Association of Glycemic Variability in Type 1 Diabetes With Progression of Microvascular Outcomes in the Diabetes Control and Complications Trial

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Diabetes Care 2017;40:777–783 | https://doi.org/10.2337/dc16-2426

OBJECTIVE

The Diabetes Control and Complications Trial (DCCT) demonstrated the beneficial effects of intensive versus conventional therapy on the development and progression of microvascular complications of type 1 diabetes. These beneficial effects were almost completely explained by the difference between groups in the levels of HbA_{1c}, which in turn were associated with the risk of these complications. We assessed the association of glucose variability within and between quarterly 7-point glucose profiles with the development and progression of retinopathy, nephropathy, and cardiovascular autonomic neuropathy during the DCCT.

RESEARCH DESIGN AND METHODS

Measures of variability included the within-day and updated mean (over time) of the SD, mean amplitude of glycemic excursions (MAGE), and M-value, and the longitudinal within-day, between-day, and total variances. Imputation methods filled in the 16.3% of expected glucose values that were missing.

RESULTS

Cox proportional hazards models assessed the association of each measure of glycemic variation, as a time-dependent covariate, with the risk of retinopathy and nephropathy, and a longitudinal logistic regression model did likewise for cardiovascular autonomic neuropathy. Adjusted for mean blood glucose, no measure of within-day variability was associated with any outcome. Only the longitudinal mean M-value (over time) was significantly associated with microalbuminuria when adjusted for the longitudinal mean blood glucose and corrected for multiple tests using the Holm procedure.

CONCLUSIONS

Overall, within-day glycemic variability, as determined from quarterly glucose profiles, does not play an apparent role in the development of microvascular complications beyond the influence of the mean glucose.

The Diabetes Control and Complications Trial (DCCT) (1) demonstrated that 6.5 years of intensive therapy markedly reduced the risks of the onset and progression of the microvascular complications of type 1 diabetes (retinopathy and nephropathy) and neuropathy. Subsequent analyses showed that the lifetime exposure to

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Received 14 November 2016 and accepted 3 March 2017.

Clinical trial reg. nos. NCT00360893 and NCT00360815, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc16-2426/-/DC1.

This article is featured in a podcast available at http://www.diabetesjournals.org/content/diabetes-core-update-podcasts.

**A complete list of investigators and members of the DCCT/EDIC Research Group appears in N Engl J Med 2015;372:1722–1733.*

hyperglycemia, represented by the mean HbA_{1c}, was the dominant determinant of the risk of complications (2), with no threshold or breakpoint above the normal range (3). Further, virtually all of the beneficial effects of intensive therapy were explained by the differences in mean levels of HbA_{1c} during the trial (4).

Brownlee and Hirsch (5) previously postulated that glycemic variables other than HbA_{1c} may contribute to diabetes complications. They specifically commented on a mechanistic article by Monnier et al. (6) that presented a case-control study in which markers of oxidative stress (free radical production) were compared with the mean amplitude of glucose excursions (MAGE) of Service et al. (7) and to the mean glucose level obtained from continuous blood glucose monitoring. Monnier et al. (6) showed that the MAGE, but not the mean level of glucose, was associated with free radical production. Brownlee and Hirsch conjectured that an additional feature of hyperglycemia not represented by the HbA_{1c} determines the risk of complications, specifically the degree of variability in blood glucose.

Kilpatrick et al. (8), with accompanying commentary (9), used the publically available DCCT data to show that the within-day variability in blood glucose, expressed as the SD of the 7-point blood glucose profile, was not significantly associated with the progression of retinopathy or nephropathy when added to the effect of the mean glucose level. However, the incompleteness of the glucose profiles calls into question the robustness of the observations and conclusions by Kilpatrick et al.

Service and O'Brien (10) avoided this pitfall by restricting their analysis to the 7-point glucose profiles of 565 of 1,441 DCCT participants, who were monitored for more than 4 years and had more than 80% of their glucose profiles complete. They observed no association among various measures of within-day glucose variability (SD, MAGE, or M-value) during the DCCT with the onset and progression of retinopathy. However, the restricted sample size potentially affects the power of these analyses.

