HbA1c variability and the risk of microvascular complications in type 1 diabetes:
data from the DCCT

Eric S. Kilpatrick a, Honorary Professor in Clinical Biochemistry,
Alan S. Rigby b, Senior Lecturer in Statistics and Cardiovascular Epidemiology and
Stephen L. Atkin c, Professor of Endocrinology and Metabolism

a Department of Clinical Biochemistry, Hull Royal Infirmary, Hull,
b Academic Department of Cardiology, University of Hull, Hull and
 c Department of Diabetes, Hull York Medical School, Hull, UK

Correspondence to:
Prof. Eric S. Kilpatrick
E-mail Eric.Kilpatrick@hey.nhs.uk

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Objective—There remains debate as to whether short or long-term glycemic instability confers a risk of microvascular complications which is in addition to that predicted by mean glycemia alone. This study has analyzed data from the Diabetes Control and Complications Trial (DCCT) to assess the effect of HbA1c variability on the risk of retinopathy and nephropathy in patients with type 1 diabetes.

Research Design and Methods—HbA1c was collected quarterly during the DCCT in 1441 individuals. The mean HbA1c and the standard deviation (SD) of HbA1c variability following stabilization of glycemia (from 6 months onwards) were compared with the risk of retinopathy and nephropathy having adjusted for age, gender, disease duration, treatment group, and baseline HbA1c.

Results—Multivariate Cox regression showed that variability in HbA1c added to mean HbA1c in predicting the risk of development or progression in both retinopathy (HR=2.26 for every 1% increase in HbA1c SD, 95% CI 1.63-3.14, p<0.0001) and nephropathy (HR=1.80, 95% CI 1.37-2.42, p<0.0001), with the relationship especially a feature in conventionally treated patients.

Conclusions—This study has shown that variability in HbA1c adds to the mean value in predicting microvascular complications in type 1 diabetes. Thus, in contrast to analyses of DCCT data investigating the effect of short-term glucose instability on complication risk, longer term fluctuations in glycemia seem to be contributory to the development of retinopathy and nephropathy in type 1 diabetes.

Abbreviations: HbA1c, haemoglobin A1c; DCCT, Diabetes Control and Complications Trial
The effect that glycemic variability may have on the risk of developing the microvascular complications of diabetes remains controversial (1). Studies such as the Diabetes Control and Complications Trial (DCCT) in type 1 and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes have left little doubt that the risk of microvascular complications rises exponentially as the mean blood glucose (assessed using HbA1c) increases (2-5). However, with regards to glycemic variability, the clinical evidence has not been consistent. For example, in the DCCT the rate of complications at a given value of HbA1c was apparently higher in the conventionally treated patients than in those treated intensively (3), leading to the suggestion that this may be a consequence of larger glycemic excursions in the former group of patients since they were on fewer injections of insulin per day (6). Nonetheless, further analyses of the DCCT dataset has shown that within-day glucose variability, using 7 point laboratory measured glucose profiles, had no additional influence on the micro- or macrovascular complication risk beyond that predicted by the mean glucose value alone (7-9). A more recent reanalysis of the HbA1c data by the DCCT group has found that the original differences between treatment groups was probably an artefact of model assumptions originally used and that no discrepancies in microvascular risk at the same HbA1c actually existed (10). Indeed, it has subsequently been suggested that the increased complication risk in conventionally treated patients was simply because their blood glucose values were higher compared to intensively treated patients at the same HbA1c (11).

It is also currently unknown whether short term (within-day) variability may have a different influence on complications compared to longer term (day-to-day or week-to-week) glucose fluctuations. Certainly, data from the Pittsburgh Epidemiology Study found that HbA1c variability seemed to be an additional risk factor for the development of macrovascular complications (12).

It is fundamental to managing diabetes to know whether a patient with glucose instability could be at higher risk of microvascular complications than one without, especially since many of the recent pharmacological advances have aimed to reduce glucose variability (as well as mean glucose) by largely targeting post prandial hyperglycemia. This current study has therefore analysed the publicly available DCCT dataset in order to investigate the potential for HbA1c variability to have an influence on microvascular complications.

RESEARCH DESIGN AND METHODS

The datasets—We used the publicly accessible datasets collected by the DCCT which was stored in SAS format (www.gcrc.umn.edu). The DCCT was a nine-year follow-up study of 1441 participants with Type 1 diabetes comparing the effect of intensive versus conventional blood glucose management on the development of the microvascular complications of diabetes. At randomisation patients were stratified into one of two cohorts. The primary prevention cohort (n=726) had no evidence of retinopathy by fundus photography and a urinary albumin excretion rate (AER) <40 mg/24 hr (28µg/min). The secondary prevention cohort (n=715) had only minimal retinopathy and a AER <200 mg/24 hr (140µg/min). The study participants were randomised into intensive (n=711) and conventional (n=730) treatment groups.

