Is HbA_{1c} Affected by Glycemic Instability?

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OBJECTIVE — HbA_{1c} is a standard clinical assessment of glycemia and the basis of most data relating glycemic control to complications. It remains unclear, however, whether HbA_{1c} is affected by glycemic variation and mean glycemia.

RESEARCH DESIGN AND METHODS — To test this question, we analyzed the statistical relationship between HbA_{1c} levels and glycemic variability as measured by self-monitoring of blood glucose (SMBG). The records of 256 subjects were studied. SMBG data for the preceding 3 months were downloaded, and HbA_{1c} was measured by ion-exchange high-performance liquid chromatography. Simple- and random-effects linear regression models were used to assess the independent contributions of mean blood glucose (BG) and SD of BG to HbA_{1c}, after adjusting for the mean BG.

RESULTS — Mean ± SD for HbA_{1c} was 7.66 ± 1.11% and for BG was 8.5 ± 1.9 mmol/l (153.3 ± 34.9 mg/dl); SD of BG for individual subjects was 3.5 mmol/l (63.3 mg/dl), varying from 0.4 mmol/l (8.1 mg/dl; very stable glycemia) to 8.4 mmol/l (152.5 mg/dl; very unstable glycemia). A close correlation between mean BG and HbA_{1c} was demonstrated ($r = 0.62$). Also, within-subject SD of BG correlated with HbA_{1c} ($r = 0.375$), indicating that people with poorer glycemic control had higher BG variance. After adjusting for mean BG in a linear regression model, however, the effect of the within-subject SD of BG on the HbA_{1c} was insignificant. Several further analyses confirmed the strength of the observation.

CONCLUSIONS — HbA_{1c} reflects mean glycemia and is not meaningfully affected by glycemic instability after adjusting for mean BG.

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HbA_{1c}, the major form of stable glycated Hb, is the product of a slow and largely irreversible reaction that occurs throughout the life span of the erythrocyte (1). Since the 1970s, it has been understood that HbA_{1c} reflects mean blood glucose (BG) levels (1–3) over the previous 2–3 months, and it has been proposed as a diagnostic criterion for diabetes (4). HbA_{1c} has, therefore, become a standard assessment of glycemia (5) and a standard part of diabetes management.

The other approach routinely used to assess glycemic control, self-monitoring of blood glucose (SMBG), provides quite different information. It can reveal patterns of highs and lows throughout the day and document hypo- or hyperglycemia. If done regularly and at representative times of day and night, SMBG results also reflect mean glycemia over time and, therefore, correlate with HbA_{1c}. Finally, SMBG results provide an indication of glycemic variability (i.e., lability or brittleness). The lability of BG can be visualized by graphic presentation of data downloads (Fig. 1) and quantified by measuring the SD of the recorded BG values.

This study asks whether the variance of BG, measured by analyzing SMBG results, contributes to HbA_{1c}. The question is significant in that it addresses whether occasional bursts of severe hyperglycemia or hypoglycemia affect the HbA_{1c} beyond their transient contribution to mean glycemia. In addition, the answer could influence our understanding of the relationship between glycemia and the complications of diabetes. Large studies of this relationship, such as the Diabetes Control and Complications Trial (DCCT) (6) and the U.K. Prospective Diabetes Study (7), used HbA_{1c} as the primary index of glycemia. If glycemic variance influences HbA_{1c}, then brief fluctuations in BG could play a significant role in the development of long-term diabetes complications.

We address whether glycemic variance affects HbA_{1c} by assessing, statistically, the contribution of SD of BG to HbA_{1c}, controlled for mean BG, in a group of people with diabetes who have performed frequent SMBG over the months before the assessment of HbA_{1c}.

