Variability in the Relationship between Mean Plasma Glucose and HbA$_{1c}$: Implications for the Assessment of Glycemic Control

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Background: Previous studies have shown a single linear relationship between mean plasma glucose (MPG) and hemoglobin A$_{1c}$ (HbA$_{1c}$). We examined the relationship in different treatment groups of patients with type 1 diabetes participating in the Diabetes Control and Complications Trial (DCCT).

Methods: Seven-point glucose profiles (premeal, postmeal, and bedtime) and HbA$_{1c}$ were measured quarterly during the DCCT. We studied measurements from (a) intensively treated patients at study commencement, (b) intensively treated patients after stabilization of their glycemia (from 6 months onward), and (c) conventionally treated patients from 6 months onward. Only complete glucose profile and HbA$_{1c}$ pairings were considered ($n$/H11549 589, 11 483, and 11 855, respectively).

Results: From 6 months into the trial, conventionally treated patients had consistently higher MPG concentrations than intensively treated patients at any given HbA$_{1c}$ value (mean difference, 1.6 mmol/L at 7% HbA$_{1c}$, increasing to 2.8 mmol/L at 11% HbA$_{1c}$). Similarly, at the same HbA$_{1c}$ the MPG of intensively treated patients at baseline was higher than in the same individuals after 6 months of intensive treatment (1.2 mmol/L difference at 7% HbA$_{1c}$, increasing to 4.6 mmol/L at 11% HbA$_{1c}$).

Conclusions: The relationship between MPG and HbA$_{1c}$ is not constant but differs depending on the glycemic control of the population being studied. Having lower mean glucose at the same HbA$_{1c}$ may help explain why intensive DCCT treatment appeared intrinsically linked to both increased hypoglycemia and decreased microvascular complications compared with conventional treatment. These findings may also have implications for expressing HbA$_{1c}$ as mean blood glucose equivalent.

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The role of hemoglobin A$_{1c}$ (HbA$_{1c}$)$^4$ in the assessment of glycemic control in patients with diabetes has been cemented by the results of the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (1, 2). These studies showed that HbA$_{1c}$ is an important marker in assessing a patient’s risk of microvascular complications and hypoglycemia. As a result, both HbA$_{1c}$ and blood glucose targets are now used in the routine management of patients with type 1 and type 2 diabetes (3).

A linear relationship between HbA$_{1c}$ and mean plasma glucose (MPG) was first established in 1984 (4) and corroborated by the feasibility study of the DCCT (5). More recently, data from the full DCCT trial were used to compare the average of every 7-point glucose day profile measured with the mean HbA$_{1c}$ of each participant (6). From this, a linear equation between the 2 measures was derived, allowing patients and healthcare workers to set plasma glucose targets based on their HbA$_{1c}$ goals. Indeed, it has been suggested that this relationship be used to express HbA$_{1c}$ as mean blood glucose equivalent so that patients can equate the test to their own self-monitored blood glucose records (7–9).

In the DCCT itself, further analysis of the data seemed to suggest that HbA$_{1c}$ predicted the risk of hypoglycemia and microvascular complications differently between the intensively and conventionally treated patients, such that intensive treatment was associated with a greater risk of

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$^4$ Nonstandard abbreviations: HbA$_{1c}$: hemoglobin A$_{1c}$; DCCT, Diabetes Control and Complications Trial; MPG, mean plasma glucose; AUC, area under the curve.
hypoglycemia (10) but a decreased risk of small vessel disease (11) for any given HbA1c value. The reasons for this observation remain speculative but are thought to relate to the treatment regimens used by the 2 treatment groups.

We reanalyzed the DCCT dataset to establish the relationship between MPG and HbA1c for each of the treatment groups to see whether this may help explain why the rate of complications differed between them.

Patients and Methods

DATASETS
We used the publicly accessible datasets stored in SAS format collected by the DCCT (www.gcrc.umn.edu). The DCCT was a 9-year follow-up study of 1441 participants with type 1 diabetes comparing the effect of intensive vs conventional blood glucose management on the development of microvascular complications. Study participants were randomized into intensive (n = 711) and conventional (n = 730) treatment groups.

Glycemic Variables and Statistical Methods
Capillary blood glucose samples were shipped on dry ice to the DCCT Central Biochemistry Laboratory and analyzed by use of a hexokinase method (12). HbA1c samples were express-transported at 4 °C to the Central HbA1c Laboratory, where samples were originally analyzed on a HPLC column (Biorex 70; Bio-Rad); within- and between-assay CVs for this HbA1c method were <6%. From November 1986 onward, a Diamat HPLC instrument (Bio-Rad) was used, with which both CVs were consistently <3% (13).