Definition of events—Severity of retinopathy was determined by the 25-point Early Diabetic Retinopathy Treatment Study (EDRTS) interim score (2). The development
and progression of sustained retinopathy was defined as a change from baseline of three or more units on the EDRTS score on any two successive annual evaluations. During the nine-years of follow-up 242 people developed sustained retinopathy, 67 of whom were in the intensive treatment group. Nephropathy was defined as an increase in albumin excretion rate (AER) $\geq$40 mg/24hr (28µg/min) on any annual evaluation providing that the baseline AER was $<$40 mg/dl (28µg/min). The mean age was 27 years (range 13-39 years). Just over half (n=761, 52.8%) were men. Average body mass index (BMI) was 23.4 kg/m$^2$; less than 2% had a BMI $>$30 kg/m$^2$. Nearly all participants were Caucasian. The median disease duration was four years. Approximately one-fifth declared themselves as current smokers.

**HbA1c measurement and statistical methods**—HbA$_{1c}$ was measured quarterly in both treatment groups. This analysis has only used data from 6 months into the trial because the intensively treated group were undergoing a period of rapidly changing glycemic control prior to reaching an HbA$_{1c}$ nadir at 6 months. The relationship between HbA$_{1c}$ and the development of diabetes complication was assessed by Cox regression from which hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. The Cox regression model is semi-parametric in the sense that no assumption concerning event-free survival times is necessary. The Cox regression model is based on the assumption that the effect of a risk factor, expressed as a hazard ratio, is constant over time. The assumption of proportionality of the Cox model covariates was tested by plotting Schoenfeld residuals. All Cox regression models were adjusted for the following baseline covariates: age (years), gender, disease duration (years), randomization treatment (conventional versus intensive), prevention cohort (primary versus secondary) and HbA$_{1c}$ at the study eligibility stage.

Three models were constructed. The first looked at the influence of solely the updated mean HbA$_{1c}$, from 6 months onwards, on the risk of subsequent microvascular complications while the second model also included the variability in HbA$_{1c}$ throughout the period expressed as standard deviations (SD) of HbA$_{1c}$ over all visits. As the number of visits an individual patient had could also influence this SD (such that few visits would make the SD apparently greater than many visits), the SD value was also divided by $\sqrt{(n/(n-1))}$ to adjust for this. The third model expressed HbA$_{1c}$ variability as an updated time-dependent SD (3).

Patients in the conventionally treated group who became pregnant were unblinded to their HbA$_{1c}$ values and treated with a goal of near-normal glycemia, before returning to the conventional treatment protocol after delivery (13). To ensure our results were not influenced by these 86 individuals, a separate analysis was made which excluded these patients.

There has been considerable interest in calculating coefficients of determination ($r^2$) in models other than for least squares regression. Many researchers have looked at developing an $r^2$ equivalent for the Cox proportional hazards model. However, the difficulty lies in how to take into account censoring. Since there is no consensus on how best to measure $r^2$ for the Cox model (14) we have not pursued it here.

The GLIM4 and SPSS statistical computer packages were used to analyse the data. An arbitrary level of 5% statistical significance (two-tailed) was assumed.

**RESULTS**

Hemoglobin A$_{1c}$ variability was greater in the conventionally treated patients than those treated intensively (HbA$_{1c}$ SD 0.86 vs. 0.59). Variability was also closely related to the mean HbA$_{1c}$ of all patients (r=0.55), being especially so in intensively treated patients.
compared to conventionally treated ones ($r=0.71$ vs. $r=0.32$).

Table 1 shows the relationship between the updated mean HbA1c and both retinopathy and nephropathy risk in the intensively, conventionally and both groups combined (Model 1). Models 2 and 3 show the effect of including HbA1c variability (either as the SD over the entire study duration or as an updated SD) within the same model. In each case where mean HbA1c was initially predictive of complications, HbA1c SD either added to (or explained) the risk indicated by the mean value of HbA1c alone. Expressing SD after dividing by $\sqrt{n/(n-1)}$ made no difference to the effect of HbA1c variability on complication risk. In addition, limiting analysis to only those conventionally treated patients who had not become pregnant during the DCCT did not alter the findings. For example, in Model 2, the hazard ratio for SD amongst non-pregnant conventionally treated patients was 2.2 (95% CI 1.4-3.4).

Figure 1a shows the relative risk of retinopathy progression using both treatment groups combined in Model 2, Table 2 when applied to DCCT patients over the range of patients from the zero to 97.5th centile of mean HbA1c after adjusting for HbA1c SD, using the 2.5th centile as a reference. Figure 1b shows the same patients over the same range of HbA1c SD after adjusting for mean HbA1c.

**DISCUSSION**

This analysis of the DCCT data has shown that in patients with type 1 diabetes increasing variability in HbA1c adds to the risk of microvascular complications over and above that predicted by the mean HbA1c value alone. This finding was present in the DCCT cohort overall and was also a feature of both treatment groups individually where the mean HbA1c alone was initially predictive. The effect was most pronounced amongst those patients who were in the conventionally treated group, presumably because the event rate, the range of variability and the spread of variability at any given mean HbA1c was much larger than in the intensively treated patients.