Kilpatrick and colleagues also reported that within-day variability in blood glucose represented by the SD and MAGE was not associated with progression of retinopathy or nephropathy (11), neuropathy (12), or cardiovascular events during DCCT (13) when adjusted for the mean glucose level; however, longitudinal variability in HbA_{1c} was associated with progression of microvascular complications during the DCCT (14). They also showed that within-day variability in blood glucose was an independent risk factor for hypoglycemia (15).

Overall compliance in the DCCT was high; however, completion of the 7-point profile collections was problematic, with many missing data points. DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) researchers (16) recently addressed this limitation by applying a modern computer-intensive statistical method, multiple imputation, to estimate missing blood glucose profile values based on other measurements that were observed, greatly increasing the statistical validity of the analyses. Using these methods, we recently evaluated the association of the within-day mean blood glucose and its components with glycated albumin and HbA_{1c}, and the association of each with hypoglycemia and microvascular outcomes in DCCT (16). However, measures of long-term glycemic variability were not explored. We now present analyses of the effects over time of the mean level of glycemia, and of measurements of variability of blood glucose, using multiple imputation methods to complete the glucose profiles, on the risk of development and progression of retinopathy, nephropathy, and cardiovascular autonomic neuropathy (CAN).

RESEARCH DESIGN AND METHODS

Patients

The DCCT enrolled 1,441 subjects with type 1 diabetes, with 711 randomly assigned to intensive therapy aimed at achieving levels of glycemia (HbA_{1c}) as close to the nondiabetic level as safely possible and 730 assigned to conventional therapy aimed at maintaining clinical well-being with no specific glucose targets (1). Two subcohorts were enrolled: a primary prevention cohort of 726 patients with 1–5 years' duration of diabetes, no retinopathy, and an albumin excretion rate (AER) $<$40 mg/24 h on study entry; and a secondary intervention cohort of 715 patients with 1–15 years of diabetes, minimal to moderate retinopathy, and AER $<$200 mg/24 h.

Measurements

These analyses are based on the measures of glycemia and the development and progression of complications during the DCCT (1983–1993).

Fundus photographs were obtained every 6 months during the DCCT, from which the severity of retinopathy for each subject was centrally graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) scale (17). Retinopathy progression was defined as a sustained progression of three or more steps from the level at baseline observed on two successive 6-month visits. The AER was measured annually from a timed urine collection during the DCCT. Onset of microalbuminuria was defined as an AER $\geq$30 mg/24 h on two successive visits among the 1,284 (1,441−157) with an AER $<$30 mg/24 h at baseline (1).

The presence of CAN was evaluated at DCCT baseline and every 2 years during DCCT using cardiovascular autonomic reflex tests that assessed the R-R response to paced breathing (R-R variation), the Valsalva maneuver, and postural changes in blood pressure (18). CAN was defined as an R-R variation $<$15, or an R-R variation 15–19.9 combined with a Valsalva ratio $\leq$1.5, or a decrease of $>$10 mmHg in diastolic blood pressure after standing for 10 min. The 66 subjects with CAN at baseline were excluded from these analyses.

HbA_{1c} was measured in all patients quarterly, from which a longitudinal mean HbA_{1c} value was computed at each 6-month visit. Quarterly, a 7-point capillary blood glucose profile (Profilsets; Boehringer-Mannheim Diagnostics, Indianapolis, IN) was collected by the participants at home with samples collected before and 90 min after each meal and at bedtime. The samples were collected in capillary tubes, placed in diluent, and shipped to the DCCT Central Biochemistry Laboratory at the University of Minnesota, where the hemolysates were analyzed for glucose as previously described (1).

