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Glycemic Variability in Type I Diabetes and Microvascular Outcomes in the Diabetes Control and Complications Trial

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1. Measures of Glycemia and Glycemic Variability:

The following are the equations for the measures of glycemia and glycemic variability employed in these analyses.

For each subject, blood glucose (BG) levels were measured quarterly at 7 time points during a day: before/after breakfast, lunch and dinner and at bedtime. Let X_{ijk} denote the k-th blood glucose value for subject *i* at quarter *j*, where *k=1,…7*, *i=1,…1441*, *j=1,…*n_i,, where n_i is the number of quarterly visits for subject *i*.

The following equations were used for the computation of the measures of within-day and between-day (longitudinal) glycemia and glycemic variability:
Mean within-day BG profile for subject *i* at quarter *j*:

$$\bar{X}_{ij} = \sum_{k=1}^{7} \frac{X_{ijk}}{7}.$$

Mean of the profile BG means up to the current visit (*j*) for the *i*th subject.

$$\bar{\bar{X}}_{ij} = \sum_{l=1}^{j} \sum_{k=1}^{7} \frac{X_{ilk}}{7j}.$$

Variance within the *j*th profile for the *i*th subject.

$$Var_{ij} = \frac{\sum_{k=1}^{7}(X_{ilk} - \bar{X}_{ij})^2}{6}$$

Standard deviation within the *j*th profile for the *i*th subject.

$$SD_{ij} = \sqrt{Var_{ij}}.$$

Coefficient of variation of the 7-point BGP profile:

$$CV_{ij} = \frac{SD_{ij}}{\bar{X}_{ij}}.$$

MAGE: There is no single equation for the MAGE. Rather an algorithm was employed. The MAGE (7) was originally described for application to continuous blood glucose monitoring and its validity was later assessed (Service et al., *Diabetes Care* 10: 225-237, 1987 for application to the 7-point glucose profile using data collected in the KROC Collaborative Study (The KROC Collaborative Study Group *NEJM* 311:365-72, 1984). We employed the program provided by F John Service and Peter C O'Brien for the computations herein. No single simple equation was employed and readers are referred to these other references for a description of the MAGE.

M-value of the 7 point profile within the *j*th profile for the *i*th subject:

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$$M_{ij} = \left[\sum_{k=1}^{7}\left|\frac{10(log_{10}\frac{X_{ij,k}}{90})^3}{7}\right|\right] + \left[\frac{Range((X_{ijk})_{k=1,\ldots 7})}{20}\right].$$

Total (within-day and between-day) variance of all values in all profiles up to and including the *j*th profile for the *i*th subject

$$(Total\ Var)_{ij} = \sum_{l=1}^{j}\frac{\sum_{k=1}^{7}(X_{ilk} - \bar{\bar{X}}_{ij})^2}{7j-1}$$

Variance between the profile mean values up to and including the *j*th profile for the *i*th subject

$$(Between\ Var)_{ij} = \sum_{l=2}^{j}\frac{\left(\bar{X}_{il} - \bar{\bar{X}}_{ij}\right)^2}{j-1},$$

Pooled variance within each profile up to and including the *j*th profile for the *i*th subject.

$$(Within\ Var)_{ij} = \sum_{l=1}^{j}\frac{\sum_{k=1}^{7}(X_{ilk} - \bar{X}_{il})^2}{6j}.$$

Mean coefficient of variation up to and including the *j*th profile for the *i*th subject:

$$\overline{CV}_{ij} = \sum_{k=1}^{j}\frac{CV_{ik}}{j}.$$

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2. Missing Data

Based on the duration of DCCT follow-up ranging from 4 to 9 years, a total of 37058 quarterly visits were expected in the 1441 subjects enrolled in the study. During the DCCT, prior to each quarterly visit, each patient was requested to collect a "profilset" of timed capillary blood collections for measurement of capillary blood glucose before and after each meal (pre-and post-prandial) and at bedtime. Of these, 32528 or 88% were conducted. However, patient compliance with the quarterly blood glucose collections was poor and many profiles were incomplete. A total of 259406 glucose values were expected but only 217197 (84%) were obtained. Of those expected, 15.4% were missing the fasting value, 15.7% the post-breakfast, 15.4% pre-lunch, 16.4% post-lunch, 15.6% pre-dinner , 16.8% post dinner and 18.5% at bedtime. Figure S1 depicts the distribution of the number of missing BG values per visit.