RESEARCH DESIGN AND METHODS

Data collection

SMBG results were downloaded routinely from 136 consecutive patients who brought their glucose meters (One Touch System; Lifescan, Milpitas, CA) during visits to a medical center diabetes clinic. Downloads recorded the 250 most recent individual BG test results, excluding any taken >90 days before the visit. BG recordings (reported as plasma glucose equivalents) had an identified date and time. For each patient, HbA_{1c} was measured on the day of the download, by ion-exchange high-performance liquid chromatography, certified by the National Glycohemoglobin Standardization Program (8). Age, sex, and race, as well as type of diabetes and diabetes treatment during the period studied, were noted for each patient. Patients could be included

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Abbreviations: BG, blood glucose; DCCT, Diabetes Control and Complications Trial; SMBG, self-monitoring of blood glucose.

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

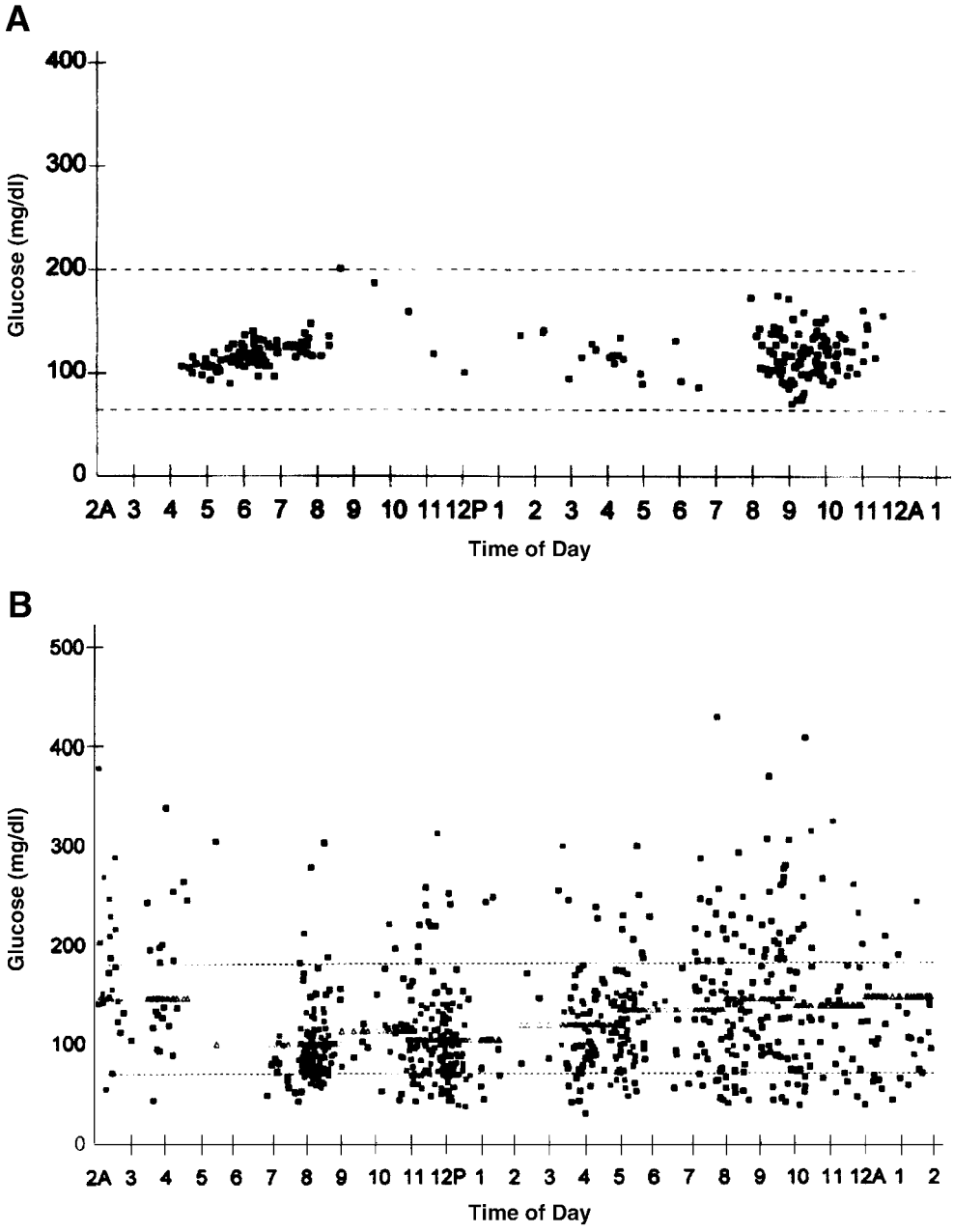

Figure 1— *Two individual SMBG downloads illustrating similar means with widely varying SDs.* A*: Mean BG 6.6 mmol/l (119 mg/dl), SD 1.1 mmol/l (20 mg/dl).* B*: Mean BG 6.7 mmol/l (121 mg/dl), SD 3.4 mmol/l (61 mg/dl). In the total dataset, means analyzed ranged from 4.6 to 15.4 mmol/l (83–218 mg/dl) and SD ranged from 0.4 to 8.8 mmol/l (7.2–153 mg/dl).*