In the DCCT, a blood glucose profile was taken at 3-month intervals. Blood glucose was assessed at 7 points throughout the day: prebreakfast (we assumed a time of 7:00 AM), postbreakfast (8:30 AM), prelunch (12:00 PM), postlunch (1:30 PM), presupper (6:00 PM), postsupper (7:30 PM), and bedtime (10:00 PM). An additional data point was collected at 3:00 AM on <1% of occasions and was used here only to validate that an absence of overnight measurement was not influencing our findings. In addition, not everybody had a complete 7-point blood glucose profile during every quarter of their participation, so we restricted the data for analysis to the time points where there was a complete glucose profile and HbA1c pairing. Mean blood glucose was calculated by the area under the curve (AUC) method using the trapezoidal rule (14). This was performed as by Rohlfing et al. (6), except that rather than assuming a constant blood glucose concentration between bedtime and the following morning, we assumed the following morning glucose value to be the same as that of the previous morning. The same 11% increase in mean blood glucose values was made to express results as an MPG equivalent (15).

The relationship between MPG and HbA1c was established for each of the treatment groups (conventional and intensive) separately and combined. HbA1c in the intensively treated group reached a nadir 6 months into the trial (1), so only data from 6 months onward were considered for both groups. We compared mean HbA1c in each patient with their average within-day MPG. We also studied the relationship between MPG and HbA1c in the intensively and conventionally treated groups at their first visit before randomization (baseline).

We analyzed the data using least-squares regression analysis weighted for the number of MPG/HbA1c pairings in each individual. Residuals were checked for gaussian distribution by plotting a histogram. Unpaired t-tests were used to compare the MPG of patient groups at the same band of HbA1c and the same test was used to compare the slopes of regression lines. Paired t-tests were used to establish differences between 7-point and 8-point glucose profiles.

Results

Seven hundred seven intensively treated and 726 conventionally treated patients had 11 483 and 11 855 complete (7-point) MPG and HbA1c pairings collected from 6 months into the study. At baseline, complete glucose profiles and HbA1c measurements were present in 589 participants who were subsequently randomized to intensive treatment and 585 individuals who were assigned to conventional treatment. There were no significant differences in age (P = 0.46), duration of diabetes (P = 0.41), sex (P = 0.30), or phase of treatment (P = 0.90) between the 589 intensively treated patients at baseline and the 122 who did not have a complete glucose profile at baseline.

The MPG values of these 4 groups categorized according to their HbA1c are shown in Table 1. After stabilization of glycemia, the intensively treated group had lower MPG values than both the conventionally treated group after stabilization and the intensively treated group at baseline (P <0.05 from 6% to 10.4% HbA1c, P <0.0001 from 6.5% to 8.4% comparing stabilized intensive patients with both groups). Only 1 of 15 comparisons between the conventionally treated group at baseline and the same group after stabilization showed any significant difference in MPG (P = 0.043 at 9% to 9.4% HbA1c).

Fig. 1 shows the relationship between MPG and HbA1c using regression in each of the treatment groups and for both groups combined after stabilization (6 months onward). The slope of the conventionally treated group [1.54 mmol/L (95% CI, 1.42–1.66)] increase in MPG for every 1% increase in HbA1c] was significantly greater than in the intensively treated patients [1.23 mmol/L (1.12–1.33), P <0.001]. The regression relationship of the combined group was skewed by the larger number of intensively treated patients with low HbA1c values and by the larger number of conventionally treated patients with higher values, so the slope in this case was greater again [1.87 mmol/L (1.81–1.94), P <0.001 compared with both individual groups]. Weighting the regression by instead using the reciprocal of each patient’s HbA1c variance provided very similar results.
Nearly identical findings were also obtained when MPG was calculated using a constant blood glucose concentration between bedtime and the following morning as per Rohlfing et al. (6) [slope of conventionally treated group, 1.69 (1.56–1.83); intensively treated group, 1.21 (1.09–1.33); combined, 1.97 (1.89–2.04); \( P < 0.001 \) between all 3 groups].

