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Visit-to-visit HbA1c variability is associated with cardiovascular disease and microvascular complications in patients with newly diagnosed type 2 diabetes

Running Title: HbA1c variability and complications in diabetes

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Abstract

Objective: To investigate the association between visit-to-visit HbA1c variability and cardiovascular events and microvascular complications in patients with newly diagnosed type 2 diabetes.

Research Design and Methods: This retrospective cohort study analyzed patients from Tayside and Fife in the Scottish Care Information-Diabetes Collaboration (SCI-DC), who were observable from the diagnosis of diabetes and had at least five HbA1c measurements before the outcomes being evaluated. We used the previously reported HbA1c variability score (HVS) calculated as the percentage of the number of changes in HbA1c over 0.5% (5.5 mmol/mol) among all HbA1c measurement within an individual. The association between HVS and ten outcomes was assessed using Cox proportional-hazards models.

Results: We included 13,111 to 19,883 patients in the analyses of each outcome. The patients with HVS over 60% were associated with elevated risks of all outcomes compared with the lowest quintile (for example, hazard ratios and 95% confidence intervals [HVS >80 to ≤100 vs. HVS ≥0 to ≤20]: 2.38 [1.61~3.53] for major adverse cardiovascular events [MACE]; 2.4 [1.72~3.33] for all-cause mortality; 2.4 [1.13~5.11] for atherosclerotic cardiovascular [ASCV] death; 2.63 [1.81~3.84] for coronary artery disease; 2.04 [1.12~3.73] for ischemic stroke; 3.23 [1.76~5.93] for heart failure; 7.4 [3.84~14.27] for diabetic retinopathy; 3.07 [2.23~4.22] for diabetic peripheral neuropathy; 5.24 [2.61~10.49] for diabetic foot ulcer; 3.49 [2.47~4.95] for the new-onset chronic kidney disease). Four sensitivity analyses, including adjustment for time-weighted average HbA1c confirmed the robustness of the results.

Conclusions: Our study shows that higher HbA1c variability is associated with increased risks of all-cause mortality, cardiovascular events and microvascular complication of diabetes independently of high HbA1c.

Keywords: HbA1c variability, cardiovascular event, all-cause mortality, heart failure, diabetic retinopathy, diabetic peripheral neuropathy, diabetic foot ulcer, chronic kidney disease

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Introduction

Although there is considerable evidence that intensive blood glucose normalization reduces the risk of both cardiovascular events and microvascular complications of diabetes (1-3), the effects were heterogeneous between trials. For example, the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial was terminated prematurely due to significantly elevated mortality and cardiovascular events (4), suggesting that the near-normalization of blood glucose should not be the only target of diabetes treatment. Glycemic variability is one factor that may explain these differences in cardiovascular outcomes.

Glycemic variability can be measured as either the glucose fluctuation within a day or the long-term visit-to-visit variability. The latter has been recently investigated in several studies, although the metrics and definition of the variability measure were inconsistent (5). Most studies evaluating HbA1c variability using the standard deviation (SD) or the coefficient of variation (CV) of HbA1c, suggested that these measures were associated with all-cause mortality and the development of the adverse outcomes of diabetes, after adjusting for the average HbA1c (6-11). However, neither SD or CV of HbA1c can be easily interpreted in clinical practice. Recently, Forbes and colleagues (12) developed a new scale, namely the HbA1c variability score (HVS) in the current study, to define the HbA1c variability. The HVS indicates how frequently the HbA1c rises or decreases by more than 0.5% (5.5mmol/mol), which is in line with the SD and CV of HbA1c but clinically more translatable (as it can be interpreted as the percentage of total HbA1c measures that vary by more than 0.5% or 5.5mmol/mol) (6,12). However, the HVS has not been widely used among the studies of HbA1c variability, with previous studies using this scale only focusing on the elderly and non-diabetic population and evaluating mainly mortality as an outcome (6,12). It is unclear whether HVS is associated with microvascular complications of diabetes and whether the increased cardiovascular risk described could be extended to real-world patients with type 2 diabetes. In this study we aimed to investigate the association between visit-to-

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visit HbA1c variability and both cardiovascular diseases and microvascular complications in a large population database of patients with newly diagnosed type 2 diabetes.

Research Design and Methods

Data source and study population

The population was selected from patients from Tayside and Fife in the Scottish Care Information-Diabetes Collaboration (SCI-DC), the electronic health record system used in Scotland for patients with diabetes. The patients were included if they: 1) were diagnosed with type 2 diabetes; 2) had their first HbA1c measurement within one year from diagnosis of diabetes; 3) were over 40 years old when first diagnosed with diabetes; 4) did not experience any study outcome before or within three years since diagnosis of diabetes; 5) had at least five records of HbA1c measurement between diagnosis of diabetes and the first episode of the study outcome. Patients were excluded where data were incomplete (details see the **Supplementary Techniques**). Data provision and linkage were carried by the University of Dundee Health Informatics Centre (HIC, https://www.dundee.ac.uk/hic), with analyses of anonymized data performed in an ISO27001 and Scottish Government accredited secure safe haven. HIC Standard Operating Procedures have been reviewed and approved by the NHS East of Scotland Research Ethics Service and consent for this study was obtained from the NHS Fife Caldicott Guardian.

Baseline parameters and follow-up

The body mass index (BMI), estimated glomerular filtration rate (eGFR), smoking status at baseline were captured from the medical record within one year from the diagnosis of diabetes (details see the **Supplementary Techniques**). The follow-up was defined by the first event of outcome or the last measurement of HbA1c before 24 April 2017 in the event-free case. Charlson Co-morbidity Index (CCI) was calculated using

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the ICD (International Classification of Diseases)-9 and ICD-10 code within the year after the diagnosis of diabetes (13), while we specifically removed the items of diabetes and cardiovascular events, which were overlapping with our population or outcomes.

Assessment of visit-to-visit HbA1c variability

To avoid the interaction between the HbA1c variability parameter with the frequency of HbA1c measurement and to better fit clinical practice, the HbA1c variability was evaluated using HVS, which was adopted from a recent publication (12). Briefly, HVS is the number of measures within an individual where the HbA1c has changed by > 0.5% (5.5mmol/mol) from the value prior, as a percentage of the total number of HbA1c measures between the diagnosis of diabetes and the outcome of interest for that individual **(Fig. S1)**. To avoid the impact of multiple HbA1c measures in a short space of time, we allocated one HbA1c measure for every three-month period, using the median of all the HbA1c measures within that time. The resulting variability measure is termed the binned HVS (b-HVS). We also calculated the time-weighted average HbA1c, which was calculated using the area under the curve (AUC) of HbA1c from the diagnosis of diabetes to the first event divided by the duration.

Outcomes

We examined ten outcomes of interest including: major adverse cardiovascular events (MACE); all-cause mortality; atherosclerotic cardiovascular death (ASCV death); hospitalization or death from coronary artery disease, ischemic stroke or heart failure; observable background diabetic retinopathy (DR), diabetic peripheral neuropathy (DPN); diabetic foot ulcer (DFU); and the new onset of chronic kidney diseases (CKD). If the event of interest occurred within the first three years from the diagnosis of diabetes, the patient was excluded from the analysis of that outcome, to avoid the outcome occurring close to diagnosis before the HVS could be defined, when the outcome would be unlikely to be related to the HVS. For full definitions of the endpoints (see the **Supplementary Techniques**).

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Statistical Analyses

The categorical variables were described using frequency and percentage. The continuous variables were described using means and SDs if normally distributed or median interquartile range (IQR) if not. Cox proportional-hazards model was used to assess the association between the HbA1c variability and each of the outcomes. The association of the adverse outcome with the HVS categories (≥0 to ≤20, >20 to ≤40, >40 to ≤60, >60 to ≤80, >80, with the ≥0 to ≤20 as reference) were adjusted for sex, index age, calendar year, Scottish Index of Multiple Deprivation (SIMD) quintiles, ever smoking, hypertension at baseline, BMI at baseline, high-density lipoprotein (HDL) cholesterol at baseline, eGFR at baseline, antiplatelet therapy at baseline and CCI (≥1 vs 0). We used Survival::cox.zph Pack in R to test the proportional hazards assumption for Cox regression models (14) for all our models. We considered the proportional hazards assumptions to be violated if the global P-value lower than 0.01. Because of the violation of proportional hazards assumptions the stage of CKD (stage 1 or 2) at baseline rather than the eGFR at baseline was stratified in the analysis of the new onset of the CKD. Five subgroup analyses were introduced based on the age (<65 years vs ≥65 years), sex, BMI at baseline (>30kg/m^2 vs ≤30kg/m^2), time-weighted mean HbA1c (>7% vs ≤7% or >53mmol/mol vs ≤53mmol/mol), and treatment at baseline (medication/insulin-treated vs. lifestyle intervention only). Five sensitivity analyses were performed for each outcome by: 1) adjusting for time-weighted average HbA1c; 2) using the b-HVS instead of HVS; 3) using the HVS based on the HbA1c measurement solely focusing on the first three years after diagnosis of diabetes, prior to the occurrence of any event; 4) using the individual-level SDs of the HbA1c instead of the HVS; 5) using individual-level CVs of HbA1c instead of the HVS. Analyses were undertaken in the SAS® 9.4 (SAS Institute Inc., Cary, NC, USA). and the RStudio for Windows (R version 3.2.5).

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Results

Baseline characteristics

As shown in **Fig. 1**, among the 79,569 patients with type 2 diabetes identified in the population, we included 21,352 patients for further analysis., The average age was 63.3 ± 11.1 years when recruited and 54.6% of them were male. The median follow-up duration was 6.8 (IQR: 4.6~11.2) years. The mean HbA1c at baseline was 7.7% ± 2.0% (60.7 ± 21.4 mmol/mol), and the median number of HbA1c measurements throughout the study period was 12 (IQR: 8~19) times during the follow-up duration. **Tab. S1** shows the baseline patient characteristics for those included for each analysis of outcomes and **Tab. 1** shows how the baseline characteristics differ across the HVS categories. 62% of the patients have an HVS below or equal to 40%; 12.5% have an HVS greater than 60%. As expected, an increasing HVS is associated with younger age of diagnosis, higher BMI, and more intensive diabetes treatment including greater insulin use.

HbA1c variability and outcomes

As shown in **Fig. 2**, between 13,111 to 19,883 patients were involved in the analyses of each outcome. Comparing with the reference (lowest HVS category, ≥0 to ≤20), patients with HVS over 60 were associated with increased risks of all outcomes in a fully adjusted Cox model. For example, those with HVS >80 to ≤100 had an increased risk of (HR [95%CI]): MACE: 2.38 [1.61~3.53]; all-cause mortality: 2.4 [1.72~3.33]; ASCV death: 2.4 [1.13~5.11]; coronary artery disease: 2.63 [1.81~3.84]; ischemic stroke: 2.04 [1.12~3.73]; heart failure: 3.23 [1.76~5.93]; DR: 7.4 [3.84~14.27]; DPN: 3.07 [2.23~4.22]; DFU: 5.24 [2.61~10.49]; CKD: 3.49 [2.47~4.95]).

Subgroup analyses and sensitivity analyses

Given the association between HVS and HbA1c we first undertook a sensitivity analysis, including time-weighted average HbA1c from diagnosis to event in the models (**Fig. 3**). The results were similar for most outcomes other than retinopathy where the

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association of HVS was diminished when adjusting for the time-weighted average HbA1c.

When comparing the subgroups with time-weighted average HBA1c more than or less than 7% (53mmol/mol) there was a stronger association between the HVS and coronary artery disease, ischemic stroke and progression to CKD in patients with time-weighted average HbA1c<7% or 53mmol/mol (**Fig. S5**). Other subgroup analyses were undertaken based on age (**Fig. S2**), sex (**Fig. S3**), obesity at baseline (**Fig. S4**) and treatment at baseline (**Fig. S6**) did not show significant differences in the trend of the association (except the cases with very small sample size). Using b-HVS instead of HVS also showed consistent results in all outcomes (**Fig. S7**). However, the sensitivity analysis using the first-three year HVS suggested a weaker association compared with the main analysis (**Fig. S8)**. The sensitivity analysis using the individual-level SD (**Fig. S9**) and CV (**Fig. S10**) of HbA1c showed a similar pattern of risk for most outcomes but not ischemic stroke for SD and CV and diabetic retinopathy for CV where weaker associations were observed.

Discussion

To our knowledge, this is the first population-based study to investigate the association between the visit-to-visit HbA1c variability and comprehensive endpoints including cardiovascular events and the microvascular complications of diabetes in patients with newly diagnosed type 2 diabetes independent of the time-weighted average HbA1c.

Our study showed clear elevated risks of adverse events in the ~12.5% of patients with a HVS higher than 60 (meaning those with 60% of their HbA1c measurements increased or decreased by > 0.5% (5.5mmol/mol) compared with the last measurement) after diagnosis of diabetes adjusted for their time-weighted average HbA1c. The results were consistent with previous studies based on trial (15,16) and observational datasets (6-12,17). Our results indicate that frequent fluctuations of HbA1c of patients with

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diabetes may be an independent risk factor of poor prognosis and more stable HbA1c control may benefit the patients in clinical practice, although it should be emphasized that our results are observational and causal inference cannot be made. Of note, a recent analysis based on the VADT trial (16) suggested that higher HbA1c variability was associated with the increased risk of cardiovascular events in the group of intensive glycemic control but not the standard control. It suggested that the increased HbA1c variability may neutralize the cardiovascular benefits of the sustained 1.5% (16.4 mmol/mol) HbA1c reduction during the study period (18). We undertook a subgroup analysis looking at HVS in those with good and poor average HbA1c. It was interesting to note that the HVS association with atherosclerotic cardiovascular events was greater in those with good HbA1c, in keeping with the VADT finding. However, we need to interpret these results with caution as we can not account for treatment intensity during the study period.