more than once, but ≥6 months was required between glucose meter downloads. Data were grouped without individual patient identifiers, and the institutional review board ruled that individual consent was not necessary.

Data inclusion/exclusion criteria

Data from patients were included if three criteria were met. First, the earliest SMBG result in the series had to occur ≥14 days before the HbA_{1c} level was drawn to include only subjects whose SMBG reflected chronic glycemia. Second, no gaps >7 days could exist between successive SMBG results, because prolonged gaps could represent periods of unrecorded changes in BG that would affect HbA_{1c}. Third, an average of ≥1.5 tests per day had to be recorded throughout the period to ensure that glycemia was being assessed at different times of day.

Individual SMBG results were also filtered in the primary analysis to exclude readings that occurred <1.5 h after the preceding reading. This was done, first, because preprandial values show intrinsic lability/stability characteristics of subjects without adding the inconsistent effect of whether, or when, postmeal glycemia was being assessed. A secondary analysis of a dataset was performed without imposing the 1.5-h exclusion, i.e., including values

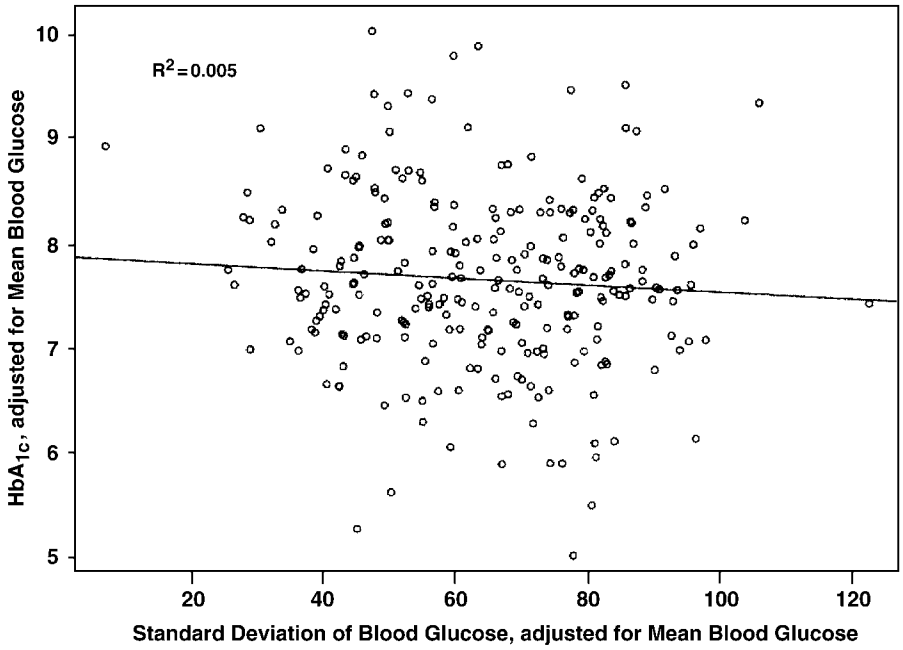

Figure 2—*Correlation of SD of BG and HbA_{1c} after adjusting for mean BG. Line reflects the regression. See text for interpretation.*