The magnitude of effect of HbA1c variability is marked, such that a 1% absolute increase in HbA1c SD results in at least a doubling in retinopathy and an 80% increase in nephropathy risk using either of our models. As shown in Figure 1b, put into the context of individuals participating in the DCCT, it means that a patient on the 97.5th centile of HbA1c variability (SD 1.87%) has over three times the retinopathy risk and over twice the nephropathy risk of a patient on the 2.5th centile (SD 0.25%) for a given HbA1c.

The findings are in contrast to a previous analysis of the DCCT data which suggested little effect of HbA1c variability on complication risk (3), but will likely have included all patient visits, including those before HbA1c stabilized at 6 months in intensively treated patients.

There are several possible reasons for the current findings. One is that medium to long term fluctuation in blood glucose truly is an additional risk for the development of microvascular complications. This is in contrast to within-day glucose variability of the same DCCT patients which showed little influence on retinopathy or nephropathy risk (7; 8). This latter finding was surprising because numerous studies have shown that short-term glycemic excursions lead to an overproduction of reactive oxygen species in cell cultures (15) as well as in patients with type 2 (16) (though not confirmed in type 1 (17)) diabetes. There is also data on the effect of longer term changes in glycemia on free radical production (18) as well as both clinical and laboratory evidence that periods of sustained hyperglycemia are ‘remembered’ and so place patients at higher subsequent long-term risk of complications (19; 20). In this regard, the detrimental effect of HbA1c
variability may be mediated through the same mechanism underlying that of the ‘metabolic memory’ phenomenon (20; 21).

An alternative possible explanation for our data relates to the fact that microvascular complication risk appears to rise exponentially, rather than linearly, as HbA1c rises (2; 3). Thus, although a patient who has a more variable HbA1c will be spending the same time above and below their mean value as another with comparatively stable HbA1c, their average risk will be higher because their periods of sustained glycemia far above their mean will be placing them at especially high complication risk. This will more than cancel out any reduction in risk caused by them also having equal periods far below their mean. If true, it would be expected that this effect would be exaggerated the higher the individual’s mean HbA1c, which may be one reason why the influence of HbA1c variability seems greater in conventionally treated patients.

Another possible reason linking HbA1c fluctuations with complication risk is the consistent observation that improving glycemic control can lead to a short-term worsening in retinopathy (22,23) before subsequently resulting in a net long-term improvement (23): the ‘normoglycemic re-entry phenomenon’. Indeed, the potential for early retinopathy worsening was one of the main concerns of improving glucose control when the DCCT was conceived. The mechanism for this paradoxical deterioration is not fully known but is thought to involve the changes in ocular blood flow and increased IGF-1 concentrations (24) consequent on improved glycemic control. Whatever the cause, the cyclical improvements in glycemic control found in patients with more variable HbA1c could result in a cyclical worsening of retinopathy which is in addition to that predicted by the mean HbA1c value alone. This excess risk, of course, could also be compounded by the intervening periods of worsening control. Counting against this hypothesis is the fact that the early worsening of nephropathy after glycemic improvement is not well recognised (with the possible exception of DCCT patients who became pregnant (13)), although this may be more a reflection of retinal changes becoming apparent before those of urinary albumin.

These explanations may go someway towards reconciling why short term (within day) glucose variability in the same patients has been found to have little effect on microvascular risk (7; 8) whereas longer term instability in the form of HbA1c has been found here to be much more predictive. Alternatively, it is possible that HbA1c is more sensitive at detecting the effect of glucose changes than daily glucose profiles, although the same DCCT data has shown it is the mean glucose, rather than glucose variability, that has the major influence on the HbA1c value (25).

There do not seem to be many limitations in this study related to the size and completeness of the dataset, which is a reflection of the rigorous protocol of the DCCT and of the dedication of the patients involved. In sum total the 1,441 individuals had 31,260 HbA1c measurements performed from 6 months into the study on which to base the conclusions. It must also not be forgotten that the study benefited by being performed before other potential confounding factors such as antihypertensives and lipid lowering agents came in to common use. Nevertheless, in interpreting this data, it should also be remembered that HbA1c in the DCCT as a whole explained no more than 23% of the risk of retinopathy progression for the entire cohort, with the risk reduction associated with intensive rather than conventional HbA1c values being just a fraction of this percentage (3).

In summary, this study has shown that longer term fluctuations in glycemia seem to
independently relate to the development of retinopathy and nephropathy in type 1 diabetes. Thus, sole measurement of mean glucose or mean HbA1c may not be the best predictor of complication risk.
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### Table 1

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<td>1.70 (1.14-2.52)</td>
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Influence of updated mean HbA1c alone (model 1), inclusive of mean HbA1c SD (model 2) or updated mean HbA1c SD (model 3) on complication risk expressed as hazard ration (HR) with 95% confidence intervals. Models adjusting for age, sex, duration, intervention group (when groups combined), HbA1c at eligibility, HbA1c time-dependent, BG time-dependent. Models from 6-months onwards.
Figure 1
Relative risk of retinopathy progression over the range of patients from the zero to 97.5th centile of mean HbA1c after adjusting for HbA1cSD, using the 2.5th centile as a reference (Figure 1a) and for HbA1cSD after adjusting for mean HbA1c (Figure 1b).