, atherosclerosis progression and hyperglycaemia-related downstream consequences [21]. The mechanisms for the link between visit-to-visit FPG and HbA_{1c} variabilities and LEA remain to be elucidated, but can be explained by three possible mechanisms. First, the interactions of inflammation and oxidative stress in the development of diabetes-related atherosclerosis may be the underlying mechanisms of glycaemic variability that are associated with LEA [22]. Hyperglycaemia, resulting in high variation in plasma glucose, is usually accompanied by inflammation and oxidative stress. Hyperglycaemia induces the

production of mitochondrial reactive oxygen species and results in the formation of intracellular advanced glycation end-products [23]. Reactive oxygen species directly increase inflammatory expression and insulin resistance [24]. Moreover, all factors contributing to hypertension have a unifying feature, oxidative stress [25], which is a risk factor for atherosclerosis. Second, glycaemic variability might be a proxy for multiple morbidities, which may have played a role in the link to LEA risk. Individuals with multi-morbidities experience difficulties in managing multiple medications and poor coordination of multiple medical care providers [26]. The mean number of chronic conditions in individuals with diabetes is 3.4, and these individuals face high demands on their time to manage their chronic conditions [27]. To rule out this possibility, we excluded individuals who suffered from chronic conditions at baseline, and the risk estimates for glycaemic variability remained similar to the original. Third, individuals with greater glycaemic variation are more likely to have neuropathy and/or ischaemia that is more susceptible to ulceration and infection, which are potential risk factors of amputation [28, 29].

Our study was the first and largest cohort study to display the independent relationships of visit-to-visit FPG and HbA_{1c} variabilities with minor LEA risk in older individuals with type 2 diabetes. FPG and HbA_{1c} were measured during follow-up care in routine clinical settings as a part of managed care in Taiwan and many other countries. Considering the availability of electronic computerised systems, deriving visit-to-visit FPG and HbA_{1c} variabilities in routine clinical practice is becoming feasible. Further studies are needed to support our findings and to determine the optimal treatment option for older individuals with type 2 diabetes and high glycaemic variability.

Several limitations should be noted. First, the design is observational. Thus, the confounding effects of some covariates without adjustment or residual confounding may exist. We made our best effort to adjust for as many confounders as possible to minimise the potential confounding effects. We also adopted a restriction strategy to exclude the confounding effects of comorbidities in sensitivity analysis by excluding those with specific comorbidities to eliminate their effects on study findings. The presence of comorbidities was associated with increased risk of minor LEA, which may confound the relationship between glycaemic variability and minor LEA risk. The multivariate adjustment for control of confounding effects may not be adequate, because the prevalence for these comorbidities was low. A restriction approach can increase the homogeneity of the study subjects and enhance the interval validity of our study findings. Our findings remained similar, indicating that our study findings are robust. Second, our study population included individuals with type 2 diabetes enrolled in the NDCMP. To assess the representativeness of the study population, we checked the age and sex distributions in our study population against those for the entire Taiwan population with type 2 diabetes for the same period. We found similar distributions, which indicated the generalisability of our findings. Our

Table 3 Sensitivity analysis for bias due to the existence of comorbidities by excluding individuals with hyperglycaemic hyperosmolar nonketotic coma, ketoacidosis, chronic hepatitis, hypoglycaemia, peripheral neuropathy and foot ulcer

Variable	Minor LEA			
	Model I	Model II	Model III	Model IV
	HR (95% CI)	p value	HR (95% CI)	p value
<i>n</i>	26,921	27,450	27,320	27,490
FPG-CV (%)				
<17.5	1.00	1.00	1.00	1.00
17.5–34.7	1.28 (0.95, 1.74)	0.11	1.32 (0.97, 1.79)	0.08
≥34.8	1.46 (1.09, 1.96)	0.01	1.56 (1.16, 2.09)	0.003
A _{1c} -CV (%)				
<8.4	1.00	1.00	1.00	1.00
8.4–16.6	1.12 (0.84, 1.49)	0.44	1.17 (0.88, 1.56)	0.28
≥16.7	1.37 (1.04, 1.81)	0.03	1.37 (1.03, 1.81)	0.03
Not adjusted for foot ulcer				
FPG-CV (%)				
<17.5	1.00	1.00	1.00	1.00
17.5–34.7	1.29 (0.95, 1.74)	0.10	1.32 (0.97, 1.80)	0.08
≥34.8	1.47 (1.10, 1.97)	0.01	1.57 (1.17, 2.11)	0.003
A _{1c} -CV (%)				
<8.4	1.00	1.00	1.00	1.00
8.4–16.6	1.13 (0.85, 1.51)	0.39	1.19 (0.89, 1.58)	0.24
≥16.7	1.37 (1.04, 1.82)	0.03	1.37 (1.03, 1.82)	0.03
Model I	HR (95% CI)	p value	HR (95% CI)	p value
Model II	HR (95% CI)	p value	HR (95% CI)	p value
Model III	HR (95% CI)	p value	HR (95% CI)	p value
Model IV	HR (95% CI)	p value	HR (95% CI)	p value
Model V	HR (95% CI)	p value	HR (95% CI)	p value
Model I	HR (95% CI)	p value	HR (95% CI)	p value
Model II	HR (95% CI)	p value	HR (95% CI)	p value
Model III	HR (95% CI)	p value	HR (95% CI)	p value
Model IV	HR (95% CI)	p value	HR (95% CI)	p value
Model V	HR (95% CI)	p value	HR (95% CI)	p value