We have previously reported that patients with high variability in HbA1c have high cardiovascular risk at baseline (19), and thus the association of HbA1c variability with risk may not be a feature of the HbA1c variability per se, but a marker of this baseline difference in patient characteristics. In this current study we have adjusted comprehensively for baseline differences in cardiovascular risk although we acknowledge there could be residual confounding. It is interesting to note that in the sensitivity analysis where we restrict our analysis to defining HbA1c variability only on the first three years of HbA1c measures, the association with micro- and macrovascular outcomes are diminished. This suggests that the HbA1c variability may continuously contribute to the clinical adverse endpoints beyond the first three years, and therefore that the risk can be less attributable to baseline differences in patient characteristics and more attributable to the HbA1c variability per se. As a recent study suggested that HbA1c variability is associated with the quality of patient care (20), it also suggests that it is never too late to reduce the HbA1c variability in clinical practice.

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Although infeasible in the current analysis, it would also be interesting to evaluate HbA1c variability on different anti-diabetic treatments to see if reduced variability can explain some of the improved outcomes with some of these agents.

Although we cannot attribute poor prognosis to the HbA1c variability per se, some underlying mechanisms may explain the association observed in our study. Although oxidative stress is suggested to be the explanation between short-term glycemic variability and adverse outcomes (5), it is not clear whether this is increased in patients with high visit-to-visit HbA1c variability. An alternative may relate to accumulated epigenetic modification induced by both high and low glycemia (21). Another explanation may simply relate to increased hypoglycemia in these individuals, since some studies suggest high HbA1c variability is linked to increased risk of severe hypoglycemic episodes (22) and patients admitted to hospital due to hypoglycemia have higher HbA1c variability (23). It will be valuable if a further study could address the frequency of overall and severe hypoglycemia among patients with different HbA1c variability.

The strengths of our study are clear. Firstly, all the included patients were tracked with their HbA1c measurement from the diagnosis of diabetes, so there is no period of the patients' diabetes journey that is not captured. Secondly, we comprehensively studied ten clinically important outcomes, including all-cause mortality, cardiovascular events and major microvascular complications of diabetes and showed consistent results across these micro- and macrovascular endpoints. Thirdly, our results were confirmed by a series of subgroup analyses and sensitivity analyses including adjusting for the time-weighted average HbA1c from the diagnosis of diabetes. Fourthly, our study was based on the real-world data of diabetes care in Scotland making these results directly translatable to clinical practice. Finally, we have used the HVS rather than SD or CV which we feel is much more clinically tractable. Although SD and CV reflect the dispersion trend of the HbA1c measures in an individual, they are no more than

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clinically meaningless statistical parameters. When considering the HVS, the clinicians can review the HbA1c profile for an individual – those where more than 60% of measures vary by more than 0.5% are at high risk.

The study does have limitations. Firstly, as a retrospective cohort study, uncorrected confounding could be possible and individuals with higher HbA1c variability may also at higher cardiovascular risks of other causes (18), and we cannot conclude an association of variability per se with the outcomes. Nevertheless, we used Cox proportional-hazards models to minimize the possible known confounding factors including CCI, smoking status and social deprivation and used a series of subgroup analyses and sensitivity analyses to confirm our findings to be robust. Secondly, we did not adjust for or evaluate the contribution of hypoglycemia, which has been reported to be associated with HbA1c variability (15) in the association between the HbA1c variability and outcomes because of the limitation of the data. Thirdly, the median follow-up duration of the study was 6.8 years and this will limit the total incident outcomes. The need to only include patients with newly diagnosed diabetes and other inclusion criteria do limit the total follow up time in this study population. This relatively short median duration does reduce the number of long-term outcome events especially for retinopathy and diabetic foot ulcer. Studies with longer follow-up duration in larger populations would be of value.

Conclusion

In conclusion, our study shows that higher HbA1c variability from the diagnosis of diabetes is independently associated with increased risks of all-cause mortality, major cardiovascular and microvascular complications of diabetes.

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Acknowledgment

SL, KZ and ERP conceived the study. SL, IN, LD and SH performed the statistical analyses. SL and ERP drafted the manuscript. ERP takes responsibility for the contents of the article as the guarantor.

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Conflict of interests: None to declare.

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Table and table legend

Table 1. Baseline characteristics of the overall study population

HVS Scores	≥0 to ≤20	>20 to ≤40	>40 to ≤60	>60 to ≤80	>80
n	7,084	6,096	5,502	2,409	261
Age of diabetes diagnosis, yrs	67.1 ± 10.3	63.5 ± 10.5	60.5 ± 10.9	58.9 ± 11.2	57.5 ± 11.2
Sex (male), n (%)	3,569 (50.4)	3,305 (54.2)	3,179 (57.8)	1,446 (60.0)	165 (63.2)
SIMD quintile, n (%)					
Q1	1,251 (17.7)	1,165 (19.1)	1,171 (21.3)	503 (20.9)	62 (23.8)
Q2	1,263 (17.8)	1,134 (18.6)	1,121 (20.4)	471 (19.6)	51 (19.5)
Q3	1,328 (18.7)	1,175 (19.3)	1,016 (18.5)	493 (20.5)	52 (19.9)
Q4	1,936 (27.3)	1,629 (26.7)	1,409 (25.6)	634 (26.3)	60 (23.0)
Q5	1,306 (18.4)	993 (16.3)	785 (14.3)	308 (12.8)	36 (13.8)
Year of diabetes diagnosis*	2010 [2005, 2012]	2008 [2002, 2011]	2008 [2002, 2011]	2009 [2003, 2011]	2010 [2006, 2013]
BMI, kg/m^2	31.3 ± 6.0	31.9 ± 6.2	32.8 ± 6.5	33.3 ± 7.1	33.2 ± 7.3
Ever smoking, n (%)	4,881 (68.9)	4,336 (71.1)	3,977 (72.3)	1,748 (72.6)	178 (68.2)
Ever regular alcohol, n (%)	4,008 (61.2)	3,345 (59.1)	2,875 (57.3)	1,185 (54.5)	131 (56.5)
Systolic blood pressure, mmHg	140.1 ± 19.0	141.2 ± 19.5	140.3 ± 19.8	139.6 ± 19.6	138.2 ± 19.4
Diastolic blood pressure, mmHg	78.9 ± 10.8	81.0 ± 10.9	82.2 ± 11.2	82.2 ± 11.4	82.2 ± 12.0
Carlson Comorbidity Index ≥1, n (%)	1,332 (18.8)	1,073 (17.6)	867 (15.8)	449 (18.6)	58 (22.2)
Hypertension, n (%)	5,505 (77.7)	4,376 (71.8)	3,786 (68.8)	1,574 (65.3)	155 (59.4)
Treatment of diabetes within the first year from the diagnosis of diabetes, n (%)					
Lifestyle intervention only	5,260 (74.3)	3,137 (51.5)	2,190 (39.8)	740 (30.7)	61 (23.4)
Anti-diabetic agents without insulin	1,770 (25.0)	2,821 (46.3)	3,153 (57.3)	1,569 (65.1)	188 (72.0)

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Treated with insulin	54 (0.8)	138 (2.3)	159 (2.9)	100 (4.2)	12 (4.6)
Receiving anti-platelet therapy, n (%)	2,465 (34.8)	1,909 (31.3)	1,598 (29.0)	667 (27.7)	67 (25.7)
Receiving statins, n (%)	4,866 (68.7)	3,716 (61.0)	3,218 (58.5)	1,373 (57.0)	161 (61.7)
HbA1c at baseline, %	6.7 ± 1.2	7.8 ± 1.9	8.4 ± 2.1	8.9 ± 2.3	9.6 ± 2.5
HbA1c at baseline, mmol/mol	49 ± 13.0	62 ± 20.3	68 ± 23.1	77.4 ± 24.6	81 ± 26.8
HDL cholesterol, mmol/L	1.3 ± 0.4	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
Non-HDL cholesterol, mmol/L	3.5 ± 1.2	3.8 ± 1.2	3.9 ± 1.3	4.0 ± 1.3	4.0 ± 1.1
ALT, IU/L*	24 [18, 34]	28 [20, 39]	30 [21, 45]	32 [22, 48]	32 [22, 48]
eGFR, mL/min/1.73m²	72.2 ± 18.7	73.7 ± 18.8	77.2 ± 19.1	80.7 ± 19.7	84.1 ± 20.8

* Presented as median [the interquartile range]

Abbreviations: ALT: alanine aminotransferase; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; SIMD: Scottish Index of Multiple Deprivation

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Figures and figure legends

Figure 1. The flow diagram of the patient selection

Abbreviations: ASCV: atherosclerotic cardiovascular; CKD: chronic kidney diseases; CV: cardiovascular; DFU: diabetic foot ulcer; DPN: diabetic peripheral neuropathy; DR: diabetic retinopathy; MACE: major adverse cardiovascular events.

Figure 2. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes

Abbreviations: CI: confidence interval; HR: hazard ratio.

Figure 3. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes after adjusting for the time-weighted average HbA1c

Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

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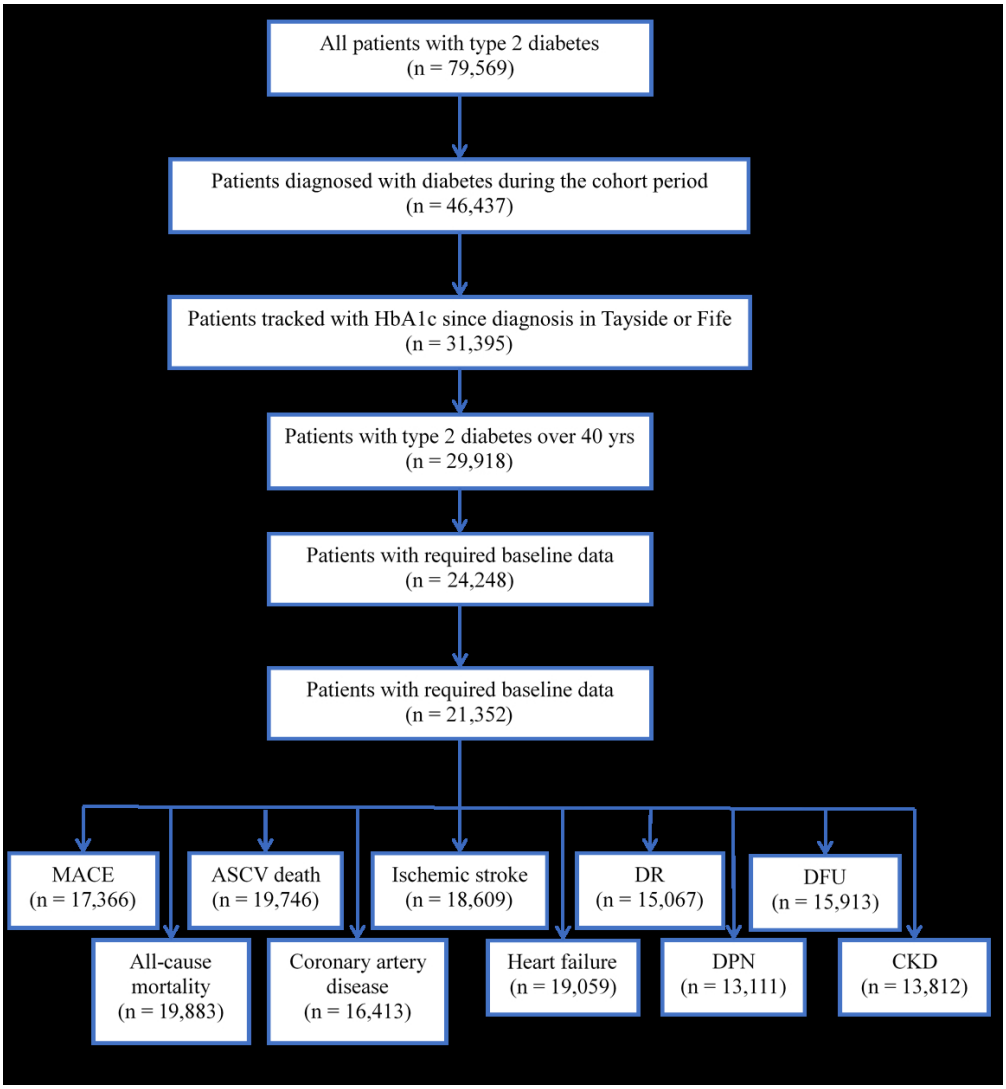

Figure 1. The flow diagram of the patient selection
Abbreviations: ASCV: atherosclerotic cardiovascular; CKD: chronic kidney diseases; CV: cardiovascular; DFU: diabetic foot ulcer; DPN: diabetic peripheral neuropathy; DR: diabetic retinopathy; MACE: major adverse cardiovascular events.

