Mean Blood Glucose and Biological Variation Have Greater Influence on HbA_{1c} Levels Than Glucose Instability

An analysis of data from the Diabetes Control and Complications Trial

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OBJECTIVE — Mean blood glucose (MBG) over 2–3 months is a strong predictor of HbA_{1c} (A1C) levels. Glucose instability, the variability of blood glucose levels comprising the MBG, and biological variation in A1C (BV) have also been suggested as predictors of A1C independent of MBG. To assess the relative importance of MBG, BV, and glucose instability on A1C, we analyzed patient data from the Diabetes Control and Complications Trial (DCCT).

RESEARCH DESIGN AND METHODS — A glucose profile set and sample for A1C were collected quarterly over the course of the DCCT from each participant (n = 1,441). The glucose profile set consisted of seven samples, one each drawn before and 90 min after breakfast, lunch, and dinner and one before bedtime. MBG and glucose instability (SD of blood glucose [SDBG]) were calculated as the arithmetic mean and SD of glucose profile set samples for each visit, respectively. A statistical model was developed to predict A1C from MBG, SDBG, and BV, adjusted for diabetes duration, sex, treatment group, stratum, and race.

RESULTS — Data from 32,977 visits were available. The overall model was highly statistically significant (log likelihood = −41,818.75, likelihood ratio $\chi^2[7] = 7{,}218.71$, $P > \chi^2 = 0.0000$). MBG and BV had large influences on A1C based on their standardized coefficients. SDBG had only 1/14 of the impact of MBG and 1/10 of the impact of BV.

CONCLUSIONS — MBG and BV have a large influence on A1C, whereas SDBG is relatively unimportant. Consideration of BV as well as MBG in the interpretation of A1C may enhance our ability to monitor diabetes management and predict complications.

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Maintenance of blood glucose levels as close as possible to the physiological range over time is an important goal in the current management of patients with type 1 diabetes. Assessment of a patient's diabetes management can be accomplished by directly analyzing the pattern of multiple blood glucose samples drawn over time (1). However, a high degree of cooperation is required on the part of the patient to collect a sufficient number of blood glucose samples that adequately represent typical diurnal glucose patterns. Once collected, statistical analysis is then necessary to assess the central tendency and variability of glucose levels. As an alternative, a patient's HbA_{1c} (A1C) level can be easily and conveniently determined from a single blood sample. A large number of studies have shown that A1C is strongly associated with the preceding mean blood glucose (MBG) level obtained from multiple blood glucose samples drawn over the preceding weeks and months (2–4). Based on the statistical relation of A1C and MBG, A1C is widely used as a clinical estimate of patient MBG (5). Monitoring MBG or A1C is an important guide in assessing diabetes management because poor glycemic control over time has been linked to the development and progression of microvascular diabetes complications (6).

Over the last 2 decades, it has been shown that factors besides MBG may also influence A1C levels in diabetic patients. Evidence of consistent between-individual biological variation in A1C (BV) that is independent of MBG has been noted in many studies (7–14). Our group has previously proposed the use of a hemoglobin glycation index (HGI) as a method to quantify BV. HGI is the difference between a patient's measured A1C and the expected A1C level predicted from the patient's MBG from measured samples of blood glucose (13,15). BV as quantified by HGI is a predictor for the development of microvascular complications in patients with type 1 (15) and type 2 (16) diabetes independent of MBG.

However, some experts have suggested that variations in the fluctuating diurnal levels of blood glucose may influence A1C in addition to the MBG. The wide variation in blood glucose levels during the course of a day that typifies the records of type 1 diabetic patients has been referred to as "glucose instability" (17). It is conceivable that between-individual differences in glucose instability might account for the previously described consistent between-individual differences in HGI that has been used as evidence of BV among type 1 diabetic patients. To assess the influence of glucose instability on A1C, we evaluated the contributions of MBG, BV, and glucose instability on A1C using data collected during

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Abbreviations: BV, biological variation in HbA_{1c}; DCCT, Diabetes Control and Complications Trial; HGI, hemoglobin glycation index; MBG, mean blood glucose; SDBG, SD of blood glucose.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Magnitude of the effect of model variables on prediction of HbA_{1c} in DCCT data*