Multivariate-adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of hypoglycaemic drugs, hypertension drug treatment, mean level of FPG, mean level of HbA_{1c}, obesity, coronary artery disease, CHF, cancer, hyperlipidaemia, hypertension, atrial fibrillation, stroke, COPD, hypoglycaemia, CKD, diabetes-related retinopathy, peripheral neuropathy, PAD and foot ulcer

Model I excluding people with hyperglycaemic hyperosmolar nonketotic coma and ketoacidosis (*n* = 653)

Model II excluding individuals with hypoglycaemia (*n* = 124)

Model III excluding individuals with peripheral neuropathy (*n* = 254)

Model IV excluding individuals with foot ulcer (*n* = 84)

Model V excluding individuals with hyperglycaemic hyperosmolar nonketotic coma, ketoacidosis, hypoglycaemia, peripheral neuropathy and foot ulcer (*n* = 1104)

Table 4 HRs of minor LEA according to the tertiles of FPG-CV and A_{1c}-CV in individuals with type 2 diabetes stratified by diabetes duration

Variable	Minor LEA							
	Model I		Model II		Model III (Not adjustment for foot ulcer)		Model IV	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Diabetes duration ≤3 years (<i>n</i> = 10,059)								
FPG-CV (%)								
<17.5	1.00		1.00		1.00		1.00	
17.5–34.7	1.07 (0.58, 1.97)	0.84	0.88 (0.48, 1.64)	0.70	0.81 (0.43, 1.50)	0.50	0.81 (0.44, 1.51)	0.51
≥34.8	1.20 (0.66, 2.19)	0.54	0.77 (0.42, 1.43)	0.41	0.64 (0.34, 1.18)	0.15	0.62 (0.33, 1.17)	0.14
<i>p</i> _{trend}	0.55		0.41		0.15		0.14	
A _{1c} -CV (%)								
<8.4	1.00		1.00		1.00		1.00	
8.4–16.6	1.40 (0.73, 2.69)	0.31	1.16 (0.61, 2.24)	0.65	1.02 (0.53, 1.97)	0.96	1.06 (0.54, 2.05)	0.87
≥16.7	1.33 (0.71, 2.50)	0.37	0.95 (0.50, 1.79)	0.87	0.72 (0.38, 1.39)	0.33	0.76 (0.39, 1.47)	0.41
<i>p</i> _{trend}	0.40		0.80		0.28		0.35	
Diabetes duration >3 years (<i>n</i> = 17,515)								
FPG-CV (%)								
<17.5	1.00		1.00		1.00		1.00	
17.5–34.7	1.81 (1.27, 2.57)	0.001	1.61 (1.13, 2.29)	0.009	1.53 (1.08, 2.18)	0.02	1.53 (1.07, 2.18)	0.02
≥34.8	2.91 (2.10, 4.05)	<0.001	2.13 (1.52, 2.98)	<0.001	1.95 (1.39, 2.74)	<0.001	1.94 (1.38, 2.72)	<0.001
<i>p</i> _{trend}	<0.001		<0.001		<0.001		<0.001	
A _{1c} -CV (%)								
<8.4	1.00		1.00		1.00		1.00	
8.4–16.6	1.44 (1.06, 1.96)	0.02	1.28 (0.94, 1.75)	0.12	1.20 (0.88, 1.64)	0.25	1.18 (0.86, 1.60)	0.31
≥16.7	1.97 (1.46, 2.66)	<0.001	1.70 (1.26, 2.30)	<0.001	1.53 (1.13, 2.07)	0.007	1.51 (1.12, 2.05)	0.008
<i>p</i> _{trend}	<0.001		<0.001		0.006		0.006	