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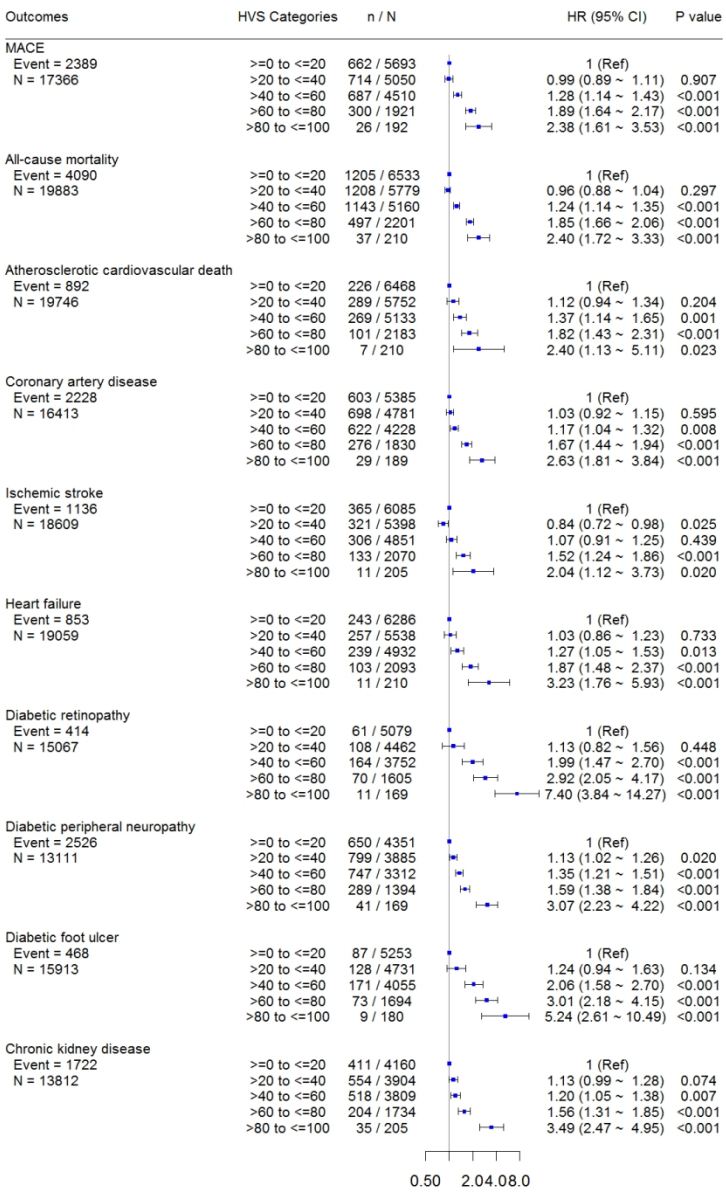

Figure 2. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes
Abbreviations: CI: confidence interval; HR: hazard ratio.

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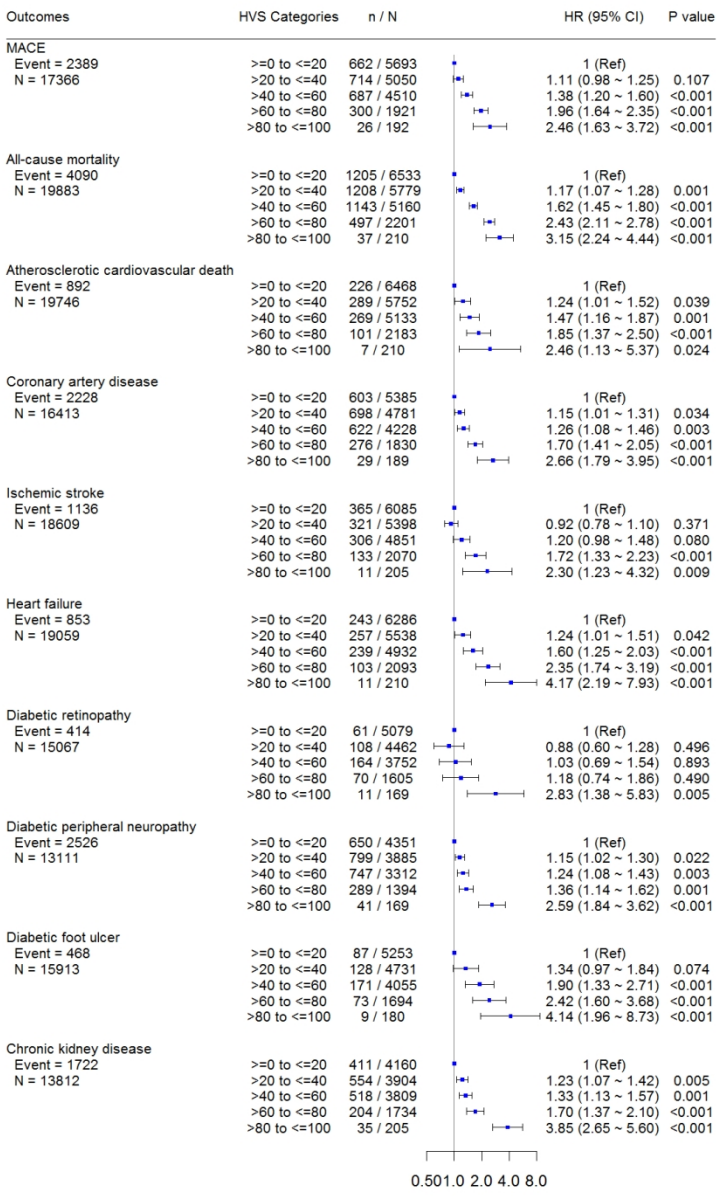

Figure 3. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes after adjusting for the time-weighted average HbA1c
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

423x635mm (72 x 72 DPI)

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Supplementary Techniques

Study exclusion criteria

They were excluded if they had: 1) unavailable data of sex, date of birth, Scottish Index of Multiple Deprivation (SIMD) or smoking records throughout the study period; 2) unavailable data of body mass index (BMI), serum creatinine or high-density lipoprotein (HDL) cholesterol within a year since diagnosis of diabetes; 3) free of the record of Scottish diabetes routine check when analyzing the outcomes of diabetic retinopathy (DR), diabetic peripheral neuropathy (DPN), diabetic foot ulcer (DFU); 5) with the estimated glomerular filtrate rate (eGFR) lower than 60 mL/min per 1.73m^2 at baseline when analyzing the outcome of new onset of chronic kidney diseases (CKD).

Defining the baseline characteristics

The patients were recognized to be ever smoking if there were any records of current or previous smoking in their the record in the electronic medical record (EMR) database. All baseline characteristics were using the data within a year since diagnosis of diabetes. BMI was extracted from the EMR database. The laboratory tests were extracted for the laboratory information systems. The eGFR was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. Hypertension at baseline was identified if there were at least two episodes of elevated blood pressures in different days (systolic blood pressure >140mmHg or diatolic blood pressure >90mmHg), or receiving at least two prescriptions of the anti-hypertensive drug (angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, beta-blockers or non-dihydroxypyridine calcium channel blockers). The baseline oral anti-diabetic agents (metformin, sulphonylureas, gliptins, acarbose, thiazolidinediones, dipeptidyl peptidase-4 inhibitors and sodium-glucose cotransporter-2 inhibitors), insulin, antiplatelet therapy and statins at baseline were identified if there were at least two prescriptions of the drug class within the first year of diagnosis. If the patients used insulin combined with oral agents, we considered insulin as a priority.

Defining the outcomes

All-cause mortality was identified as any death record in the General Registry Office or the Community Health Index (CHI) registry. Cardiovascular death was identified as the death due to ischemic stroke (defined as ICD-9: 433, 434, 435 or 436; ICD-10: I63, I64, I65 or I66) or coronary artery disease (defined as ICD-9: 410, 411, 412, 413 or 414; ICD-10: I20, I21, I22, I23, I24 or I25). The cardiovascular outcomes were identified if the patients were hospitalized or died due to the coronary artery disease, ischemic stroke or heart failure (defined as ICD-9: 428 or ICD10: I50), respectively. Major adverse cardiovascular events (MACE) was defined as a composite outcome of cardiovascular death, ischemic stroke and coronary artery diseases. DR was identified as the first episode of observable background or more advanced retinopathy according to the annual retinal photograph taken as part of the routine care or receiving a laser treatment based on the records in the Scottish Care Information-Diabetes Collaboration (SCI-DC). DPN and DFU were identified as the first episode of impaired monofilament test and the first record of active foot ulcer according to the record of the SCI-DC system as part of the routine care in Scotland, respectively. CKD was identified for the first episode of persistently reduced eGFR (all eGFR values in and between two nonadjacent months were below 60 mL/min per 1.73m^2).

Table S1. Baseline characteristics of patients for those included for each analysis of outcomes.

	MACE (n = 17,366)	**All-cause mortality (n = 19.883)**	**Cardiovascular death (n = 19,746)**	**Coronary artery disease (n = 16,413)**	**Ischemic stroke (n = 18,609)**	**Heart failure (n = 19,059)**	**Diabetic retinopathy (n = 15,067)**	**Diabetic peripheral neuropathy (n = 13,111)**	**Diabetic foot ulcer (n = 15,913)**	**Chronic kidney disease (n = 13,812)**
Age of diabetes diagnosis, yrs	62.7 ± 11.0	63.2 ± 11.0	63.1 ± 11.0	62.5 ± 11.0	62.9 ± 10.9	62.9 ± 10.9	62.8 ± 10.7	62.3 ± 10.6	63.0 ± 10.8	59.5 ± 9.8
Sex (male), n (%)	9,202 (53.0)	10,854 (54.6)	10,777 (54.6)	8,594 (52.4)	10,080 (54.2)	10,329 (54.2)	8,206 (54.5)	7,061 (53.9)	8,577 (53.9)	8,418 (60.9)
SIMD quintile, n (%)										
Q1	3,246 (18.7)	3,818 (19.2)	3,784 (19.2)	3,073 (18.7)	3,534 (19.0)	3,636 (19.1)	2,863 (19.0)	2,459 (18.8)	3,028 (19.0)	2,724 (19.7)
Q2	3,265 (18.8)	3,755 (18.9)	3,738 (18.9)	3,015 (18.4)	3,526 (18.9)	3,597 (18.9)	2,812 (18.7)	2,424 (18.5)	2,960 (18.6)	2,590 (18.8)
Q3	3,282 (18.9)	3,789 (19.1)	3,756 (19.0)	3,130 (19.1)	3,518 (18.9)	3,620 (19.0)	2,863 (19.0)	2,440 (18.6)	3,005 (18.9)	2,597 (18.8)
Q4	4,710 (27.1)	5,315 (26.7)	5,275 (26.7)	4,467 (27.2)	5,003 (26.9)	5,114 (26.8)	4,061 (27.0)	3,627 (27.7)	4,343 (27.3)	3,667 (26.5)
Q5	2,863 (16.5)	3,206 (16.1)	3,193 (16.2)	2,728 (16.6)	3,028 (16.3)	3,092 (16.2)	2,468 (16.4)	2,161 (16.5)	2,577 (16.2)	2,234 (16.2)
Year of diabetes diagnosis*	2009 [2003, 2011]	2009 [2003, 2011]	2009 [2003, 2011]	2009 [2003, 2011]	2009 [2003, 2011]	2009 [2003, 2011]	2008 [2003, 2010]	2006 [2002, 2010]	2006 [2002, 2010]	2009 [2004, 2011]
BMI, kg/m^2	32.2 ± 6.4	32.1 ± 6.4	32.1 ± 6.4	32.2 ± 6.5	32.2 ± 6.4	32.1 ± 6.4	32.2 ± 6.3	32.0 ± 6.3	32.0 ± 6.3	32.6 ± 6.5