Variable	Regression coefficient	SE	*z* value	$P > z$	95% CI
MBG	0.00638	0.000083	77.11	0.000	0.00622–0.00654
SDBG	0.00093	0.000172	5.39	0.000	0.00059–0.00127
BV	0.90016	0.017602	51.14	0.000	0.86566–0.93466
Duration of diabetes from time of diagnosis	−0.00305	0.000721	−4.23	0.000	−0.00446 to −0.00164
Treatment group*	−1.34005	0.048737	−27.50	0.000	−1.43557 to −1.24452
Stratum†	0.17919	0.071616	2.50	0.012	0.03882–0.31955
Race	0.36171	0.132290	2.73	0.006	0.10242–0.62099
Sex	−0.11453	0.048563	−2.36	0.018	−0.20971 to −0.01934

z value or standardized coefficient was calculated as the regression coefficient divided by its SE. *Intensive versus conventional treatment group; †primary or secondary prevention cohort.

the Diabetes Control and Complications Trial (DCCT) (6).

RESEARCH DESIGN AND METHODS

DCCT dataset

We used data collected during the DCCT and stored in SAS datasets on magnetic tape (National Technical Information Service, Washington, DC). The DCCT was a 9-year study of 1,441 participants with type 1 diabetes to determine the effect of intensive versus conventional blood glucose control on the development and progression of diabetes complications (6). At randomization, all participants were free of advanced micro- or macrovascular complications of diabetes. Patients were stratified into two cohorts: the "primary prevention cohort" ($n = 726$), in which subjects had no evidence of retinopathy by fundus photography and a urinary albumin excretion rate <40 mg/24 h (18), and the "secondary intervention cohort" ($n = 715$), in which subjects had minimal-to-moderate retinopathy and a urinary albumin excretion rate <200 mg/24 h (18). At entry into the study, participants were randomized into a conventional or intensive treatment arm. They were then followed at their study site quarterly throughout the course of the DCCT. Detailed descriptions of the design and outcome of the DCCT have been previously published (6,18,19).

Glucose and A1C measurements in the DCCT

During the DCCT, glucose control was monitored at each quarterly visit by collection of a 1-day, seven-sample glucose profile set and a sample for A1C (2,20). The glucose profile set consisted of seven capillary samples, one each drawn before and 90 min after the main meals (breakfast, lunch, and dinner) and one at bedtime (2). Glucose profile set results were available from 95% of the scheduled pre- and postmeal time slots and 92% of the bedtime time slots. The protocol also called for 3:00 A.M. glucose measurements, but these were available in <1% of the profiles; thus, analysis of profile set data omitted this eighth sample. Glucose concentrations in samples from the profile sets and A1C levels from all participants were determined at a central laboratory (20).

Calculation of MBG and glucose instability

After first evaluating the distribution of blood glucose assessments to check for normality, the MBG was calculated as the arithmetic mean of glucose concentrations from the associated glucose profile set for each quarterly visit. Glucose instability was calculated as the SD of blood glucose (SDBG) around the mean from the glucose profile set for each quarterly visit.

Statistical modeling and assessment of between-individual biological variation

We previously developed a statistical model to assess between-individual BV in diabetic patients (13). A similar statistical approach was applied to the DCCT data (15). As briefly described, a longitudinal linear response model was developed from all measured A1C values and the corresponding MBG values from the glucose profile sets. This model enabled us to adjust variance estimates for the correlation of multiple blood glucose measurements on the same individual over time. The appropriateness of a linear model was confirmed by a spline fitting algorithm that made no prior assumptions regarding the shape of the relation. Akaike's information criterion (21) indicated that a random intercept provided the best fit for the data.