Model I adjusted for age and sex

Model II adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of glucose-lowering drugs and hypertension drug treatment

Model III adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of hypoglycaemic drug, hypertension drug treatment, mean level of FPG, mean level of HbA_{1c}, obesity, coronary artery disease, CHF, cancer, hyperlipidaemia, hypertension, atrial fibrillation, stroke, COPD, hypoglycaemia, CKD, diabetes-related retinopathy, peripheral neuropathy and PAD

Model IV adjusted for age, sex, smoking, alcohol consumption, diabetes duration, type of hypoglycaemic drug, hypertension drug treatment, mean level of FPG, mean level of HbA_{1c}, obesity, coronary artery disease, CHF, cancer, hyperlipidaemia, hypertension, atrial fibrillation, stroke, COPD, hypoglycaemia, CKD, diabetes-related retinopathy, peripheral neuropathy, PAD and foot ulcer

findings can also be generalised to a population with type 2 diabetes that has similar characteristics to ours. Third, we did not apply time-varying proportional hazards models due to the unavailability of the NDCMP database for deriving glucose variation for subsequent years of follow-up blood tests. We only had NDCMP datasets for 2002–2004, which allowed us to derive 1 year glucose variability measures for those who had been followed up for more than 1 year during this period as baseline measures. Fourth, the mean value for the number of outpatient visits within 1 year is about 3, which is less than 4, the expected mean number under the guidelines of NDCMP in Taiwan for diabetes outpatient care. These

mean values, in general, are consistent with guideline regulations. Although the data on glucose variability in this analysis are limited, they still can capture the association between glucose variability and minor LEA. After thorough evaluation of potential bias, our study's findings remain similar, indicating the robustness of the results. A future study measuring glucose variability over a longer period and with more outpatient visits is needed to verify our study findings. Fifth, the results of the restricted multivariable cubic spline plot for A_{1c}-CV indicate that the CI of the HR for A_{1c}-CV ≥45% was wide, and the HR went down to 1, which implied that the number of individuals with A_{1c}-CV ≥45% was not large enough (*n* = 1331, 4.95%) or its effect had a high variation.

By categorising A_{1c} -CV on the basis of tertiles, individuals with A_{1c} -CV $\geq 45\%$ were classified into the third category because the cut-off point value for the third tertile was 16.7%. Given the small number of individuals with A_{1c} -CV $\geq 45\%$, our study was not able to detect its effect. By considering those with A_{1c} -CV $\geq 45\%$ and those with 16.7%–45% together, we underestimated the effect of the third category for A_{1c} -CV, which was a minor threat to the validity of our findings. Finally, misclassification in the variation of glucose levels might be possible because of variation due to inter-clinical settings and accuracy. However, no evidence showed that this type of measurement error was differential. If an association existed between glucose variation and minor LEA, the non-differential misclassification error may underestimate the effect, which is a minor threat to the validity of our findings.

Several studies have identified the risk factors associated with LEA [2–7]. However, most of these studies were conducted in individuals with diabetes-related foot ulcers [29–32] or foot syndrome [33]. Few studies have been performed in individuals with diabetes [6, 34, 35], which could provide clues about LEA prevention for a broader spectrum of the population with diabetes. Moreover, these studies focused on glucose control status, but none of them explored the potential effects of glycaemic variation on LEA. Therefore, our findings provide new insights into the adverse effects of visit-to-visit HbA_{1c} and FPG variations on minor LEA in older individuals with type 2 diabetes and suggest that older individuals with type 2 diabetes and high visit-to-visit variations in HbA_{1c} and FPG are more likely to have minor LEA incidence.

Our study was the first to demonstrate that FPG and HbA_{1c} variabilities are associated with incident minor LEA risk. Older individuals who have had diabetes for more than 3 years are at substantial risk of minor LEA.

Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement TCL and CCL were responsible for the conception and design of the study. TCL and SYJ acquired the data and CIL analysed the data. HMC, CSL, WYL, CHL and MCW interpreted the data. CIL and TCL were responsible for drafting the article. All authors revised the manuscript and approved the final version. TCL and CCL are responsible for the integrity of the work as a whole.

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