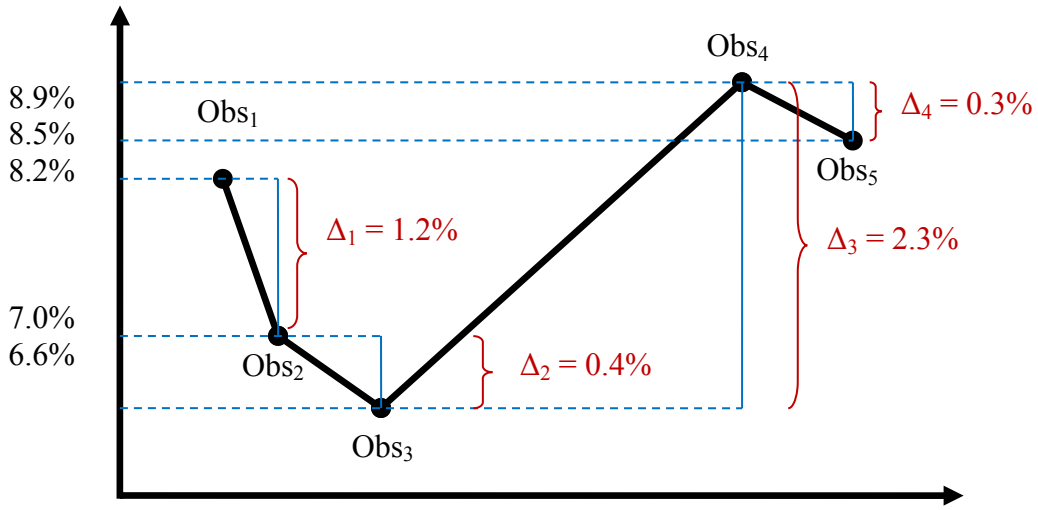
Ever smoking, n (%)	12,065 (69.5)	14,122 (71.0)	14,019 (71.0)	11,310 (68.9)	13,125 (70.5)	13,452 (70.6)	10,774 (71.5)	9,402 (71.7)	11,421 (71.8)	9,790 (70.9)
Ever regular alcohol, n (%)	9,420 (58.5)	10,823 (58.7)	10,771 (58.8)	8,840 (58.3)	10,177 (58.9)	10,410 (58.9)	8,378 (59.0)	7,389 (59.6)	8,733 (58.3)	8,043 (63.0)
Systolic blood pressure, mmHg	141.1 ± 19.3	140.6 ± 19.5	140.6 ± 19.4	141.4 ± 19.3	140.7 ± 19.3	140.8 ± 19.3	140.8 ± 19.2	141.4 ± 19.5	141.2 ± 19.6	139.9 ± 18.9
Diastolic blood pressure, mmHg	81.4 ± 10.9	80.9 ± 11.1	80.9 ± 11.0	81.7 ± 10.9	81.1 ± 11.0	81.1 ± 11.0	81.1 ± 10.9	81.5 ± 11.0	81.2 ± 11.0	82.0 ± 10.9
Carlson Comorbidity Index ≥1, n (%)	2,599 (15.0)	3,369 (16.9)	3,328 (16.9)	2,326 (14.2)	2,952 (15.9)	3,064 (16.1)	2,364 (15.7)	1,829 (14.0)	2,424 (15.2)	1,867 (13.5)
Hypertension, n (%)	12,166 (70.1)	14,323 (72.0)	14,214 (72.0)	11,311 (68.9)	13,282 (71.4)	13,600 (71.4)	10,916 (72.4)	9,425 (71.9)	11,522 (72.4)	9,282 (67.2)
Treatment of diabetes within the first year from the diagnosis of diabetes, n (%)										
Lifestyle intervention only	9,259 (53.3)	10,685 (53.7)	10,618 (53.8)	8,662 (52.8)	9,951 (53.5)	10,255 (53.8)	8,175 (54.3)	7,116 (54.3)	8,601 (54.1)	7,034 (50.9)
Anti-diabetic agents without insulin	7,789 (44.9)	8,791 (44.2)	8,727 (44.2)	7,443 (45.3)	8,279 (44.5)	8,430 (44.2)	6,585 (43.7)	5,724 (43.7)	6,990 (43.9)	6,522 (47.2)
Treated with insulin	318 (1.8)	407 (2.0)	401 (2.0)	308 (1.9)	379 (2.0)	374 (2.0)	307 (2.0)	271 (2.1)	322 (2.0)	256 (1.9)
Receiving anti-platelet therapy, n (%)	4,510 (26.0)	6,232 (31.3)	6,170 (31.2)	3,832 (23.3)	5,459 (29.3)	5,769 (30.3)	4,790 (31.8)	4,049 (30.9)	5,052 (31.7)	3,555 (25.7)
Receiving statins, n (%)	10,385 (59.8)	12,333 (62.0)	12,252 (62.0)	9,592 (58.4)	11,391 (61.2)	11,743 (61.6)	9,238 (61.3)	7,680 (58.6)	9,513 (59.8)	8,472 (61.3)

HbA1c, %	7.7 ± 2.0	7.7 ± 2.0	7.7 ± 2.0	7.8 ± 2.0	7.7 ± 2.0	7.7 ± 2.0	7.7 ± 2.0	7.8 ± 2.0	7.8 ± 2.0	7.8 ± 2.0
HbA1c, mmol/mol	61 ± 21.8	61 ± 21.6	61 ± 21.5	61 ± 22.0	61 ± 21.6	61 ± 21.5	61 ± 21.5	61 ± 22.1	61 ± 21.9	62 ± 22.0
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
Non-HDL cholesterol, mmol/L	3.9 ± 1.2	3.8 ± 1.3	3.8 ± 1.3	3.9 ± 1.2	3.8 ± 1.2	3.8 ± 1.3	3.8 ± 1.2	3.9 ± 1.3	3.9 ± 1.3	3.9 ± 1.3
ALT, IU/L*	28 [20, 41]	28 [20, 40]	28 [20, 40]	28 [20, 41]	28 [20, 41]	28 [20, 40]	28 [20, 41]	28 [20, 41]	28 [20, 41]	30 [22, 44]
eGFR, mL/min/1.73m^2	75.5 ± 18.7	74.7 ± 19.0	74.8 ± 18.9	75.8 ± 18.8	75.1 ± 18.8	75.1 ± 18.7	73.3 ± 18.1	73.4 ± 17.8	73.1 ± 18.1	83.0 ± 14.5

* Presented as median [the interquartile range]

Abbreviations: ALT: alanine aminotransferase; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; SIMD: Scottish Index of Multiple Deprivation

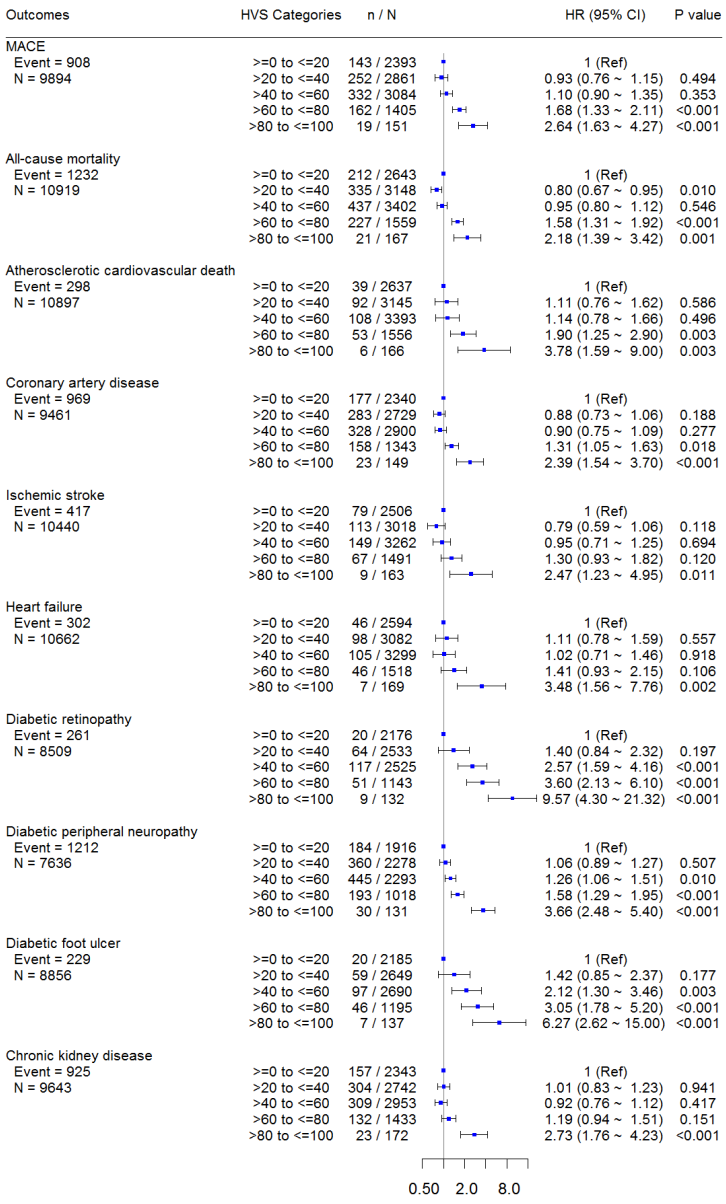
Figure S1. The definition of HbA1c Variability Score (HVS)



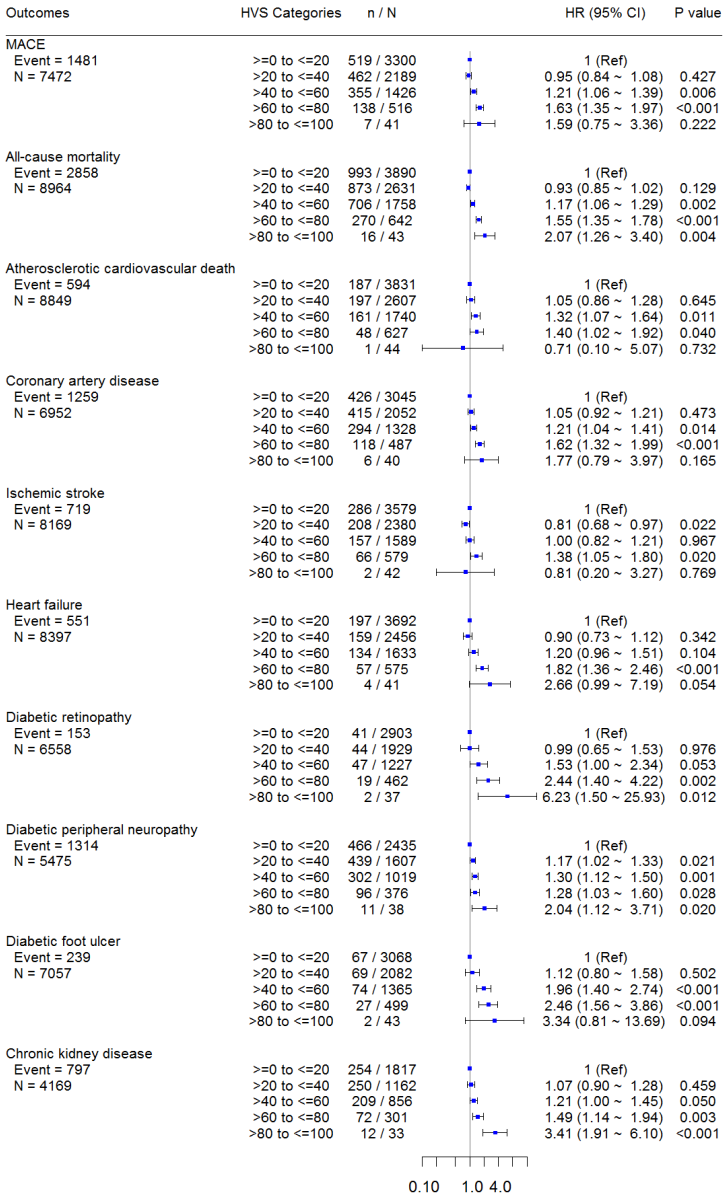
HbA1c Variability Score (HVS) = Number of HbA1c fluctuation events (Δ>0.5%) / (Total number of HbA1c measurements – 1) × 100
In this case, there are 2 fluctuation events (Δ_1 & Δ_3) in 5 HbA1c measurements (4 Δs). ∴ HVS = 2 / (5 - 1) × 100 = 50

Figure S2. The subgroup analysis based on the age

A.



B.

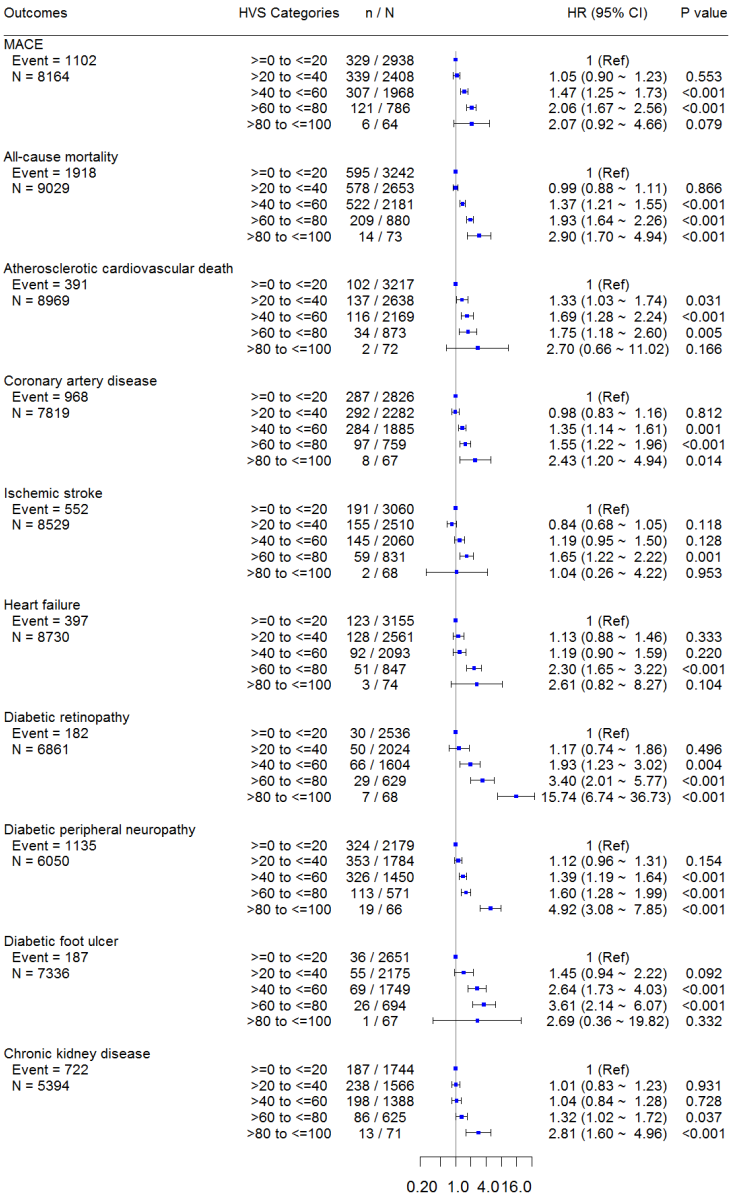


A. the subgroup of patients younger than 65; B. the subgroup of patients aged 65 or older

Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S3. The subgroup analysis based on sex

A.