The chosen model was then used to predict A1C from the MBG across the years of the DCCT. Model variance estimates were adjusted to account for the correlation between multiple measurements on the same individuals over time. Other variables taken into account in the model were diabetes duration, sex, treatment group (intensive versus conventional therapy), stratum (primary or secondary intervention arm), and race. Statistical analysis was performed using Stata 8 software. The relative impact of each variable on A1C was assessed by comparing the variable's standardized coefficients (*z* value) in the model, which was calculated as the regression coefficient divided by its SE. The standardized coefficient was chosen to facilitate comparison of independent variables that differ in unit scaling from one another. Unstandardized coefficients represent the amount of change in the regression variable associated with one unit of change by the scale in the independent variable. Calculation of standardized coefficients uniformly converts the scales of each variable to comparable SE units.

RESULTS — A total of 32,977 A1C and profile set pairs were collected at quarterly visits over the 9-year course of the DCCT. The overall statistical model used to predict A1C was highly statistically significant (log likelihood = −41,818.75, likelihood ratio $\chi^2[7] = 7{,}218.71$, $P > \chi^2 = 0.0000$).

Table 1 presents the data from the model for the major independent variables used to predict A1C. All three variables of particular interest in this study (MBG, SDBG, and BV) were found to be statistically significant when controlled

for the presence of the other covariates. MBG and BV had the largest influence on A1C based on their standardized coefficients (coefficients divided by their SEs) derived from the statistical model. Glucose variability expressed as SDBG had only ~1/14 of the impact of MBG and ~1/10 of the impact of BV on A1C. Because of the large number of observations in the dataset, SDBG was found to be statistically significant even though its influence on A1C was minor. As might be expected, other covariates included in the model (e.g., intensive versus conventional treatment group) also had an influence on A1C levels.

CONCLUSIONS — The goal of this study was to evaluate clinically important influences on A1C using the extensive dataset collected from 1,441 participants in the DCCT who were closely followed for as long as 9 years. Besides the well-known influence of MBG, we specifically were interested in assessing the relative influences of glucose instability and between-individual BV in the same statistical model.

The number of glucose profile sets that could be paired with A1C for analysis was impressively large, numbering 32,977 pairs. The arithmetic MBG computed from the glucose profile sets was highly correlated with A1C, as previously reported (2,22), and accounted for the greatest effect on A1C. When we compared the relative impact of MBG, BV, and SDBG on A1C by computing the ratios of standardized coefficients, the influence of BV was substantial (66% as great as MBG), whereas the impact of SDBG on A1C was minor (7% as great as MBG), Based on these results, the effect of BV is independent of and greater than the influence attributable to glucose instability. In a somewhat similar analysis, Derr et al. (17) previously reported that the SDBG levels obtained during self-monitoring among adult patients attending a large academic center diabetes clinic had no influence on A1C levels.

We confirmed that individual-specific differences unrelated to glycemia were an important predictor of A1C in addition to the influence of MBG. Individual-specific differences in A1C are also called interindividual or between-individual biological variation (11,14, 23). Besides this study using the DCCT study population, our group and many others have previously reported the presence of between-individual BV from many different populations of individuals both with and without diabetes (7–14,16,24,25). In the present analysis of data from the DCCT, BV had a rather strong influence on A1C: its standardized coefficient for prediction of A1C was ~67% of that of MBG. Measurement of between-individual BV is clinically relevant because these differences have been associated with microvascular complications (15,16,24).

The presence of BV levels found in nondiabetic and diabetic populations suggests that some individuals may be more susceptible to nonenzymatic glycation than others. Factors influencing the membrane influx/egress of glucose, or glucose binding to hemoglobin, could influence the accumulation of intracellular A1C (9,26). Other potential mechanisms for between-individual differences in A1C may involve differences in glycolytic enzyme activity, which might facilitate the glycation of hemoglobin (9,10,12,27) or enhance deglycation of hemoglobin (28,29). There is preliminary evidence that individual glycation differences, such as those measured by BV, are under genetic control (30).

The relatively large influences of MBG and BV on A1C compared with the influence of variation in glucose levels (SDBG) may help explain why there frequently is discordance between A1C levels and the results of oral glucose tolerance testing in the diagnosis of impaired glucose tolerance and early diabetes (31). In conclusion, MBG and between-individual BV are the two major components determining a patient's level of A1C. Glucose instability (the diurnal fluctuations in glucose levels) has minimal impact on A1C and does not account for the observed biological variation in A1C.

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