In addition to mean blood glucose, three well established metrics of glucose variability that differed in characterization of glycemic behavior were computed from each quarterly profile: *1*) SD, an expression of variability in glucose exposure; *2*) MAGE (7), a measure of glucose excursions reflecting glycemic

variability independent of glucose exposure; and *3*) M-value of Schlichtkrull et al. (19), as modified (10), a hybrid of glucose exposure and glycemic variability. The MAGE was originally developed for use with continuous in vivo blood glucose analysis using an autoanalyser that preceded continuous glucose monitoring (CGM). Its value is provided by an algorithm, not a single equation. The MAGE values reported here were computed using a program provided by Service and O'Brien (10) for application to 7-point blood glucose profiles as used in their prior report. Our computations of the M-value used a reference blood glucose value of 90 mg/dL, which reflects mean nondiabetic glycemia using modern methodology.

In addition to the values at each visit, the longitudinal mean of each measure was computed from all prior visit profile values up to each 6-month visit (e.g., the mean of the within-day MAGE up to 6 months, then up to 12 months, etc.). The longitudinal variances of the glucose values within and between profiles and the longitudinal total variance of all glucose values were also computed up to each 6-month visit. These between-day or longitudinal measures of variability were also included because high within-day variability might contribute to longitudinal variability and thus provide greater power to detect an association. These 6-month values were used as predictors of progression of retinopathy, the annual values were used as predictors of progression of nephropathy, and the biennial values were used as predictors of CAN.

The Supplementary Data includes equations that define each measure, except for the MAGE, for which there is no single equation. The pooled within-day variance was used in lieu of the mean of the within-day SD, the variance being a quadratic function of the SD.

Statistical Methods

During the DCCT, 37,058 quarterly visits were conducted, from which 259,406 profile values were expected; however, 42,209 (16.3%) were not collected. At each of the seven time points, between 15 and 18% of glucose values were missing. Only 67% (24,866) of the quarterly 7-point profiles were complete, 15.3% were missing a single time point, 5.4% were missing between 2 and 6 time points, and 12.2% were missing the complete profile (Supplementary Fig. 1). However, the HbA_{1c} data were virtually complete.

To address the missing glucose data, the statistical technique of multiple imputation (20,21) was used to provide 10 estimates of each missing value, yielding 10 complete data sets. Multiple imputations were generated using chained equations (MICE [22]) and the method of Schafer and Yucel (23). The results of the two methods were consistent, and those using MICE are presented here. A given analysis was then conducted using each of the 10 complete data sets, and the results were averaged using the methods of Rubin and Schenker (24). For the analysis of each outcome, the resulting CI and *P* value accounted for the overall extent of the original missing data. A more thorough description of these methods is provided in the Supplementary Data, which also includes a validation of the multiple imputation approach for dealing with missing values in the DCCT data.

Baseline characteristics are described using mean (SD) or frequency (%). The discrete Cox proportional hazards regression model (25), adjusted for tied event times, assessed the effects of measures of variation on relative risk of a three-step progression of retinopathy and the onset of microalbuminuria. A logistic regression model for repeated measures using generalized estimating equations (26) assessed the effects of covariates on the odds of CAN over 2, 4, 6, and 8 years of follow-up. Updated values of each measure of variation were used as a time-dependent covariate, meaning that at a given point in time, the most recent measure of the covariate was used as the value of the covariate for each subject at risk at that time. The 157 subjects with prevalent microalbuminuria at baseline were excluded from analyses of nephropathy, as were the 66 subjects with prevalent CAN at baseline who were excluded from the analyses of CAN.

Analyses were performed using SAS software (SAS Institute, Inc., Cary, NC) and R software. Along with *P* values, the test statistic *Z* value is presented to represent the strength of the association of the covariate with the outcome, with absolute values of $Z \geq 1.96$ representing a nominally significant *P* value ≤ 0.05. The Holm procedure was used to provide corrected levels of significance to account for the multiple tests conducted in the analyses of all outcomes (27).

The multiply imputed data sets used as the basis for these analyses can be obtained from the NIDDK Data Repository at https://www.niddkrepository.org/home/.

RESULTS

Baseline characteristics of the DCCT cohort have been presented previously (1) and are summarized in Supplementary Table 1. The DCCT cohort was evenly divided, by design, into the primary prevention and secondary intervention cohorts, based on the absence or presence of complications at baseline, respectively, with average diabetes duration of 2.6 and 9 years, respectively. The mean age was 27 years, slightly more than half were men, and the mean HbA_{1c} was 8.9%, with no difference between treatment groups.