From 6 months into the study, there were 1361 glucose points collected at 3:00 AM, 1068 of which were at the 6-month visit itself (528 intensive and 540 conventional treatment). Using these 6-month data, we calculated each patient’s 8-point AUC and compared this with what their 7-point profile would have shown had the 3:00 AM sample not been collected. The 8-point AUC was, on average, 0.06 mmol/L greater than when just 7 points were used (\( P < 0.001 \)). In the intensive group alone, the 8-point AUC was 0.02 mmol/L lower than the 7-point, and in the conventional group it was 0.13 mmol/L greater (\( P < 0.001 \), intensive vs conventional).

### Discussion

This study shows that the relationship between MPG and HbA1c in the DCCT was different between intensively and conventionally treated patients such that the former group had clinically significantly lower MPG concentrations at the same HbA1c values. The difference became greater as the HbA1c increased, being 1.6 mmol/L at 7% HbA1c and 2.8 mmol/L at 11% HbA1c. This observation does not seem to be a consequence of any differences between treatment groups, as a very similar finding was identified among just the intensively treated patients when their glycemic control was improved.

### Table 1. MPG values at different mean HbA1c values.

<table>
<thead>
<tr>
<th>HbA1c, %</th>
<th>Intensive from 6 months</th>
<th>Conventional from 6 months</th>
<th>Intensive at baseline</th>
<th>Conventional at baseline</th>
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<tr>
<td></td>
<td>MPG, mmol/L</td>
<td>n</td>
<td>MPG, mmol/L</td>
<td>n</td>
</tr>
<tr>
<td>5–5.4</td>
<td>7.9 (1.4)</td>
<td>3</td>
<td>8.9 (1.0)</td>
<td>2</td>
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<td>5.5–5.9</td>
<td>8.2 (0.8)</td>
<td>29</td>
<td>9.2 (1.4)</td>
<td>5</td>
</tr>
<tr>
<td>6–6.4</td>
<td>8.0 (1.1)</td>
<td>110</td>
<td>9.8 (1.8)</td>
<td>26</td>
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<tr>
<td>6.5–6.9</td>
<td>8.8 (1.0)</td>
<td>183</td>
<td>11.3 (1.9)</td>
<td>38</td>
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<td>9.4 (1.4)</td>
<td>173</td>
<td>12.0 (1.8)</td>
<td>72</td>
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<td>13.7 (2.0)</td>
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<td>15.1 (2.6)</td>
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<td>1</td>
<td>24.4 (7.9)</td>
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* Data are mean (SD).

\( MPG = 1.54 \times \text{HbA1c} - 0.10; r = 0.69; \cdots \cdots \), intensively treated
\( MPG = 1.23 \times \text{HbA1c} + 0.47; r = 0.67; \cdots \cdots \), and both patient groups combined (\( MPG = 1.87 \times \text{HbA1c} - 3.61; r = 0.82; \cdots \cdots \) after stabilization. All \( P < 0.0001 \).
A plausible explanation can be found for this difference if the concept of high and low glycators is assumed (16). High glycators have consistently higher HbA$_{1c}$ than expected for their MPG, whereas low glycators have lower HbA$_{1c}$ than their MPG would suggest. The proposed reasons for this between-individual variability in hemoglobin glycation rate include differences in erythrocyte survival and other genetic elements (17, 18). In relation to the DCCT, the majority of patients at study baseline had HbA$_{1c}$ values between 8% and 10%. A proportion of those at 10% probably found it difficult to achieve a lower HbA$_{1c}$ because they were high glycators and so had HbA$_{1c}$ values that were more pessimistic than their MPG values. By comparison, more of those starting at 8% were probably able to achieve the lower HbA$_{1c}$ because they were low glycators. Conventionally treated patients continued at these same HbA$_{1c}$ levels throughout the study but if the HbA$_{1c}$ was to improve by 2%, as happened with intensive treatment, then the larger proportion of high glycators who started at 10% would remain being high glycators but would now have the same HbA$_{1c}$ (8%) as the conventionally treated population, with more low glycators at this HbA$_{1c}$. This means that high glycators who improve their glycemic control end up being compared with more low glycators, so at every chosen HbA$_{1c}$ level they would, on average, have the lower MPG that this study has shown.

An alternative explanation posits that a component of DCCT measured HbA$_{1c}$ not only unrelated to glycation but also variable in concentration between individuals. The term high glycator would then be a misnomer, because these individuals would simply have more of this component than a low glycator. Certainly, it is accepted that the original DCCT assay is not entirely specific for HbA$_{1c}$ because HbA$_{1c}$ values are 1.5% to 2% lower using the standardized IFCC method (19). What is not so clear is whether this difference in HbA$_{1c}$ values between the 2 assays is constant between individuals, despite there being a good overall correlation between the 2 (19). If this non-HbA$_{1c}$ component was indeed the cause for our findings, it might be expected that future studies will show that MPG relates more closely to the IFCC standardized HbA$_{1c}$ method but not necessarily to any routine (possibly less specific) HbA$_{1c}$ method calibrated from it.