that may have been postprandial or confirmatory. Also, secondary data calculations were performed simulating postprandial increases of 4.4 mmol/l (80 mg/dl) and 7.8 mmol/l (140 mg/dl). The second reason for excluding values taken in proximity to each other from the primary analysis is that they may reflect repeat testing performed to confirm highs or lows, weighting them excessively and biasing results.

Statistical analysis

Linear regression models were used to assess the associations between HbA_{1c}, the mean of the BG levels, and the SD of the BG levels. To account for the correlation between more than one visit for the same individuals, random-effects linear regression models were used (9). Initial models examined the independent contributions of mean BG and SD of BG to HbA_{1c} and the association between the mean BG and the SD of BG. At this point, the functional forms of the variables were considered and assumptions of linear regression were assessed. There seemed to be no need for transformation or inclusion of higher-order terms due to variance instability or nonlinearity. Data were then fit to a model predicting HbA_{1c} by both the mean and SD of BG to determine the additional effect of SD on HbA_{1c} after adjusting for the mean.

To present data graphically (Fig. 2), an adjusted variable approach was taken to graphically show the relationship between HbA_{1c} and SD of BG after adjusting for mean BG. Two random-effects linear regressions were fit: HbA_{1c} on mean BG and SD of BG on mean BG. The residuals from each of these regressions represent HbA_{1c} adjusted for mean BG and SD of BG adjusted for mean BG, respectively. The mean of HbA_{1c} and SD of BG were then added to the residuals to appropriately rescale them to the metric of interest. We plot these residuals versus each other to provide an indication of the magnitude of the association and the level of correlation between the variables of interest after adjustment for mean BG.

All statistical analyses were performed using Stata 7.0 statistical package (StataCorp, College Station, TX) (10).

RESULTS

Patient characteristics

There were 256 visits with simultaneous HbA_{1c} determinations and SMBG meter downloads. Some patients were studied more than once, such that data from 136 different patients who met inclusion criteria and had simultaneous HbA_{1c} and SMBG downloads were included. When a patient's data were used more than once, there was an interval of ≥6 months between visits. Demographic information for the patients is presented in Table 1. Our subjects were a cross-section of adults with diabetes representing a range of characteristics of interest (SMBG variability and HbA_{1c}) and were not necessarily representative of people with diabetes as a whole.

SMBG and HbA_{1c} characteristics

Table 2 shows the number of days with SMBG readings (73.6 ± 20.3 days) and the number of readings per day (mean 2.4). This indicates monitoring over a sufficient duration and frequency to be well reflected in HbA_{1c}. Data in Table 3 indicate that the patients included had a wide range of glycemia, as evidenced by the range of mean BG (4.6–15.4 mmol/l [83.1–277.1 mg/dl]) and HbA_{1c} (4.8–11.4%). Some patients were apparently normoglycemic, whereas others were in very poor glycemic control.

Of equal importance in addressing our question was a wide range of glycemic variance (lability) represented in our dataset. The lowest SD of BG (0.4 mmol/l [8.1 mg/dl]) indicates extremely stable diabetes, whereas the highest SD of BG (8.4 mmol/l [152.5 mg/dl]) indicates very unstable diabetes. The mean SD of BG for our patients (3.5 mmol/l [63.3 mg/dl]) indicates what might be considered moderately unstable diabetes.