A summary of the distributions of the measures of glycemia, glycemic variability, and long-term variation at baseline, at 1 year, and over follow-up are reported in Table 1. The correlations among the measures over all follow-up visits are presented in Supplementary Table 2. No two measures of variation were colinear or redundant, with all such correlations $\leq$0.94. Correlations of measures of variability with the mean blood glucose ranged typically as high as 0.73, except for the within-day and the longitudinal mean M-value, with correlations of 0.94 and 0.88 with the mean within-day and longitudinal glucose values, respectively.

Multiple measures of glucose variability were significantly associated with each of the three outcomes in analyses that did not adjust for the mean blood glucose or multiple tests of significance (Supplementary Table 3). In these analyses, retinopathy progression was nominally significantly associated with the within-day SD, M-value, and with all of the longitudinal mean measures of variability; the onset of microalbuminuria with some of the within-day variance measures (SD and M-value), and all of the longitudinal measures of variability; and CAN with the MAGE, M-value, and all of the measures of

Table 1—Measures of glycemia and glycemic variability in the DCCT cohort at baseline, at 1 year of follow-up, and over all visits (*N* = 1,441); incident cases of retinopathy and microalbuminuria and prevalent cases of CAN over all visits

	Baseline	Year 1	All visits
Measure of average glucose, median (quartiles 1, 3), rSD*			
Within-day mean blood glucose (mg/dL)	223.1 (172.2, 282.1) 81.5	177.1 (134.8, 231.5) 71.7	182.7 (139.7, 241.7) 75.6
Longitudinal mean of the profile mean glucose (mg/dL)	223.1 (172.2, 282.1) 81.5	183.4 (148.4, 232.3) 62.2	182.0 (149.1, 229.6) 59.7
Longitudinal mean HbA_{1c} (%)	8.8 (7.9, 10.1) 1.6	7.6 (6.9, 8.9) 1.5	7.9 (7.0, 9.1) 1.6
Longitudinal mean HbA_{1c} (mmol/mol)	72.7 (62.8, 86.9) 17.9	59.6 (51.9, 73.8) 16.2	62.8 (53.0, 76.0) 17.0
Measures of glucose variability, median (quartiles 1, 3), rSD*			
Within-day standard deviation (mg/dL)	77.2 (60.2, 98.7) 28.5	72.0 (52.6, 92.2) 29.4	74.6 (55.2, 97.7) 31.5
Within-day MAGE (mg/dL)	163.8 (117.0, 214.5) 72.3	143.0 (103.0, 201.5) 73.0	154.5 (109.0, 213.5) 77.5
Within-day M-value	96.9 (56.1, 154.5) 72.9	53.2 (27.1, 103.3) 56.5	61.68 (31.82, 114.3) 61.2
Longitudinal mean MAGE (mg/dL)	163.8 (117.0, 214.5) 72.3	150.4 (122.3, 183.3) 45.2	158.6 (133.4, 187.2) 39.9
Longitudinal mean M-value	96.9 (56.1,154.5) 72.9	58.3 (36.0, 97.9) 45.9	71.14 (45.64, 112.1) 49.3
Total variance† within and between days $(mg/dL)^2$	6,165.7 (3,770.6, 9,893.2) 4,538.7	6,093.9 (4,239.0, 8,709.4) 3,314.0	7,129.1 (4,902.4, 10,000.4) 3,779.2
Between-day variance† $(mg/dL)^2$	—	1,465.1 (700.5, 3,013.9) 1,714.9	1,980.6 (1,040.7, 3,605.6) 1,901.3
Pooled within-day variance† $(mg/dL)^2$	6,165.7 (3,770.6, 9,893.2) 4,538.7	5,694.5 (3,960.8, 7,983.8) 2,982.2	6,213.1 (4,491.4, 8,438.9) 2,926.3
Incident and prevalent outcomes‡			
Retinopathy (*N* = 1,441), incidence, *n* (%)	—	—	271 (18.8)
Microalbuminuria (*N* = 1,284), incidence, *n* (%)	—	—	118 (9.2)
CAN (*N* = 1,375), prevalence at years 2, 4, 6, and 8 (*n*)	—	—	49, 79, 60, 24