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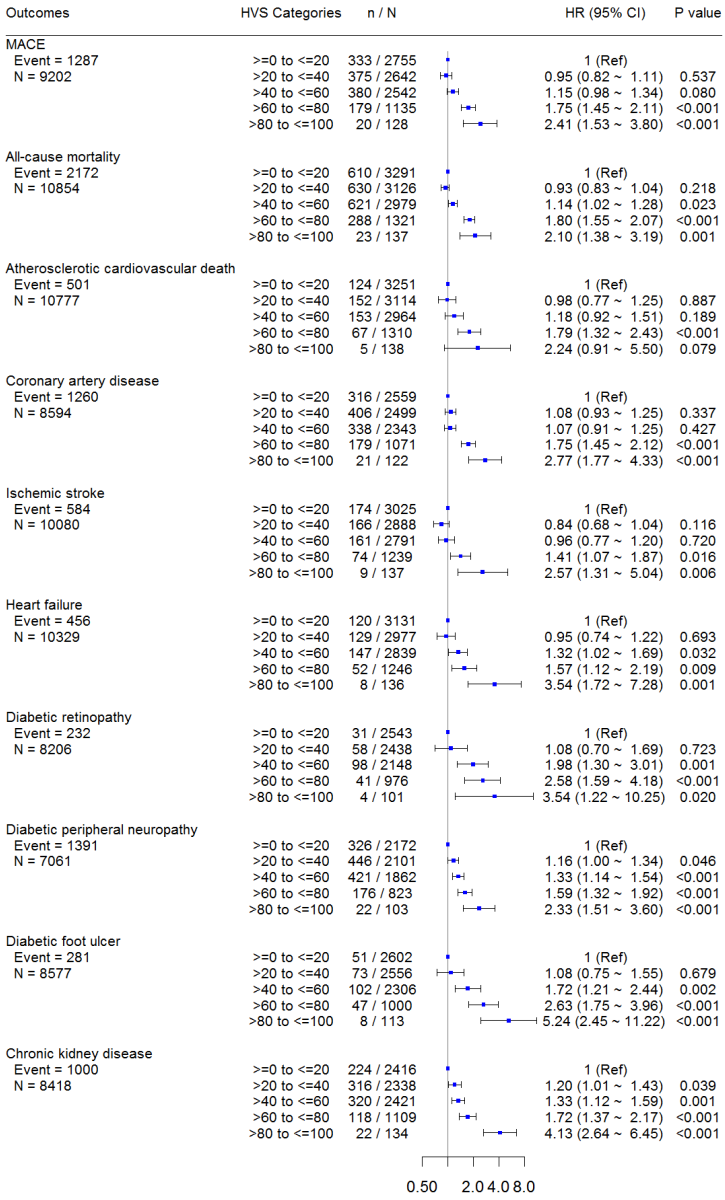
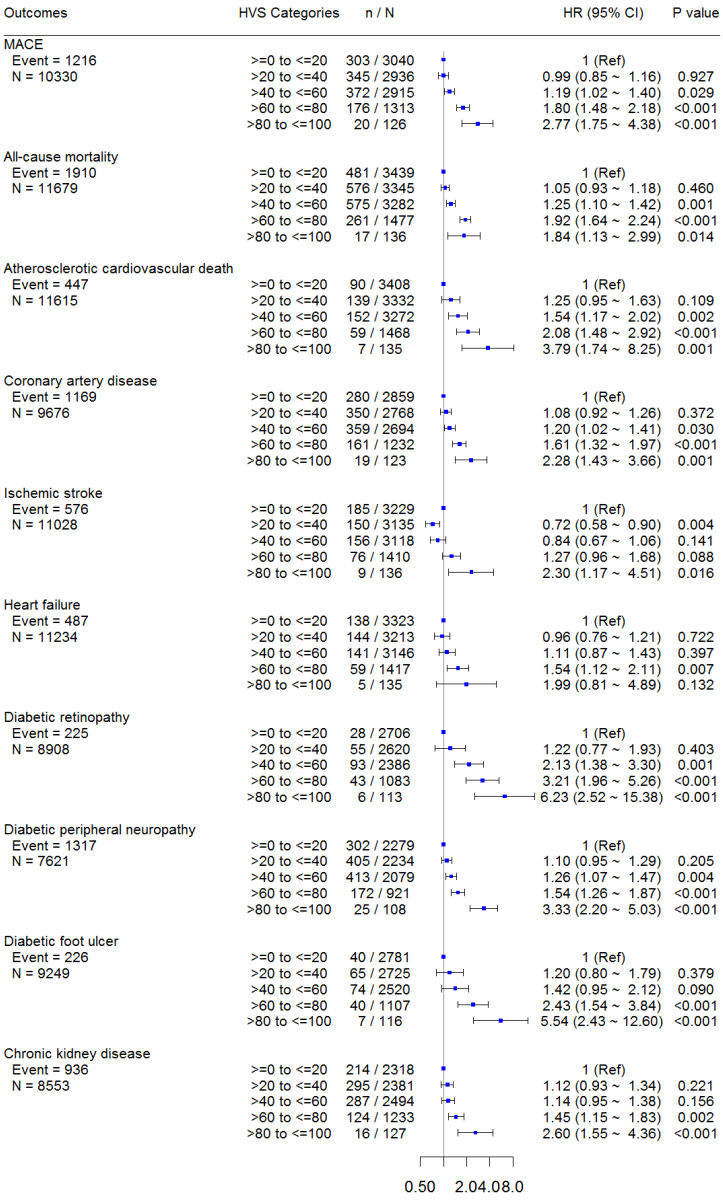


A. the subgroup of female patients; B. the subgroup of male patients

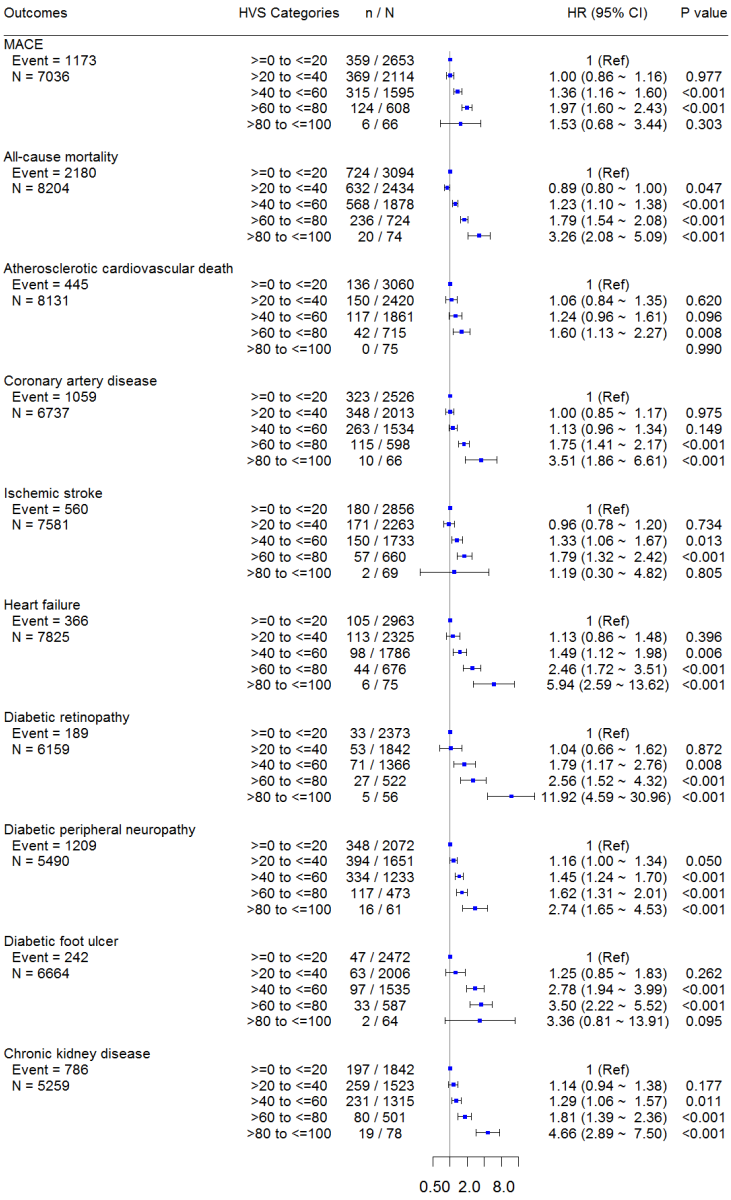
Abbreviations: CI: confidence interval; HR: hazard ratio.

Figure S4. The subgroup analysis based on the baseline body mass

A.



B.

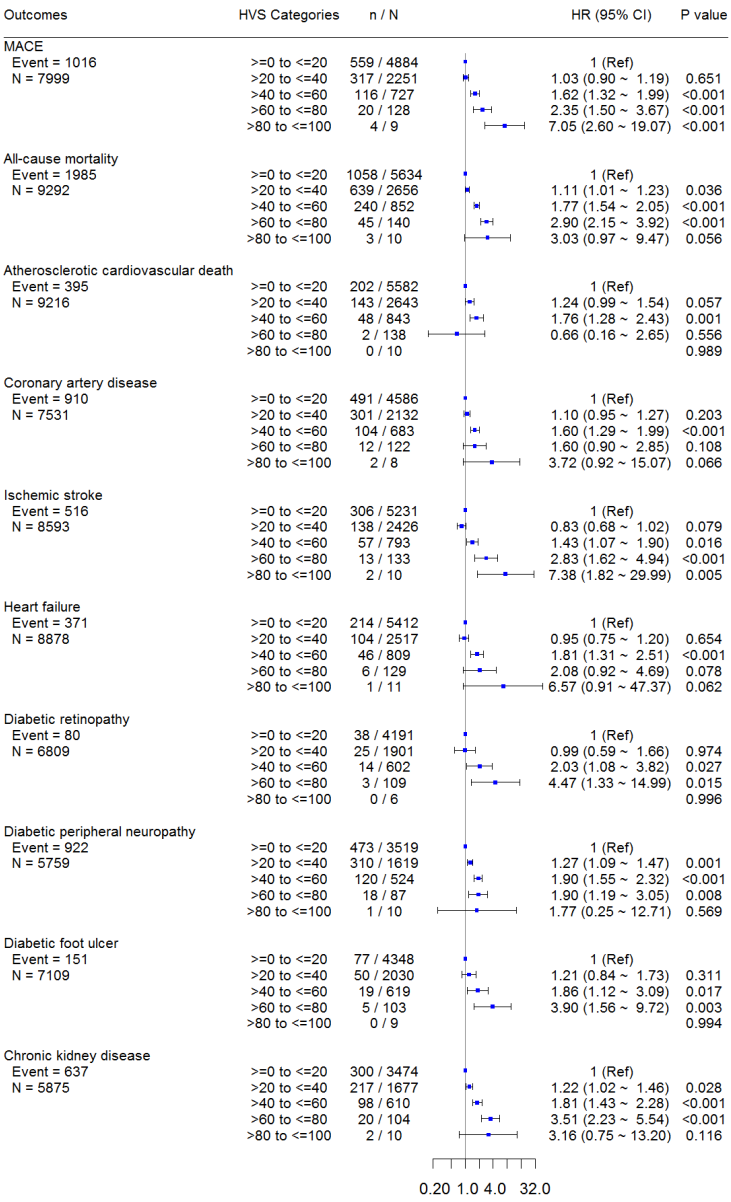


A. the subgroup of non-obese patients at baseline; B. the subgroup of obese patients at baseline

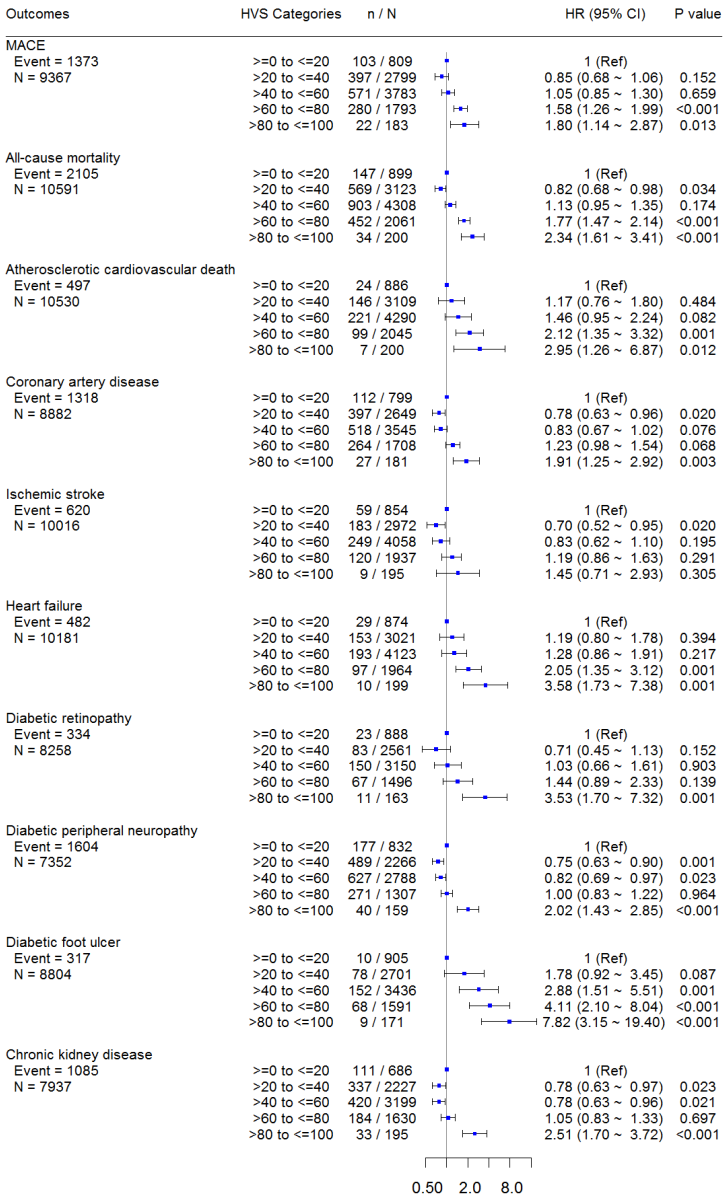
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S5. The subgroup analysis based on the time-weighted average HbA1c

A.