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3. Multiple Imputation

The statistical technique of multiple imputation (20, 21) was employed to address the impact of the missing profilset blood glucose data. Multiple imputation uses regression models for the associations among the observed data to impute a value for a missing data point by adding a random error to the conditional expectation estimated from all the other observed data for that subject.

Herein statistical models regressed the observed HbA1c and profile-derived glucose values, simultaneously, on each other and other covariates using all available data for each subject, including values from different visits. The imputation models were employed separately by DCCT treatment group (intensive vs. conventional), and included age group (adult versus not), sex, duration of diabetes all evaluated at baseline, the quarterly mean updated HbA1c, annual natural log albumin excretion rate, hypoglycemia status (rate of coma, rate requiring assistance and prior hypoglycemia) , and binary indicator variables to designate whether or not the patient experienced retinopathy, nephropathy, cardiovascular disease, neuropathy and the time of each. Additionally, all the quarterly 7 profilset capillary blood glucose values, quarterly HbA1c and BMI and the annual total insulin, were included in the imputation models their correlation with the outcome was > 0.2. For example, in addition to the other covariates, a missing post-lunch glucose concentration at year 2 was estimated from the other profile values measured at any visit if their correlation was >0.2.

Multiple imputation was implemented using chained equations (MICE) (22, 23) to generate 10 imputed complete data sets. Because the imputation model provides a random imputed value for each missing value, the resulting imputed data sets are different. A given analysis was then conducted separately for the 10 data sets and the summary statistics were then combined into a single overall estimate that takes account of the variation between imputations as well as within (24).

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4. Analyses of the Coefficient of Variation

The coefficient of variation (*CV*) within each profile and the longitudinally-updated mean of the *CV* were also included as measures of variability in these analyses. The mathematical expressions are shown in Section 1 above. The *CV* within a profile is the SD within the profile divided by the profile mean. However, at least in these analyses, the *CV* more strongly represents the mean level of glycemia than it does glycemic variability, and for this reason the results of those analyses are presented herein, with further explanations, and not in the main manuscript.

Table S2 shows that the *CV* has a modest correlation with other factors, some of them inverse such as a correlation of -0.29 with the mean blood glucose.

Table S4 presents the results of analyses using the within profile and updated mean *CV* that largely show *inverse* associations, meaning that the hazard ratio for the risk of a complication per unit increase in the *CV* is less than 1, or that the risk of the complication *decreases* as the *CV* increases. In the unadjusted analyses the within profile *CV* is strongly significantly inversely associated with the risk of retinopathy but not the other complications, and the updated mean *CV* is nominally significantly associated with all complications, strongly with retinopathy and microalbuminuria, and weakly with CAN. In analyses adjusted for the mean blood glucose the updated mean *CV* has a nominally significant but weaker inverse association with the risk of retinopathy and microalbuminuria. Herein we show statistically that these inverse associations with the *CV* are a reflection of the association of the mean blood glucose, not the SD, with outcomes.

The hazard ratios (HRs, Table S4) are computed per standard deviation of *CV* (0.08, Table 1) so that a value of the HR < 1 indicates that the risk of the complication decreases per standard deviation increase in the mean *CV*. Herein we demonstrate that this inverse association with the *CV* occurs because an increase in the profile mean (the denominator of the *CV*) has a stronger effect on risk than does the within profile *SD*. In this case, an increase in the *CV* reflects a decrease in the mean rather than an increase in the *SD*. Thus an increase in the *CV* does not represent an increase in glycemic variation.

For the analysis of retinopathy, with no adjustment for other factors, the *HR* per *SD* change in the within profile standard deviation, say *S*, is 1.117 with p=0.047, and the *HR* per *SD* change in the within profile mean, say *M*, is 1.49 with p<0.0001 (Table S3), much stronger than the effect of the effect of the standard deviation. Then the *HR* per *SD* change in the *CV*=*S/M*, with no adjustment, is 0.768 with p<0.0001 (Table S4). Thus, the risk of retinopathy increases as *S* increases, as *M* increases (more so), but as *CV* decreases.