*All analyses of glucose-based values are based on multiply imputed data sets. Values for the quartiles of each measure are calculated using all imputations. The SDs are calculated using Rubin's variance formula (20) averaged over imputations (except for the longitudinal variances). †For the longitudinal variances, the rSD is a robust estimator of the SD obtained as 0.7413 × (quartile 3 − quartile 1) from a randomly selected imputation. ‡Incident retinopathy: ≥3-step change on the ETDRS scale at a 6-month visit in the full cohort (*N* = 1,441); incident microalbuminuria: AER ≥30 mg/24 h on two successive annual visits among the 1,284 subjects with AER <30 mg/24 h at baseline; prevalent CAN as described in RESEARCH DESIGN AND METHODS among the 1,375 subjects without CAN at baseline.

longitudinal variation. Many of these remained statistically significant when corrected for the total of 24 statistical tests by the Holm procedure (Supplementary Table 3).

After adjusting for the within-day mean blood glucose, no measure of within-day variability remained nominally significantly associated with any of the three outcomes (Table 2). However, after adjusting for the longitudinal mean blood glucose, the mean M-value remained nominally significantly associated with microalbuminuria, and the total and between-day variance with CAN (Table 2).

Applying the Holm correction for 8 tests for a given complication would require that the largest $|Z|$ value be ≥2.734 for significance, two-sided, at the 0.05 level; and that the second largest be ≥2.69. Only the effect of the longitudinal total variance on CAN (Z = 3.03) and that of the longitudinal M-value on microalbuminuria (Z = 4.23, $P < 0.0001$) meet this criterion. The association of the between-day variance with CAN does not meet the Holm criterion (Z = 2.63 to <2.69). Further, applying the Holm procedure for the complete set of 24 tests conducted in aggregate for the three outcomes would require a $|Z|$ value ≥3.08 for significance. Only the association of the longitudinal M-value with microalbuminuria (Z = 4.23) meets this criterion.

Analyses were also conducted separately within the DCCT intensive and conventional groups that showed a like paucity of significant associations in both groups when adjusted for the corresponding measure of mean blood glucose (within-profile or longitudinally).

The Supplementary Data presents additional analyses using the coefficient of variation (CV = SD/mean) that curiously showed an inverse association with retinopathy and microalbuminuria. The Supplementary Data also presents statistical derivations which show that an increasing coefficient of variation is a marker of decreasing blood glucose, not increasing glycemic variability.

CONCLUSIONS

The current analyses differ from previous analyses examining the potential effects of glycemic variability, independent from mean glycemia, on complications during the DCCT (8,10–12) by including additional assessments of glycemic

Table 2—Association of measures of glucose variability over a mean of 6.5 years of quarterly follow-up in the DCCT with progression of complications

		Adjusted for mean blood glucose*		
	Hazard ratio	95% CL	*Z* value	*P* value†
Retinopathy				
Within-day				
SD	0.937	0.834, 1.054	−1.08	0.28
MAGE	0.938	0.837, 1.050	−1.11	0.27
M-value	0.804	0.582, 1.112	−1.32	0.19
Longitudinal				
Total blood glucose variance	0.951	0.844, 1.072	−0.83	0.41
Between-day variance	0.920	0.839, 1.009	−1.76	0.08
Within-day variance	0.970	0.872, 1.080	−0.55	0.59
Mean MAGE	0.966	0.853, 1.095	−0.54	0.60
Mean M-value	0.972	0.792, 1.191	−0.28	0.79
Microalbuminuria				
Within-day				
SD	1.021	0.842, 1.238	0.21	0.84
MAGE	1.01	0.834, 1.213	0.062	0.96
M-value	0.899	0.517, 1.564	−0.38	0.71
Longitudinal				
Total blood glucose variance	1.084	0.838, 1.401	0.61	0.54
Between-day variance	1.132	0.999, 1.283	1.95	0.06
Within-day variance	0.904	0.698, 1.172	−0.76	0.45
Mean MAGE	0.812	0.621, 1.062	−1.52	0.13
Mean M-value	2.142	1.505, 3.048	4.23	<0.0001
	Odds ratio			
Cardiovascular autonomic neuropathy				
Within-day				
SD	1.098	0.952, 1.268	1.29	0.20
MAGE	1.138	0.999, 1.298	1.93	0.06
M-value	1.336	0.953, 1.874	1.68	0.10
Longitudinal				
Total blood glucose variance	1.357	1.114, 1.655	3.03	0.0025
Between-day variance	1.221	1.052, 1.416	2.63	0.0087
Within-day variance	1.132	0.946, 1.355	1.35	0.18
Mean MAGE	1.155	0.925, 1.444	1.27	0.21
Mean M-value	1.011	0.690, 1.483	0.06	0.96