Regression analysis

Table 4 shows the results of regression analysis of the major variables considered. HbA_{1c} correlated strongly with the mean BG ($R = 0.62$, $P < 0.001$), confirming the known relationship between mean glycemia over the previous months and HbA_{1c}. In addition, the SD of BG was significantly correlated with HbA_{1c} ($R = 0.375$, $P < 0.001$). There was also a significant correlation between the SD of BG and mean BG ($R = 0.65$, $P < 0.001$). The latter associations indicate that poorer glycemic control correlates not only with HbA_{1c} but also with a wider fluctuation in BG.

Multiple regression analysis (Table 4), however, indicated that after controlling for the mean BG, there was a minimal, insignificant effect of the SD of BG on HbA_{1c}. This result is not unexpected, due to the strong correlation between SD of BG and mean of BG. When such collinearity is present in linear regression models, predictors often become insignificant or

Table 1—*Patient demographics*

n	136
Age (years)	
Mean	55.0
Median	54
SD	16.9
Range	18–101
Sex	
Female	58 (43)
Male	78 (57)
Race	
White	114 (84)
Black	11 (8)
Asian	1 (1)
Hispanic	2 (1)
Other/unknown	8 (6)
Type of diabetes	
Type 1	79 (58)
Type 2	57 (42)
Diabetes treatment	
Insulin only	106 (78)
Oral agents only	17 (12)
Both insulin and oral agents	7 (5)
Diet modification only	5 (4)
Unknown	1 (1)

Data are *n* (%) unless otherwise indicated.

Table 2—*Downloaded SMBG data characteristics*

	Mean	Median	SD	Range
Days analyzed	73.6	84.2	20.3	14–90
Number of readings	179.3	183	73.6	23–179
Readings per day	2.4	2.2	—	—

Table 3—*SMBG and HbA_{1c} characteristics*

	Mean	Median	SD	Range
Mean BG (mmol/l)	8.5	8.3	1.9	4.6–15.4
SD of BG (mmol/l)	3.5	3.6	1.3	0.4–8.5
HbA_{1c} (%)	7.66	7.60	1.11	4.8–11.4

the direction of the association changes after adjustment. Model 1 shows the correlation of mean BG to HbA_{1c}, whereas model 2 relates SD of BG to HbA_{1c}. In model 3, the addition of SD of BG after adjustment for mean of BG increases the r^2 only from 0.3807 to 0.3837, showing that the SD of BG adds virtually no predictive ability for HbA_{1c} after adjusting for mean BG. Additionally, the effect for mean of BG in model 3 is only slightly different than the effect of mean BG in model 1, whereas the effect for SD of BG in model 3 compared with model 2 is much smaller (half the size) and has changed direction. Taken together, these results imply that SD of BG adds minimally to a predictive model for HbA_{1c} that already includes mean BG.

In Fig. 2, the correlation of HbA_{1c} is plotted against SD of BG after adjustment for mean BG (as described in RESEARCH DESIGN AND METHODS). There is a slight, insignificant inverse correlation, with an *r* of only 0.071.

We found that patients with a large number of readings tended to be better controlled (i.e., had lower mean BG) than those with fewer readings ($r = -0.29$). However, it seems likely that important causal factors could contribute to this, e.g., that individuals with better control are more likely to take readings more frequently whereas those who test less often are in worse control as a result. Therefore, including the number of readings in our regression model would likely lead to spurious collinearity effects, irrelevant to the influence of lability on HbA_{1c}, our fundamental question.

Further analyses also tested various other potential predictors of glycemic variation and change in HbA_{1c}. For example, the question was asked whether individuals with daily trajectories of increasing BG over the course of the day would influence our results. In this case, the diurnal pattern of BG would increase SD of BG although the individual was consistent in his pattern, not "labile." To test for this, we estimated the average daily slope for each individual and considered this to be a predictor of HbA_{1c}. There was no association. We also considered the square root of the mean square error from the same analysis (i.e., the SD of the residuals) considering whether this was a predictor of HbA_{1c}. Again, the results did not change.