Let *Y* denote the outcome, in this case the log(*HR*) so that a *HR*<1 means log(*HR*)<0, or a negative association. Then assume that (*Y, M, S*) are jointly distributed with mean vector (μ_Y, μ_M, μ_S) and covariance matrix Σ with components σ_{ij}, i,j=1,2,3. Asymptotically, the covariance between *Y* and *CV*=*S/M* can be obtained using the delta method, and it is given by

$$Cov(Y, CV) = \frac{\sigma_{13}}{\mu_M} - \frac{\sigma_{12}}{\mu_M^2},$$

Where $\sigma_{12} = Cov(Y, M)$ and $\sigma_{13} = Cov(Y, S)$. Thus, $Cov(Y, CV)$ is negative if

$$\frac{Cov(Y, M)}{\mu_M} > \frac{Cov(Y, S)}{\mu_S}.$$

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In our case *Y* and *M* are strongly correlated while the correlation between *Y* and *S* is weak, and the above equation applies, which leads to the observed negative correlation between *Y* and *CV*.

An additional derivation is available from the authors that applies specifically to the Cox PH model used in these analyses.

Statistically, therefore, increasing CV is not, at least in these analyses, a measure of increasing glycemic variability.

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5. Supplementary Tables and Figures

Supplementary Table S1. Distribution of characteristics at baseline.

Covariate	**N=1441**
Cohort	
Primary Prevention, No. (%)	726 (50)
Age (yr.), mean (SD)	26.8 (7.1)
Male, No. (%)	760 (53)
Duration of T1D (yr.), mean (SD)	
Primary Cohort	2.6(1.4)
Secondary Cohort	8.7(3.7)
HbA1c (%) at Baseline, mean (SD)	8.9(1.6)

Mean (SD) or Mean ± SE or %

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Supplementary Table S2. Pearson correlation coefficients among measures for all subjects and quarterly visits combined.

	Mean BG	SD	MAGE	M-value	CV	Updated Mean Glucose	Total Variance	Variance of MBG	Within Variance	Mean MAGE	Mean M-value	Mean CV
Mean BG	1	0.476	0.434	0.943	−0.291	0.732	0.549	0.37	0.489	0.475	0.680	−0.344
SD		1	0.887	0.621	0.639	0.33	0.503	0.161	0.552	0.448	0.345	0.189
MAGE			1	0.565	0.571	0.306	0.46	0.159	0.499	0.431	0.320	0.163
M-value				1	−0.065	0.681	0.597	0.385	0.535	0.489	0.659	−0.237
CV					1	−0.225	0.089	−0.109	0.178	0.092	−0.164	0.513
Updated Mean BG						1	0.725	0.502	0.651	0.598	0.885	−0.48
Total Variance							1	0.576	0.906	0.716	0.730	0.054
Variance of MBG								1	0.348	0.378	0.543	−0.241
Within Variance									1	0.741	0.636	0.246
Mean MAGE										1	0.742	0.096
Mean M-value											1	−0.364
Mean CV												1

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Supplementary Table S3. Association of measures of glucose variability over a mean of 6.5 years of quarterly follow-up in the DCCT with progression of microvascular complications in models with no adjustments for either the within-day mean blood glucose or the longitudinal updated mean blood glucose.

	Unadjusted for Mean BGP			
	Hazard Ratio	**95% Confidence Limits**	**Z-value**	**P-Value***
Retinopathy				
Within-Day:				
Mean BG	1.490	1.348, 1.648	7.79	<0.0001
SD	1.117	1.001, 1.247	1.99	0.048
MAGE	1.102	0.990, 1.227	1.77	0.08
M-value	1.327	1.230, 1.431	7.33	<0.0001
Longitudinal:				
Mean BG	1.960	1.783, 2.155	13.94	<0.0001
Total Variance BG	1.379	1.298, 1.465	10.40	<0.0001
Between Profile Variance	1.264	1.195, 1.338	8.12	<0.0001
Within Profile Variance	1.292	1.225, 1.364	9.36	<0.0001
Mean MAGE	1.471	1.339, 1.615	8.05	<0.0001
Mean M-value	1.755	1.599, 1.925	11.88	<0.0001