B.

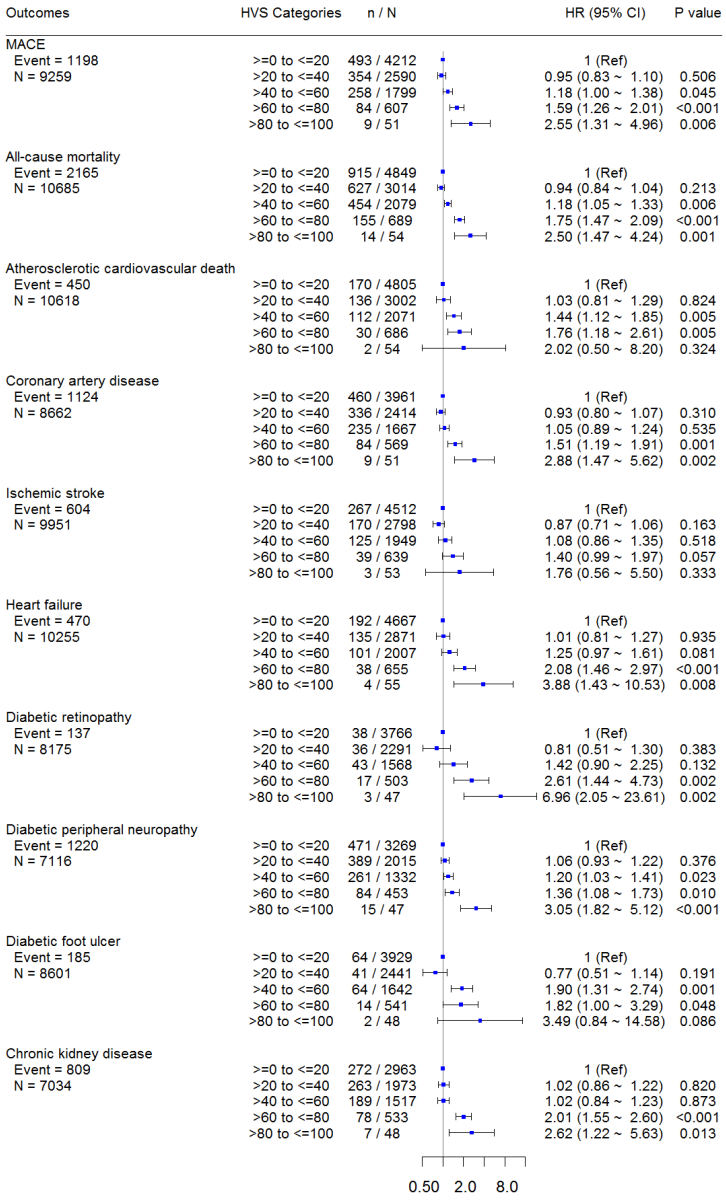


A. the subgroup of the time-weighted average HbA1c ≤7% (53 mmol/mol); B. the subgroup of the time-weighted average HbA1c >7% (53 mmol/mol)

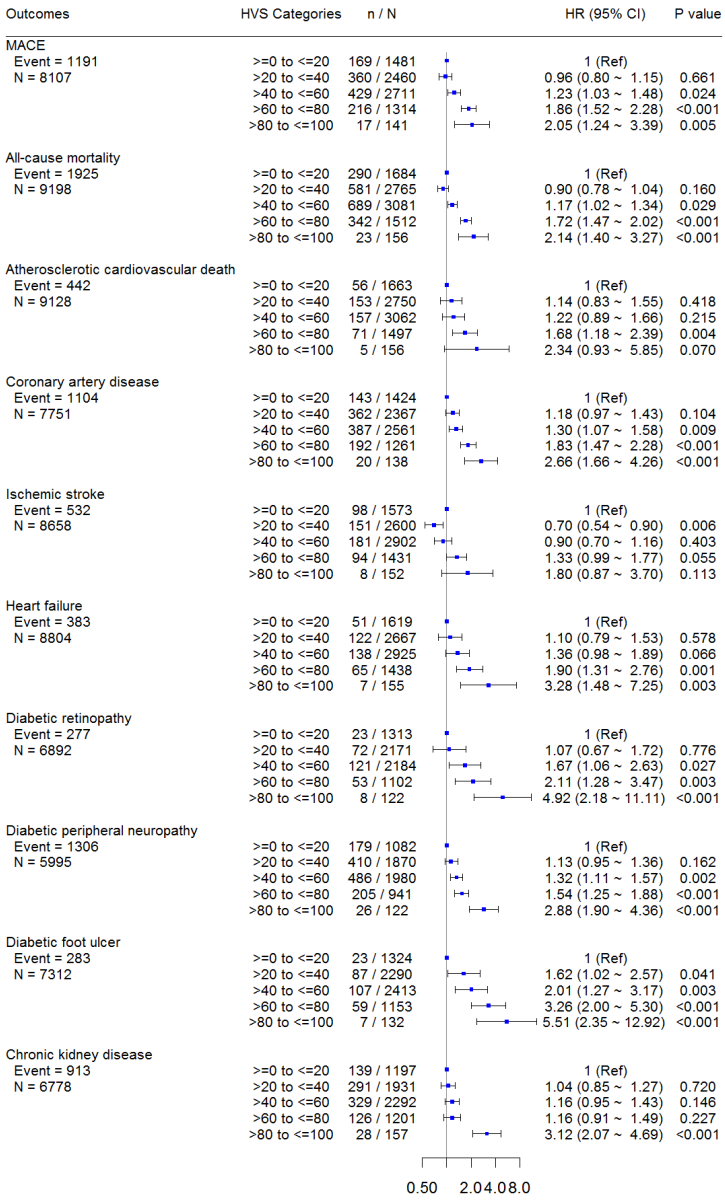
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S6. The subgroup analysis based on the treatment of diabetes at baseline

A.



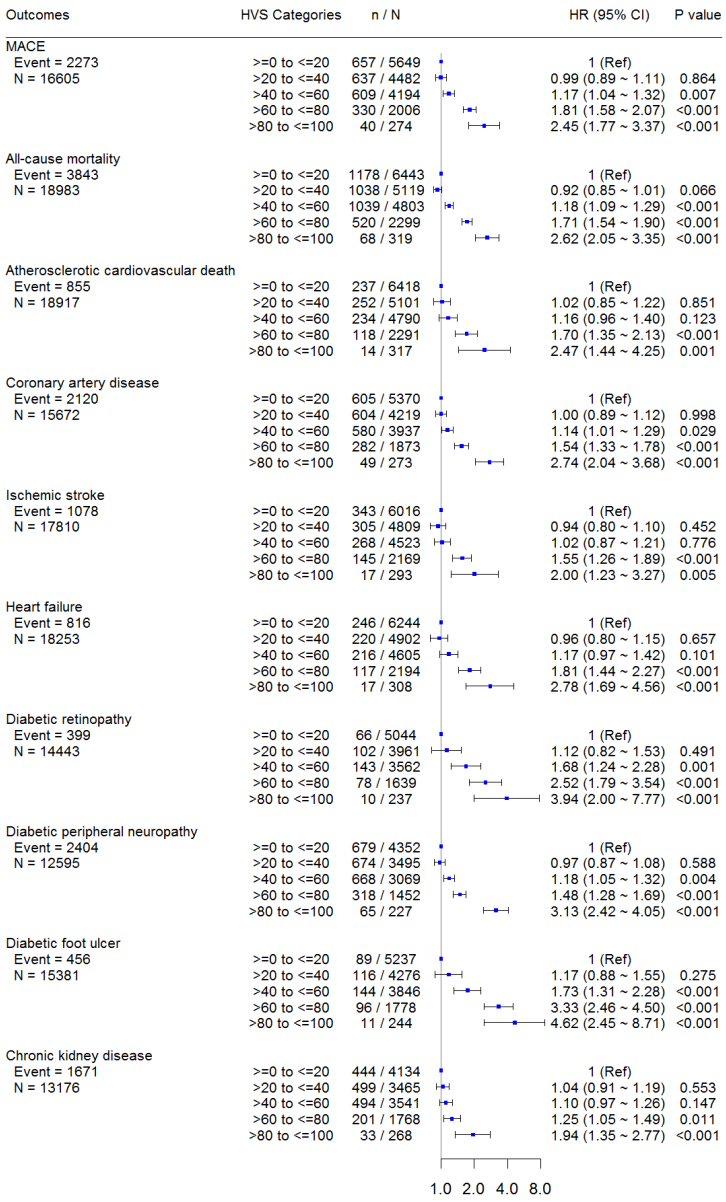
B.



A. the subgroup of patients receiving lifestyle intervention only at baseline; B. the subgroup of patients receiving anti-diabetic medication or insulin at baseline

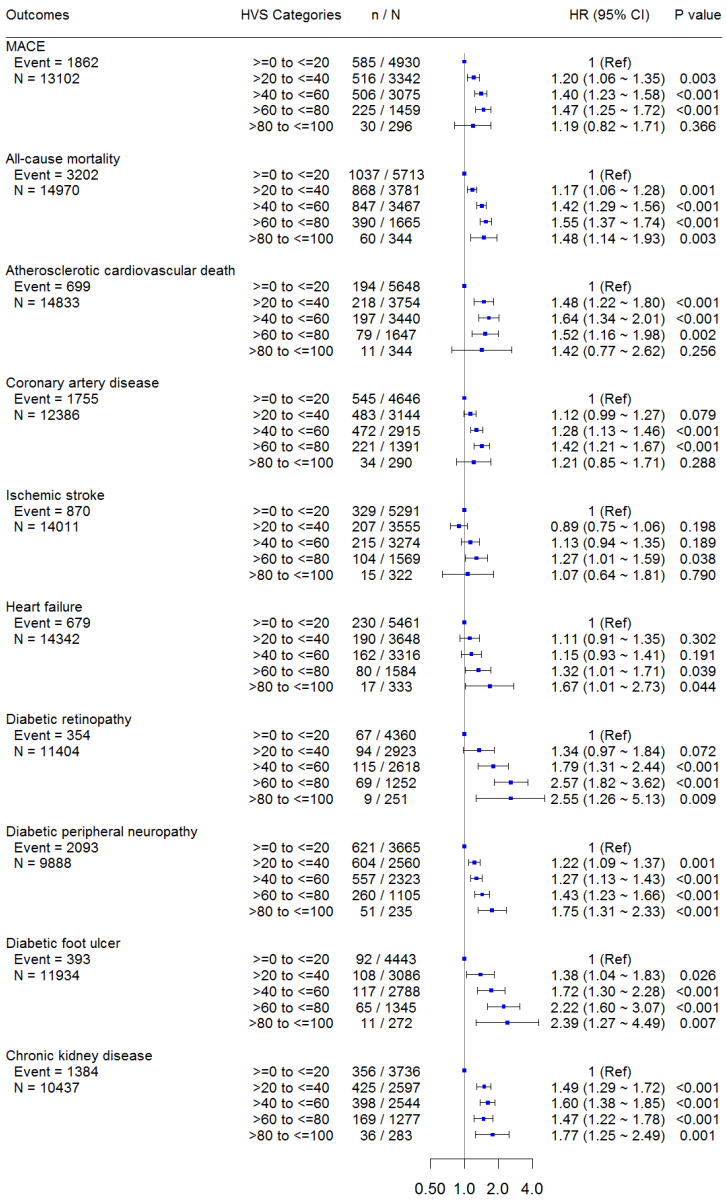
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S7. The sensitivity analysis using the binned HbA1c variability score (b-HVS)



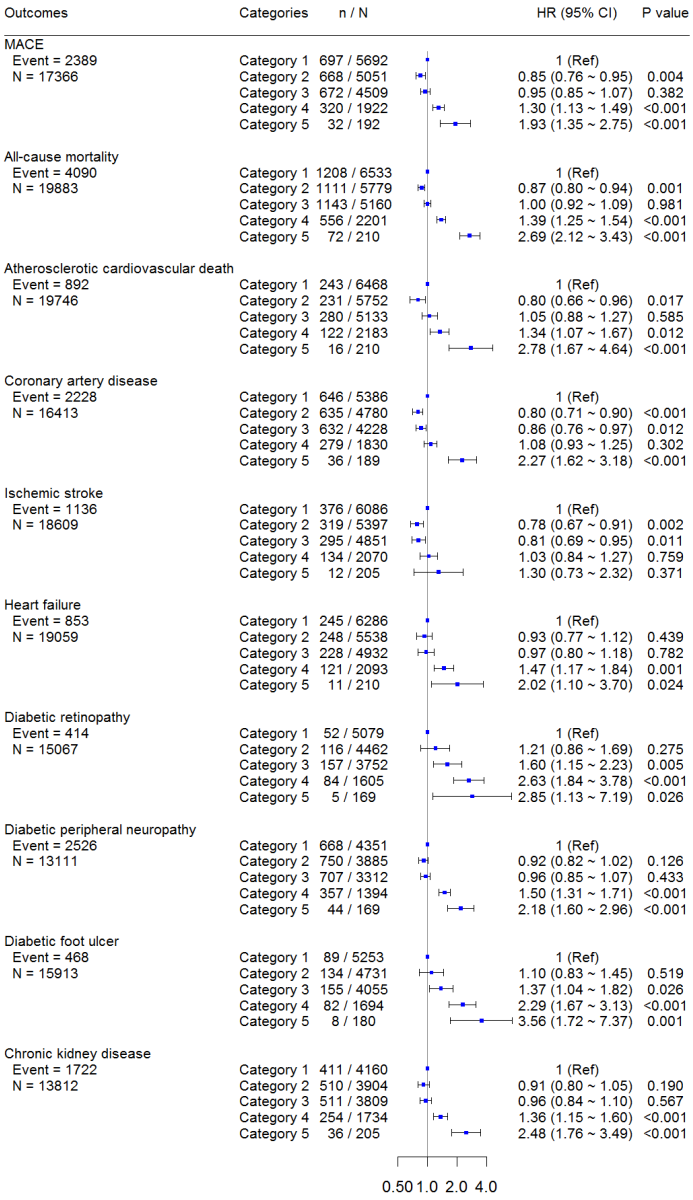
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S8. The sensitivity analysis using the HbA1c variability score (HVS) based on the HbA1c measurement in the first three years since diagnosis