Finally, a series of secondary, confirmatory analyses were performed. We considered the impact of including multiple readings within a short time period. To do this, we created a new dataset (similar to the primary dataset) in which we allowed observations within 1.5 h of previous readings. This analysis included all values, whether pre- or postprandial. Inclusion of postmeal glucose values does not change the result. The effect of SD of BG on HbA_{1c} was very similar to that presented in Table 4: the model with mean BG had an R^2 of 0.3220 and the inclusion of SD of BG only increased the R^2 to 0.3224, a change in R^2 of 0.0004.

Next, two arbitrary sets of BG values (4.4 and 7.8 mmol/l [80 and 140 mg/dl]) were added to each actual result as an additional reading, to simulate postmeal values in all subjects. Again, the conclusions were not altered. Adding 4.4 mmol/l, the R^2 for relationship of BG-corrected mean BG to HbA_{1c} was 0.3842 ($P = 0.35$, with SD range 1.67–8.9 mmol/l); adding 7.8 mmol/l to every BG value, the R^2 for relationship of BG-corrected mean BG to HbA_{1c} was 0.3847 ($P = 0.30$, with SD range 2.7–9.2 mmol/l). These correlations are virtually the same as values found in the primary analysis (Table 2), where R^2 is 0.3837 ($P = 0.39$), and the range of SD is, of course, somewhat higher than in the primary analysis (0.4–8.5 mmol/l; Table 3).

CONCLUSIONS — The strong relationship we have found between mean BG and HbA_{1c} reconfirms what has been well established. Our finding that people with greater glycemic variance have poorer diabetic control is also expected, because patients with widely fluctuating BG will have periods of high glycemia that increase their mean BG, and less stable glycemia is more difficult to control well to a mean level approaching normal. Possible causal relationships between poor mean glycemia and labile glycemia are not addressed by our data. However, it is evident from our data that an assessment of whether glycemic lability affects HbA_{1c} cannot ignore the relationship between mean glycemia and HbA_{1c}.

Our data refute the hypothesis that glycemic lability has an important independent effect on HbA_{1c}. In particular, we do not find that spikes of glycemia into

Table 4—*Regression model*

	Model 1			Model 2			Model 3		
	β	SE	*P*	β	SE	*P*	β	SE	*P*
Mean BG	0.019	0.0016	<0.001				0.020	0.0021	<0.001
SD of BG				0.016	0.0027	<0.001	−0.0026	0.0030	0.39
Model[2]		0.3807			0.1403			0.3837	

β-Parameters are the slope coefficients from the linear regression models where outcome variable is HbA_{1c}. Models 1 and 2 are simple linear regression models, including only one covariate each. Model 3 is a multiple linear regression model, including both mean BG and SD of BG.

high ranges increase HbA_{1c} beyond their contribution to mean BG. Rather, the statistical relationship between glycemic variance and HbA_{1c} is, for all practical purposes, entirely accounted for by the tendency of people with increased fluctuations in BG to have increased mean BG.

Hb is continuously glycated during the 120-day life span of the erythrocyte, such that the proportion of Hb that is in the form of HbA_{1c} increases throughout the erythrocyte's lifetime (11). This reaction, in which the NH_2-terminal amino acid of the β-chain of Hb forms an unstable Schiff base and then undergoes an Amadori rearrangement to form a stable ketoamine, is post-translational, nonenzymatic, and relatively slow. Driven by the nucleophilic nature of the NH_2-terminal amino group of Hb that condenses with glucose found in the erythrocyte, kinetic principles suggest, and in vitro and in vivo studies confirm, that the cumulative amount of HbA_{1c} in a erythrocyte is directly proportional to the time-averaged concentration of glucose within the erythrocyte (11–14). Given this relationship, it stands to reason that brief periods of high blood glucose are unlikely to have a significant impact on Hb glycation.