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Microalbuminuria				
Within-Day:				
Mean BG	1.438	1.233, 1.677	4.63	<0.0001
SD	1.199	1.009, 1.424	2.06	0.040
MAGE	1.164	0.986, 1.374	1.78	0.08
M-value	1.309	1.165, 1.471	4.54	<0.0001
Longitudinal:				
Mean BG	1.772	1.476, 2.126	6.15	<0.0001
Total Variance BG	1.476	1.264, 1.724	4.92	<0.0001
Between Profile Variance	1.300	1.194, 1.415	6.04	<0.0001
Within Profile Variance	1.317	1.098, 1.579	2.97	0.003
Mean MAGE	1.247	1.016, 1.529	2.12	0.034
Mean M-value	1.796	1.556, 2.074	7.99	<0.0001

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	Odds Ratio	95% Confidence Limits	Z-value	P-Value*
Cardiovascular Autonomic Neuropathy				
Within-Day:				
Mean BG	1.125	0.959, 1.321	1.45	0.15
SD	1.127	0.993, 1.280	1.85	0.07
MAGE	1.160	1.028, 1.307	2.40	0.017
M-value	1.125	1.002, 1.262	2.00	0.046
Longitudinal:				
Mean BG	1.398	1.163, 1.682	3.56	0.0004
Total Variance BG	1.391	1.213, 1.595	4.73	<0.0001
Between Profile Variance	1.299	1.156, 1.460	4.40	<0.0001
Within Profile Variance	1.283	1.127, 1.462	3.76	0.0002
Mean MAGE	1.347	1.135, 1.599	3.41	0.0007
Mean M-value	1.336	1.120, 1.594	3.22	0.0013

HR: Hazard ratio from a Cox proportional hazards model; OR: Odds ratio from a GEE logistic model of the prevalence of CAN at 2, 4, 6 and 8 years of follow-up.

* After applying the Holm procedure to correct for the total of 24 tests, the 16 smallest p-values < 0.017 meet the criteria for significance at the 0.05 level.

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Supplementary Table S4. Association of the coefficient of variation (CV) over a mean of 6.5 years of quarterly follow-up in the DCCT with progression of microvascular complications in models with no adjustments for other covariates, and after adjustment for the updated mean level of blood glucose.

	Unadjusted for Mean BGP†			
Retinopathy	**Hazard Ratio**	**95% Confidence Limits**	**Z-value**	**P-Value**
Within-Day CV	0.768	0.673, 0.877	-3.90	<0.0001
Longitudinal CV	0.587	0.520, 0.662	-8.69	<0.0001
Microalbuminuria				
Within-Day CV	0.851	0.699, 1.037 9	-1.60	0.11
Longitudinal CV	0.563	0.452, 0.700 4	-5.16	<0.0001
Cardiovascular Autonomic Neuropathy	**Odds Ratio**	**95% Confidence Limits**	**Z-value**	**P-Value**
Within-Day CV	1.027	0.866, 1.217	0.30	0.77
Longitudinal CV	0.808	0.700, 0.976	-2.22	0.027
	Adjusted for Mean BG†			
Retinopathy	**Hazard Ratio**	**95% Confidence Limits**	**Z-value**	**P-Value**
Within-Day CV	0.897	0.782, 1.028	-1.56	0.12
Longitudinal CV	0.861	0.744, 0.997	-2.01	0.045
Microalbuminuria				
Within-Day CV	1.028	0.829, 1.274	0.25	0.81
Longitudinal CV	0.728	0.549, 0.967	-2.19	0.028
Cardiovascular Autonomic Neuropathy	**Odds Ratio**	**95% Confidence Limits**	**Z-value**	**P-Value**
Within-Day CV	1.081	0.912, 1.281	0.90	0.38
Longitudinal CV	0.992	0.789, 1.248	-0.06	0.95

HR: Hazard ratio from a Cox proportional hazards model; OR: Odds ratio from a GEE logistic model of the prevalence of CAN at 2, 4, 6 and 8 years of follow-up.

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Supplementary Figure S1. Distribution of the number of missing BG values per visit. For example, 24892 (67.1%) visits had complete BG profile, 5688 (15.3%) visits had only one BG value missing, and 4530 (12.2%) visits had all 7 BG values missing.