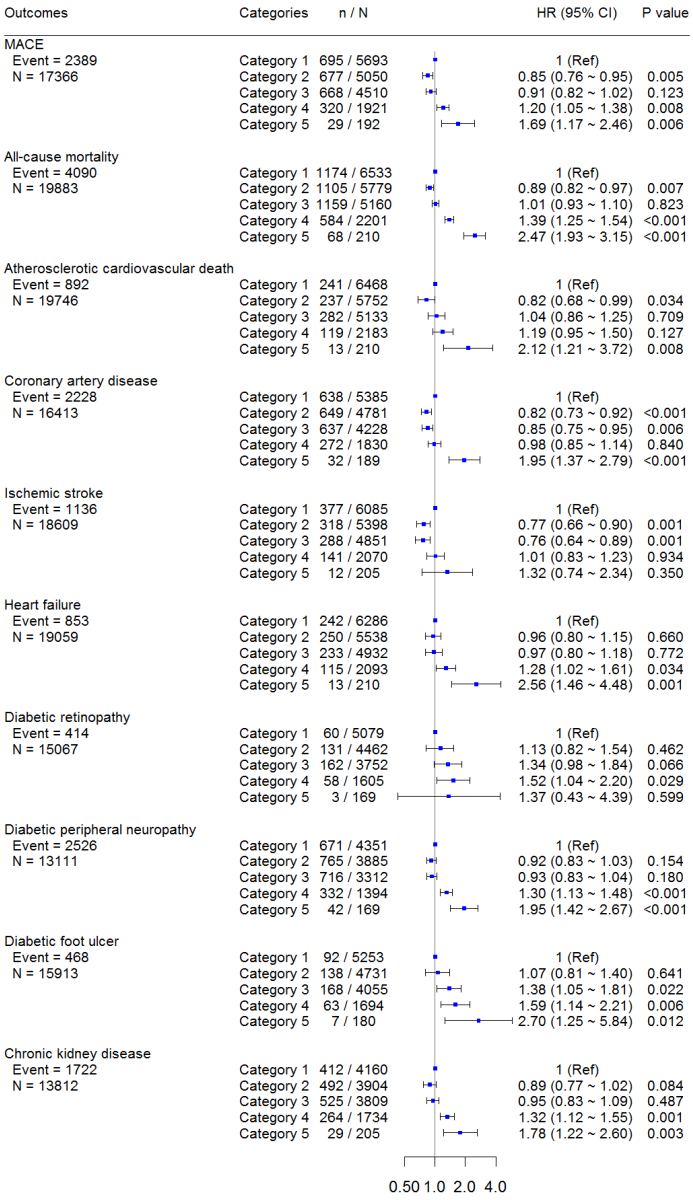
Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S9. The sensitivity analysis using the standard deviation (SD) of the HbA1c levels in accordance with the HVS category



Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Figure S10. The sensitivity analysis using the coefficients of variance (CV) of the HbA1c levels in accordance with the HVS category



Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

Visit-to-visit HbA1c variability is associated with cardiovascular disease and microvascular complications in patients with newly diagnosed type 2 diabetes

Running Title: HbA1c variability and complications in diabetes

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Abstract

Objective: To investigate the association between visit-to-visit HbA1c variability and cardiovascular events and microvascular complications in patients with newly diagnosed type 2 diabetes.

Research Design and Methods: This retrospective cohort study analyzed patients from Tayside and Fife in the Scottish Care Information-Diabetes Collaboration (SCI-DC), who were observable from the diagnosis of diabetes and had at least five HbA1c measurements before the outcomes being evaluated. We used the previously reported HbA1c variability score (HVS) calculated as the percentage of the number of changes in HbA1c over 0.5% (5.5 mmol/mol) among all HbA1c measurement within an individual. The association between HVS and ten outcomes was assessed using Cox proportional-hazards models.

Results: We included 13,111 to 19,883 patients in the analyses of each outcome. The patients with HVS over 60% were associated with elevated risks of all outcomes compared with the lowest quintile (for example, hazard ratios and 95% confidence intervals [HVS >80 to ≤100 vs. HVS ≥0 to ≤20]: 2.38 [1.61~3.53] for major adverse cardiovascular events [MACE]; 2.4 [1.72~3.33] for all-cause mortality; 2.4 [1.13~5.11] for atherosclerotic cardiovascular [ASCV] death; 2.63 [1.81~3.84] for coronary artery disease; 2.04 [1.12~3.73] for ischemic stroke; 3.23 [1.76~5.93] for heart failure; 7.4 [3.84~14.27] for diabetic retinopathy; 3.07 [2.23~4.22] for diabetic peripheral neuropathy; 5.24 [2.61~10.49] for diabetic foot ulcer; 3.49 [2.47~4.95] for the new-onset chronic kidney disease). Four sensitivity analyses, including adjustment for time-weighted average HbA1c confirmed the robustness of the results.

Conclusions: Our study shows that higher HbA1c variability is associated with increased risks of all-cause mortality, cardiovascular events and microvascular complication of diabetes independently of high HbA1c.

Keywords: HbA1c variability, cardiovascular event, all-cause mortality, heart failure, diabetic retinopathy, diabetic peripheral neuropathy, diabetic foot ulcer, chronic kidney disease

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Introduction

Although there is considerable evidence that intensive blood glucose normalization reduces the risk of both cardiovascular events and microvascular complications of diabetes (1-3), the effects were heterogeneous between trials. For example, the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial was terminated prematurely due to significantly elevated mortality and cardiovascular events (4), suggesting that the near-normalization of blood glucose should not be the only target of diabetes treatment. Glycemic variability is one factor that may explain these differences in cardiovascular outcomes.

Glycemic variability can be measured as either the glucose fluctuation within a day or the long-term visit-to-visit variability. The latter has been recently investigated in several studies, although the metrics and definition of the variability measure were inconsistent (5). Most studies evaluating HbA1c variability using the standard deviation (SD) or the coefficient of variation (CV) of HbA1c, suggested that these measures were associated with all-cause mortality and the development of the adverse outcomes of diabetes, after adjusting for the average HbA1c (6-11). However, neither SD or CV of HbA1c can be easily interpreted in clinical practice. Recently, Forbes and colleagues (12) developed a new scale, namely the HbA1c variability score (HVS) in the current study, to define the HbA1c variability. The HVS indicates how frequently the HbA1c rises or decreases by more than 0.5% (5.5mmol/mol), which is in line with the SD and CV of HbA1c but clinically more translatable (as it can be interpreted as the percentage of total HbA1c measures that vary by more than 0.5% or 5.5mmol/mol) (6,12). However, the HVS has not been widely used among the studies of HbA1c variability, with previous studies using this scale only focusing on the elderly and non-diabetic population and evaluating mainly mortality as an outcome (6,12). It is unclear whether HVS is associated with microvascular complications of diabetes and whether the increased cardiovascular risk described could be extended to real-world patients with type 2 diabetes. In this study we aimed to investigate the association between visit-to-

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visit HbA1c variability and both cardiovascular diseases and microvascular complications in a large population database of patients with newly diagnosed type 2 diabetes.

Research Design and Methods

Data source and study population

The population was selected from patients from Tayside and Fife in the Scottish Care Information-Diabetes Collaboration (SCI-DC), the electronic health record system used in Scotland for patients with diabetes. The patients were included if they: 1) were diagnosed with type 2 diabetes; 2) had their first HbA1c measurement within one year from diagnosis of diabetes; 3) were over 40 years old when first diagnosed with diabetes; 4) did not experience any study outcome before or within three years since diagnosis of diabetes; 5) had at least five records of HbA1c measurement between diagnosis of diabetes and the first episode of the study outcome. Patients were excluded where data were incomplete (details see the **Supplementary Techniques**). Data provision and linkage were carried by the University of Dundee Health Informatics Centre (HIC, https://www.dundee.ac.uk/hic), with analyses of anonymized data performed in an ISO27001 and Scottish Government accredited secure safe haven. HIC Standard Operating Procedures have been reviewed and approved by the NHS East of Scotland Research Ethics Service and consent for this study was obtained from the NHS Fife Caldicott Guardian.

Baseline parameters and follow-up

The body mass index (BMI), estimated glomerular filtration rate (eGFR), smoking status at baseline were captured from the medical record within one year from the diagnosis of diabetes (details see the **Supplementary Techniques**). The follow-up was defined by the first event of outcome or the last measurement of HbA1c before 24 April 2017 in the event-free case. Charlson Co-morbidity Index (CCI) was calculated using

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the ICD (International Classification of Diseases)-9 and ICD-10 code within the year after the diagnosis of diabetes (13), while we specifically removed the items of diabetes and cardiovascular events, which were overlapping with our population or outcomes.

Assessment of visit-to-visit HbA1c variability

To avoid the interaction between the HbA1c variability parameter with the frequency of HbA1c measurement and to better fit clinical practice, the HbA1c variability was evaluated using HVS, which was adopted from a recent publication (12). Briefly, HVS is the number of measures within an individual where the HbA1c has changed by > 0.5% (5.5mmol/mol) from the value prior, as a percentage of the total number of HbA1c measures between the diagnosis of diabetes and the outcome of interest for that individual **(Fig. S1)**. To avoid the impact of multiple HbA1c measures in a short space of time, we allocated one HbA1c measure for every three-month period, using the median of all the HbA1c measures within that time. The resulting variability measure is termed the binned HVS (b-HVS). We also calculated the time-weighted average HbA1c, which was calculated using the area under the curve (AUC) of HbA1c from the diagnosis of diabetes to the first event divided by the duration.

Outcomes

We examined ten outcomes of interest including: major adverse cardiovascular events (MACE); all-cause mortality; atherosclerotic cardiovascular death (ASCV death); hospitalization or death from coronary artery disease, ischemic stroke or heart failure; observable background diabetic retinopathy (DR), diabetic peripheral neuropathy (DPN); diabetic foot ulcer (DFU); and the new onset of chronic kidney diseases (CKD). If the event of interest occurred within the first three years from the diagnosis of diabetes, the patient was excluded from the analysis of that outcome, to avoid the outcome occurring close to diagnosis before the HVS could be defined, when the outcome would be unlikely to be related to the HVS. For full definitions of the endpoints (see the **Supplementary Techniques**).

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Statistical Analyses

The categorical variables were described using frequency and percentage. The continuous variables were described using means and SDs if normally distributed or median interquartile range (IQR) if not. Cox proportional-hazards model was used to assess the association between the HbA1c variability and each of the outcomes. The association of the adverse outcome with the HVS categories (≥0 to ≤20, >20 to ≤40, >40 to ≤60, >60 to ≤80, >80, with the ≥0 to ≤20 as reference) were adjusted for sex, index age, calendar year, Scottish Index of Multiple Deprivation (SIMD) quintiles, ever smoking, hypertension at baseline, BMI at baseline, high-density lipoprotein (HDL) cholesterol at baseline, eGFR at baseline, antiplatelet therapy at baseline and CCI (≥1 vs 0). We used Survival::cox.zph Pack in R to test the proportional hazards assumption for Cox regression models (14) for all our models. We considered the proportional hazards assumptions to be violated if the global P-value lower than 0.01. Because of the violation of proportional hazards assumptions the stage of CKD (stage 1 or 2) at baseline rather than the eGFR at baseline was stratified in the analysis of the new onset of the CKD. Five subgroup analyses were introduced based on the age (<65 years vs ≥65 years), sex, BMI at baseline (>30kg/m^2 vs ≤30kg/m^2), time-weighted mean HbA1c (>7% vs ≤7% or >53mmol/mol vs ≤53mmol/mol), and treatment at baseline (medication/insulin-treated vs. lifestyle intervention only). Five sensitivity analyses were performed for each outcome by: 1) adjusting for time-weighted average HbA1c; 2) using the b-HVS instead of HVS; 3) using the HVS based on the HbA1c measurement solely focusing on the first three years after diagnosis of diabetes, prior to the occurrence of any event; 4) using the individual-level SDs of the HbA1c instead of the HVS; 5) using individual-level CVs of HbA1c instead of the HVS. Analyses were undertaken in the SAS® 9.4 (SAS Institute Inc., Cary, NC, USA). and the RStudio for Windows (R version 3.2.5).