*Models for the association of within-day measures of variation with the risk of progression of microvascular complications are also adjusted for the within-day mean blood glucose; models for longitudinal measures of variation are adjusted for the longitudinal mean level of blood glucose. The hazard ratio is from a Cox proportional hazards model of the incidence of retinopathy and nephropathy progression over time, and the odds ratio is from a general estimating equation logistic regression model of prevalence of CAN at 2, 4, 6, and 8 years of follow-up. The association of all measures with progression of complications without adjustment for the mean blood glucose is shown in the Supplementary Data. CL, confidence limits.
†After applying the Holm procedure to correct for the total of 24 tests, only the effect of the mean M-value on risk of microalbuminuria ($Z = 4.23$) meets the criteria for significance at the 0.05 level.

variability and more complete glucose profiles through multiple imputation methods. Although the prior analyses of these data (8,10–12) also showed no association of within-day measures of variability with long-term complications, they suffered from the large fraction of incomplete quarterly profiles. The prior reports used simple analyses in which there was no attempt to correct for the large fraction of missing glucose values other than to eliminate subjects from the analysis (10) or to compute profile summary measures from incomplete profiles (8,11,12), which could have contributed to reduced power to detect associations. For example, prior reports simply computed the SD and other measures from the available values. This strategy introduces errors into the estimates for each profile that in turn reduce power. For example, if a preponderance of preprandial values are missing, the within-day SD is overestimated, whereas if more postprandial values are missing, the SD is underestimated.

Further, there was a modest correlation, ranging up to 0.73, between measures of variability and the mean glucose level, except for the M-value, which had a strong correlation of 0.94 that reflects the glycemic exposure component of this metric, and the mean M-value with a correlation of 0.88 with the updated mean blood glucose. Because the within-day variance (or SD) is correlated with the mean, the analysis adjusted for the mean provides the more relevant assessment of the role of glycemic variability. Thus, it was prespecified that analyses of the effects of glycemic variability on outcomes would adjust for the relevant measure of mean glycemia, either the within-day mean blood glucose for within-day measures of glycemic variation or the updated mean blood glucose over time for longitudinal measures of between-day variation. However, unadjusted and adjusted analyses for the mean blood glucose are both presented.

In this report using multiply imputed complete data sets in simple analyses not adjusted for the mean level of blood glucose, various measures of within-day and longitudinal (average or between-day) variability were associated with each microvascular complication during the DCCT (Supplementary Table 3). However, after adjusting for the mean glucose, few associations remained nominally significant. The longitudinal mean M-value was strongly associated with microalbuminuria ($Z = 4.23$) when adjusted for the longitudinal mean glucose ($P < 0.0001$), in part a reflection of its high correlation with the longitudinal mean blood glucose ($r = 0.88$). The total variance and the between-day variance were both associated with CAN, with Z values of 3.03 ($P = 0.0025$) and 2.63 ($P = 0.0087$), respectively, when adjusted for the longitudinal mean glucose. These associations with microalbuminuria and CAN remained nominally significant when also adjusted for the mean HbA_{1c}. However, when also corrected for 24 total tests using the Holm procedure (28), only the adjusted longitudinal mean M-value association with microalbuminuria remained statistically significant. Separate analyses within the DCCT intensive and conventional group produced similarly negative results, as above, within each group.