A previous analysis of the DCCT data to establish the relationship between MPG and HbA$_{1c}$ combined data from both treatment groups into 1 (6). In so doing, as shown in Fig. 1, this may have overestimated the slope of the regression equation, i.e., the increase in MPG for a given increase in HbA$_{1c}$. In our analysis, the slope showed a 1.23 mmol/L increase in plasma glucose for every 1% increase in HbA$_{1c}$ in the intensively treated population, which was significantly different from the 1.54 mmol/L increase in the conventionally treated group, which in turn was significantly lower than the 1.87 mmol/L increase when the 2 groups were combined.

These findings have several clinical implications, the first of which relates to our current interpretation of the DCCT itself. In that trial, the major problem associated with intensive treatment was the greater risk of hypoglycemia compared with conventionally treated patients with the same HbA$_{1c}$ (10). In showing that intensively treated patients had consistently lower MPG than conventionally treated ones at the same HbA$_{1c}$ (equivalent to an HbA$_{1c}$ difference of 0.75% to 1.5%), this study is likely to have at least partly explained the discrepancy in risk between the patient groups.

Intensive treatment was also associated with an unexplained decrease in the rate of retinopathy progression at the same HbA$_{1c}$ value (11). For example, an intensively treated patient with 9% HbA$_{1c}$ had an event rate similar to that of a conventionally treated patient at 8% HbA$_{1c}$. Increased glucose variability in the latter group has been postulated as the cause (20), although a recent analysis of DCCT data seems to discount this as a possibility (21). Again, however, the lower MPG in intensively treated patients at the same HbA$_{1c}$ provides a simple alternative explanation.

Our data analysis could also be of relevance in the current debate on how to report standardized IFCC HbA$_{1c}$ values. Some advocate continuing to report IFCC HbA$_{1c}$ using existing DCCT numbers; others advocate expressing HbA$_{1c}$ as an average blood glucose equivalent (7, 8). The latter is seen as a preferred option (9), but our study suggests doubt about what constitutes the average blood glucose for any given HbA$_{1c}$ value, given the fact that this can change substantially between (and even within) groups of patients. Researchers in the ongoing prospective study organized by the American Diabetes Association/European Association for the Study of Diabetes/International Diabetes Federation Working Group for the HbA$_{1c}$ Assay to further clarify the relationship between MPG and HbA$_{1c}$ (Mean Blood Glucose Study) (9) need to be aware that the overall glycemia of patients chosen for the study could have a major influence on the outcome.

Last, our findings may help physicians understand why many intensively managed patients seem to have broadly acceptable self-monitored glucose records but comparatively high HbA$_{1c}$ results. Rather than questioning the monitoring technique (or honesty) of their patients, the MPG values in Table 1 show that in intensively treated patients the increase in mean glucose (1.23 mmol/L) per percentage HbA$_{1c}$ is not nearly as large as has been previously assumed. Thus, many may be close to their glucose target, although they appear some way from their HbA$_{1c}$ goal.

The DCCT dataset is not without its limitations. Although more than 163 000 laboratory-measured glucose samples were used in this analysis, newer techniques such as a continuous glucose monitoring system could give a clearer indication of glycemia, especially overnight. However, it is reassuring that when glucose samples were
collected at 3:00 AM they showed that the 7-point MPG was just 0.06 mmol/L different from the 8-point MPG. In fact, the differences between 7- and 8-point measurement in the 2 DCCT treatment groups indicated that our findings would be further reinforced if an overnight sample had been collected on every occasion. It is also reassuring that preliminary data from the Mean Blood Glucose Study have demonstrated a remarkably good correlation \((r = 0.94)\) between continuous glucose monitoring system and 8-point AUCs (22).

In summary, this study has shown that the relationship between MPG and HbA1c can differ substantially depending on the glycemic control of the population studied. The lower mean glucose at any given HbA1c value in intensively treated DCCT patients may go some way toward explaining why they appeared more prone to hypoglycemia and less likely to develop microvascular complications when HbA1c was used as the sole marker of glycemia. Researchers investigating the feasibility of expressing HbA1c as an MPG equivalent need to be aware of this finding, as do healthcare staff who are currently relating MPG to HbA1c in their clinic patients.

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References