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Results

Baseline characteristics

As shown in **Fig. 1**, among the 79,569 patients with type 2 diabetes identified in the population, we included 21,352 patients for further analysis., The average age was 63.3 ± 11.1 years when recruited and 54.6% of them were male. The median follow-up duration was 6.8 (IQR: 4.6~11.2) years. The mean HbA1c at baseline was 7.7% ± 2.0% (60.7 ± 21.4 mmol/mol), and the median number of HbA1c measurements throughout the study period was 12 (IQR: 8~19) times during the follow-up duration. **Tab. S1** shows the baseline patient characteristics for those included for each analysis of outcomes and **Tab. 1** shows how the baseline characteristics differ across the HVS categories. 62% of the patients have an HVS below or equal to 40%; 12.5% have an HVS greater than 60%. As expected, an increasing HVS is associated with younger age of diagnosis, higher BMI, and more intensive diabetes treatment including greater insulin use.

HbA1c variability and outcomes

As shown in **Fig. 2**, between 13,111 to 19,883 patients were involved in the analyses of each outcome. Comparing with the reference (lowest HVS category, ≥0 to ≤20), patients with HVS over 60 were associated with increased risks of all outcomes in a fully adjusted Cox model. For example, those with HVS >80 to ≤100 had an increased risk of (HR [95%CI]): MACE: 2.38 [1.61~3.53]; all-cause mortality: 2.4 [1.72~3.33]; ASCV death: 2.4 [1.13~5.11]; coronary artery disease: 2.63 [1.81~3.84]; ischemic stroke: 2.04 [1.12~3.73]; heart failure: 3.23 [1.76~5.93]; DR: 7.4 [3.84~14.27]; DPN: 3.07 [2.23~4.22]; DFU: 5.24 [2.61~10.49]; CKD: 3.49 [2.47~4.95]).

Subgroup analyses and sensitivity analyses

Given the association between HVS and HbA1c we first undertook a sensitivity analysis, including time-weighted average HbA1c from diagnosis to event in the models (**Fig. 3**). The results were similar for most outcomes other than retinopathy where the

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association of HVS was diminished when adjusting for the time-weighted average HbA1c.

When comparing the subgroups with time-weighted average HBA1c more than or less than 7% (53mmol/mol) there was a stronger association between the HVS and coronary artery disease, ischemic stroke and progression to CKD in patients with time-weighted average HbA1c<7% or 53mmol/mol (**Fig. S5**). Other subgroup analyses were undertaken based on age (**Fig. S2**), sex (**Fig. S3**), obesity at baseline (**Fig. S4**) and treatment at baseline (**Fig. S6**) did not show significant differences in the trend of the association (except the cases with very small sample size). Using b-HVS instead of HVS also showed consistent results in all outcomes (**Fig. S7**). However, the sensitivity analysis using the first-three year HVS suggested a weaker association compared with the main analysis (**Fig. S8)**. The sensitivity analysis using the individual-level SD (**Fig. S9**) and CV (**Fig. S10**) of HbA1c showed a similar pattern of risk for most outcomes but not ischemic stroke for SD and CV and diabetic retinopathy for CV where weaker associations were observed.

Discussion

To our knowledge, this is the first population-based study to investigate the association between the visit-to-visit HbA1c variability and comprehensive endpoints including cardiovascular events and the microvascular complications of diabetes in patients with newly diagnosed type 2 diabetes independent of the time-weighted average HbA1c.

Our study showed clear elevated risks of adverse events in the ~12.5% of patients with a HVS higher than 60 (meaning those with 60% of their HbA1c measurements increased or decreased by > 0.5% (5.5mmol/mol) compared with the last measurement) after diagnosis of diabetes adjusted for their time-weighted average HbA1c. The results were consistent with previous studies based on trial (15,16) and observational datasets (6-12,17). Our results indicate that frequent fluctuations of HbA1c of patients with

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diabetes may be an independent risk factor of poor prognosis and more stable HbA1c control may benefit the patients in clinical practice, although it should be emphasized that our results are observational and causal inference cannot be made. Of note, a recent analysis based on the VADT trial (16) suggested that higher HbA1c variability was associated with the increased risk of cardiovascular events in the group of intensive glycemic control but not the standard control. It suggested that the increased HbA1c variability may neutralize the cardiovascular benefits of the sustained 1.5% (16.4 mmol/mol) HbA1c reduction during the study period (18). We undertook a subgroup analysis looking at HVS in those with good and poor average HbA1c. It was interesting to note that the HVS association with atherosclerotic cardiovascular events was greater in those with good HbA1c, in keeping with the VADT finding. However, we need to interpret these results with caution as we can not account for treatment intensity during the study period.

We have previously reported that patients with high variability in HbA1c have high cardiovascular risk at baseline (19), and thus the association of HbA1c variability with risk may not be a feature of the HbA1c variability per se, but a marker of this baseline difference in patient characteristics. In this current study we have adjusted comprehensively for baseline differences in cardiovascular risk although we acknowledge there could be residual confounding. It is interesting to note that in the sensitivity analysis where we restrict our analysis to defining HbA1c variability only on the first three years of HbA1c measures, the association with micro- and macrovascular outcomes are diminished. This suggests that the HbA1c variability may continuously contribute to the clinical adverse endpoints beyond the first three years, and therefore that the risk can be less attributable to baseline differences in patient characteristics and more attributable to the HbA1c variability per se. As a recent study suggested that HbA1c variability is associated with the quality of patient care (20), it also suggests that it is never too late to reduce the HbA1c variability in clinical practice.

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Although infeasible in the current analysis, it would also be interesting to evaluate HbA1c variability on different anti-diabetic treatments to see if reduced variability can explain some of the improved outcomes with some of these agents.

Although we cannot attribute poor prognosis to the HbA1c variability per se, some underlying mechanisms may explain the association observed in our study. Although oxidative stress is suggested to be the explanation between short-term glycemic variability and adverse outcomes (5), it is not clear whether this is increased in patients with high visit-to-visit HbA1c variability. An alternative may relate to accumulated epigenetic modification induced by both high and low glycemia (21). Another explanation may simply relate to increased hypoglycemia in these individuals, since some studies suggest high HbA1c variability is linked to increased risk of severe hypoglycemic episodes (22) and patients admitted to hospital due to hypoglycemia have higher HbA1c variability (23). It will be valuable if a further study could address the frequency of overall and severe hypoglycemia among patients with different HbA1c variability.

The strengths of our study are clear. Firstly, all the included patients were tracked with their HbA1c measurement from the diagnosis of diabetes, so there is no period of the patients' diabetes journey that is not captured. Secondly, we comprehensively studied ten clinically important outcomes, including all-cause mortality, cardiovascular events and major microvascular complications of diabetes and showed consistent results across these micro- and macrovascular endpoints. Thirdly, our results were confirmed by a series of subgroup analyses and sensitivity analyses including adjusting for the time-weighted average HbA1c from the diagnosis of diabetes. Fourthly, our study was based on the real-world data of diabetes care in Scotland making these results directly translatable to clinical practice. Finally, we have used the HVS rather than SD or CV which we feel is much more clinically tractable. Although SD and CV reflect the dispersion trend of the HbA1c measures in an individual, they are no more than

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clinically meaningless statistical parameters. When considering the HVS, the clinicians can review the HbA1c profile for an individual – those where more than 60% of measures vary by more than 0.5% are at high risk.

The study does have limitations. Firstly, as a retrospective cohort study, uncorrected confounding could be possible and individuals with higher HbA1c variability may also at higher cardiovascular risks of other causes (18), and we cannot conclude an association of variability per se with the outcomes. Nevertheless, we used Cox proportional-hazards models to minimize the possible known confounding factors including CCI, smoking status and social deprivation and used a series of subgroup analyses and sensitivity analyses to confirm our findings to be robust. Secondly, we did not adjust for or evaluate the contribution of hypoglycemia, which has been reported to be associated with HbA1c variability (15) in the association between the HbA1c variability and outcomes because of the limitation of the data. Thirdly, the median follow-up duration of the study was 6.8 years and this will limit the total incident outcomes. The need to only include patients with newly diagnosed diabetes and other inclusion criteria do limit the total follow up time in this study population. This relatively short median duration does reduce the number of long-term outcome events especially for retinopathy and diabetic foot ulcer. Studies with longer follow-up duration in larger populations would be of value.

Conclusion

In conclusion, our study shows that higher HbA1c variability from the diagnosis of diabetes is independently associated with increased risks of all-cause mortality, major cardiovascular and microvascular complications of diabetes.

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Acknowledgment

SL, KZ and ERP conceived the study. SL, IN, LD and SH performed the statistical analyses. SL and ERP drafted the manuscript. ERP takes responsibility for the contents of the article as the guarantor.

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Conflict of interests: None to declare.

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Table and table legend

Table 1. Baseline characteristics of the overall study population

HVS Scores	≥0 to ≤20	>20 to ≤40	>40 to ≤60	>60 to ≤80	>80
n	7,084	6,096	5,502	2,409	261
Age of diabetes diagnosis, yrs	67.1 ± 10.3	63.5 ± 10.5	60.5 ± 10.9	58.9 ± 11.2	57.5 ± 11.2
Sex (male), n (%)	3,569 (50.4)	3,305 (54.2)	3,179 (57.8)	1,446 (60.0)	165 (63.2)
SIMD quintile, n (%)					
Q1	1,251 (17.7)	1,165 (19.1)	1,171 (21.3)	503 (20.9)	62 (23.8)
Q2	1,263 (17.8)	1,134 (18.6)	1,121 (20.4)	471 (19.6)	51 (19.5)
Q3	1,328 (18.7)	1,175 (19.3)	1,016 (18.5)	493 (20.5)	52 (19.9)
Q4	1,936 (27.3)	1,629 (26.7)	1,409 (25.6)	634 (26.3)	60 (23.0)
Q5	1,306 (18.4)	993 (16.3)	785 (14.3)	308 (12.8)	36 (13.8)
Year of diabetes diagnosis*	2010 [2005, 2012]	2008 [2002, 2011]	2008 [2002, 2011]	2009 [2003, 2011]	2010 [2006, 2013]
BMI, kg/m^2	31.3 ± 6.0	31.9 ± 6.2	32.8 ± 6.5	33.3 ± 7.1	33.2 ± 7.3
Ever smoking, n (%)	4,881 (68.9)	4,336 (71.1)	3,977 (72.3)	1,748 (72.6)	178 (68.2)
Ever regular alcohol, n (%)	4,008 (61.2)	3,345 (59.1)	2,875 (57.3)	1,185 (54.5)	131 (56.5)
Systolic blood pressure, mmHg	140.1 ± 19.0	141.2 ± 19.5	140.3 ± 19.8	139.6 ± 19.6	138.2 ± 19.4
Diastolic blood pressure, mmHg	78.9 ± 10.8	81.0 ± 10.9	82.2 ± 11.2	82.2 ± 11.4	82.2 ± 12.0
Carlson Comorbidity Index ≥1, n (%)	1,332 (18.8)	1,073 (17.6)	867 (15.8)	449 (18.6)	58 (22.2)
Hypertension, n (%)	5,505 (77.7)	4,376 (71.8)	3,786 (68.8)	1,574 (65.3)	155 (59.4)
Treatment of diabetes within the first year from the diagnosis of diabetes, n (%)					
Lifestyle intervention only	5,260 (74.3)	3,137 (51.5)	2,190 (39.8)	740 (30.7)	61 (23.4)
Anti-diabetic agents without insulin	1,770 (25.0)	2,821 (46.3)	3,153 (57.3)	1,569 (65.1)	188 (72.0)

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Treated with insulin	54 (0.8)	138 (2.3)	159 (2.9)	100 (4.2)	12 (4.6)
Receiving anti-platelet therapy, n (%)	2,465 (34.8)	1,909 (31.3)	1,598 (29.0)	667 (27.7)	67 (25.7)
Receiving statins, n (%)	4,866 (68.7)	3,716 (61.0)	3,218 (58.5)	1,373 (57.0)	161 (61.7)
HbA1c at baseline, %	6.7 ± 1.2	7.8 ± 1.9	8.4 ± 2.1	8.9 ± 2.3	9.6 ± 2.5
HbA1c at baseline, mmol/mol	49 ± 13.0	62 ± 20.3	68 ± 23.1	77.4 ± 24.6	81 ± 26.8
HDL cholesterol, mmol/L	1.3 ± 0.4	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
Non-HDL cholesterol, mmol/L	3.5 ± 1.2	3.8 ± 1.2	3.9 ± 1.3	4.0 ± 1.3	4.0 ± 1.1
ALT, IU/L*	24 [18, 34]	28 [20, 39]	30 [21, 45]	32 [22, 48]	32 [22, 48]
eGFR, mL/min/1.73m²	72.2 ± 18.7	73.7 ± 18.8	77.2 ± 19.1	80.7 ± 19.7	84.1 ± 20.8

* Presented as median [the interquartile range]

Abbreviations: ALT: alanine aminotransferase; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; SIMD: Scottish Index of Multiple Deprivation

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Figures and figure legends

Figure 1. The flow diagram of the patient selection

Abbreviations: ASCV: atherosclerotic cardiovascular; CKD: chronic kidney diseases; CV: cardiovascular; DFU: diabetic foot ulcer; DPN: diabetic peripheral neuropathy; DR: diabetic retinopathy; MACE: major adverse cardiovascular events.

Figure 2. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes

Abbreviations: CI: confidence interval; HR: hazard ratio.

Figure 3. The association between HbA1c variability score and adverse outcomes in patients with newly diagnosed type 2 diabetes after adjusting for the time-weighted average HbA1c

Abbreviations: CI: confidence interval; HR: hazard ratio; HVS: HbA1c variability score.

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