The major weakness of these data is that the 7-point glucose profiles may be

insufficient to characterize glucose variability correctly when compared, for example, with CGM. The M-value and MAGE, in particular, were originally developed for use with continuous data. One study has reported that measurements of glucose variability from the 7-point glucose profile differed from those derived from CGM (29). Further, it has been reported that even one hyperglycemic spike may cause continued overproduction of reactive oxygen species for days in the setting of subsequent normal glucose homeostasis by activating a multicomponent feedback loop (30). Thus, variability in the 7-point glucose profiles may not capture the full degree of variability that would have been observed had CGM been used. Of course, this assumes that CGM is the gold standard to capture variability, and that may not be the case.

Conversely, the large DCCT data sets available with the longitudinal assessment of diabetes complications, and the careful implementation of modern statistical analyses to address missing glucose data, may offset this weakness. We used multiple imputed complete data sets that would correct the association of measures of variation with outcomes for any bias introduced by missing data and should yield more definitive or reliable results than an analysis based only on the incomplete observed data. Although proving that we have completely addressed the shortcomings of prior analyses is not possible, the previously demonstrated lack of associations of within-profile and longitudinal measures of glycemic variability with outcomes has largely been confirmed.

We previously reported that the level of HbA_{1c} over time in the DCCT explained virtually all of the difference in the risk of complications between the intensive and conventional groups, but that in the combined cohort, the HbA_{1c} as a risk factor for complications explained only ~10–15% of the variation in risk (2–4). The results reported here using blood glucose values show that neither diurnal variation nor longitudinal variation based on quarterly 7-point profiles appears to contribute substantial additional effects beyond those of the mean blood glucose.

In summary, analyses that fully account for the missing and incomplete blood glucose profiles and the inclusion of additional measures of variability fail to show that the within-day variability in blood glucose, when adjusted for the mean glucose, is associated with the development or progression of retinopathy, nephropathy, or CAN. Moreover, the longitudinal measures of variability are not associated with the risk of complications when adjusted for the mean glucose and corrected for multiple tests, with the exception of the longitudinal M-value association with microalbuminuria. Overall, the measures of glycemic variability based on the complete quarterly 7-point glucose profile data sets fail to provide strong or consistent evidence that glycemic variability contributes to the risk of development or progression of microvascular complications beyond that contributed by the mean level of glucose.

Funding. The DCCT/EDIC has been supported by cooperative agreement grants (1982–1993, 2012–2017) and contracts (1982–2012) with the Division of Diabetes, Endocrinology, and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases (current grant numbers U01-DK-094176 and U01-DK-094157) and by the National Eye Institute, the National Institute of Neurological Disorders and Stroke, the General Clinical Research Centers Program (1993–2007), and the Clinical Translational Science Center Program (2006–present), Bethesda, MD.

Industry contributors have had no role in the DCCT/EDIC study but have provided free or discounted supplies or equipment to support participants' adherence to the study: Abbott Diabetes Care (Alameda, CA), Animas (Westchester, PA), Bayer Diabetes Care (North America Headquarters, Tarrytown, NY), Becton Dickinson (Franklin Lakes, NJ), Eli Lilly (Indianapolis, IN), Extend Nutrition (St. Louis, MO), Insulet Corporation (Bedford, MA), LifeScan (Milpitas, CA), Medtronic Diabetes (Minneapolis, MN), Nipro Home Diagnostics (Ft. Lauderdale, FL), Nova Diabetes Care (Billerica, MA), Omron (Shelton, CT), Perrigo Diabetes Care (Allegan, MI), Roche Diabetes Care (Indianapolis, IN), and Sanofi (Bridgewater, NJ).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. J.M.L. wrote the initial draft of the manuscript. I.B. assisted in writing the initial draft of the manuscript and conducted all analyses. R.M.B., R.P.-B., F.J.S., B.Z., and D.M.N. contributed to the specification of the analyses and critically reviewed and edited the manuscript. J.M.L. and I.B. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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