Our findings could have potentially important implications for understanding the development of long-term complications of diabetes. Because the major long-term studies of control and complications use HbA_{1c} to assess glycemia, and because we have shown that HbA_{1c} is not importantly affected by glycemic variability, our results could be interpreted as compatible with the hypothesis that glycemic variability has little independent role in causing microvascular complications. The DCCT found that although HbA_{1c} was the predominant determinant of risk, it did not account for the entire risk of retinopathy progression; other unidentified factors contributed to a difference between the conventionally and the intensively treated groups (15). The question of whether lability, as such, contributes to incidence of complications would ultimately be settled only by large, long-term, prospective clinical trials, presumably using continuous glucose monitoring. Our data, however, indicate that lability of blood glucose does not, at least, contribute to complications by affecting glycation of the protein Hb.

Clinical experience suggests that people have widely varying levels of intrinsic lability, the most obvious determinant being whether the person has type 1 or type 2 diabetes, i.e., whether there is residual endogenous insulin secretion. Prescribed treatment and patterns of self-care also affect lability. Data automatically stored in the glucose monitoring device allowed us to calculate SD of BG for patients that reflects their degree of manifested lability, whatever the cause. We required a frequency of ≥1.5 measures per day, so that we were assessing glycemia at several times of day, not only, for example, fasting glycemia. Our analyses excluded the chance that a stable, systematic increase, for example at the second test of the day, resulted in perceived increased variance as a function of treatment, without reflecting intrinsic lability. We also excluded patients with gaps in monitoring of >7 days, "vacation" periods during which glycemia could have affected the HbA_{1c} without being reflected in SMBG results. In our primary analysis, we excluded values taken within 1.5 h of a previous value, and when results were calculated using a dataset that included all data (which would include pre- and postprandial values), the conclusions do not change.

There are several limitations to our data and its analysis. The dataset is not chosen to be representative of any particular population of people with diabetes, and some individuals were included more than once. However, the patient population is not critical to addressing the hypothesis of this study because the association we addressed focused on two objective predictors, mean blood glucose levels and glycemic variability. The critical characteristic of our dataset is the very wide range of data points presented for each variable. Figure 1 demonstrates the difference between relatively stable and relatively labile diabetes with similar mean glycemia, and even the patients illustrated in Fig. 1 are not at the extremes of the mean or SD range studied.

A second limitation is that self-monitoring, no matter how frequently done, falls well short of quantifying glycemic lability with the accuracy that would be possible with continuous glucose monitoring. Ultimately, as continuous glucose monitoring technology matures, this study question could be addressed by data collected from a large number of patients wearing reliable continuous blood glucose monitors for lengthy periods of time. Also, using continuous blood glucose analysis could allow for use of other indexes of glycemic lability. Such measures as mean amplitude of glycemic excursions (MAGE), as described by Service et al. (16) in their classic study, could be calculated with continuous monitoring but not with SMBG. One report found SD of BG to be the most useful of several measures of glycemic stability (17).

The problem of a type II error in our results (missing a relationship that actually exists) would be an issue if our study were underpowered. However, it is evident from Fig. 2 that there is essentially no correlation between SD and HbA_{1c} after adjusting for mean BG, and this conclusion is statistically confirmed with the observed r of 0.07 and r^2 of 0.005. The 95% CIs encompass what would be, at most, a very poor correlation ($R = 0.24$), and overlaps 0, meaning that any correlation could be direct or indirect. If type II

error were a problem, this CI would overlap a region of strong correlation.

There is evidence that HbA_{1c} does not reflect the mean blood glucose equally over the prior 3 months but weights the more recent period more heavily (18). This important observation should not introduce any important systematic bias into our data analysis, however, unless glycemic variability were significantly changed over the month before the visit, compared with the full 90 days before the visit.

In conclusion, we have found that variation in BG does not affect HbA_{1c}. We have reconfirmed the strong correlation between mean BG and HbA_{1c} and found a close correlation between glycemic variance and mean BG. When these associations are taken into account, we identified no contribution of glycemic variance to HbA_{1c}.

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