Articles

The relation of glycaemia to the risk of development and progression of retinopathy in the Diabetic Control and Complications Trial

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Abstract

Aims/hypothesis. We have assessed the relation between the quarterly capillary glucose profile and the risk of the development and progression of retinopathy in the DCCT.

Methods. Seven point (preprandial and 90-min postprandial for each meal and bedtime) capillary glucose data were analysed from volunteers whose collections were complete in 80 %, or more, of quarterly periods and who were in the study longer than 4 years ($n = 296$, conventional therapy; $n = 269$, intensive therapy). The study cohort differed from excluded patients in having more women and lower HbA_{1c} at baseline and fewer adolescents, older age and lower baseline mean blood glucose in the intensive therapy group.

Results. Univariate analysis showed significant ($p < 0.01$) associations to sustained 3-step change in retinopathy of each updated glycaemic parameter: mean blood glucose, mean preprandial glucose, mean postprandial glucose, each preprandial, postprandial and bedtime glucose; range glucose, stan-

dard deviation glucose; M-value of Schlichtkrull and mean amplitude of glycaemic excursions, albeit with relatively small hazard ratios. Multivariate analyses showed updated mean blood glucose to be the primary risk factor ($p < 0.001$) with a weak contribution of mean amplitude of glycaemic excursions at baseline ($p < 0.005$); no other variables added significantly to the model. The association between updated mean blood glucose and risk for retinopathy was nonlinear: risk progressively increased above updated mean blood glucose of 8.3 mmol/l. A gradient of risk could not be determined below this level because events were few.

Conclusion/interpretation. Within the limitations provided by quarterly 7-point capillary glucose measurements as an expression of overall glycaemic behaviour, the major risk for progression of retinopathy is conveyed by updated mean blood glucose especially above 8.3 mmol/l. [Diabetologia (2001) 44: 1215–1220]

Keywords DCCT, glycaemia, retinopathy, HbA_{1c}, mean blood glucose.

The achievement of near-normal blood glucose concentrations from intensified therapy has been shown to retard the development and progression of the mi-

crovascular complications of Type I (insulin-dependent) and Type II (non-insulin-dependent) diabetes mellitus (DCCT [1] and the Kumamoto study [2] UKPDS [3] respectively). Because of its unique characteristic as an integrated measure of glycaemic exposure, HbA_{1c} was the primary parameter studied in relation to the microvascular complications in all three clinical trials [1–3]. Additional analyses of other potential confounding factors on the relation between HbA_{1c} and microvascular complications were conducted by the DCCT [4], but none involving the 7-point capillary glucose profiles collected quarterly

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Abbreviations: MBG, Mean blood glucose; MAGE, mean amplitude of glycaemic excursions

Table 1. Demographic features at baseline of patients with complete vs incomplete capillary glucose data

	Conventional therapy			Intensive therapy		
	Complete	Incomplete	<i>p</i> -value	Complete	Incomplete	<i>p</i> -value ^b
<i>n</i>	296	434		269	442	
Age (years)	27 ± 7	27 ± 7	0.86	29 ± 7	26 ± 7	0.0001
Adolescent (13–18 years)	47	71	0.86	22	85	0.001
Male	138	257	0.001	122	244	.011
Female	158	177		147	198	
Duration of Type I (months) diabetes	69	64	0.20	76	66	0.014
Pregnant ^d	44	50	0.19	40	58	0.51
Primary ^a	148	230	.43	124	224	.24
Secondary ^a	148	204		145	218	
HbA _{1c} (%)	8.7	9.0	0.04	8.7	9.0	0.006
Mean blood glucose (mmol/l)	13.0	12.7	0.24	12.1	13.4	0.002
Retinal status of secondary cohort						
mild NPDR ^c	134	180	0.552	131	200	0.427
moderate NPDR	14	24		18	18	

Complete = 7-point capillary glucose collection was complete in ≥ 80 % of quarterly periods and duration in study ≥ 4 years. These patients were entered into study. Patients with incomplete data were excluded

^a Primary cohort had 1–5 years duration Type I diabetes at baseline, no retinopathy on fundus photography and albuminuria < 40 mg/24 hours; secondary cohort had 1–15 years dura-

tion Type I diabetes at baseline, very mild to moderate nonproliferative retinopathy and albuminuria < 200 mg/24 h

^b *p*-value = comparison of complete vs incomplete

^c NPDR, nonproliferative diabetic retinopathy

^d Number of patients pregnant on one or more occasions during the study

by DCCT volunteers. The purpose of this study was to assess the associations among the components of the 7-point capillary glucose profiles in the DCCT and measures of glucose variability generated from those profiles with the risk for a sustained 3-step change in retinopathy, data for which are in the public domain.

Subjects and methods

Subjects. Each volunteer enrolled in the DCCT was expected to collect at quarterly intervals, a 7-point set of capillary specimens preprandially and 90 min postprandially for each of the three major meals and before the bedtime snack using the Profiset (Boehringer-Mannheim Diagnostics, Indianapolis, Ind., USA) for glucose analyses in the central laboratory [5]. Among the 1441 volunteers with Type I diabetes enrolled in the DCCT, only those whose 7-point capillary glucose profiles were complete in 80 %, or more, of quarterly collections and who were in the study for 4 years, or longer, qualified for this study. Using these criteria, 565 volunteers (296 assigned to conventional therapy and 269 assigned to intensive therapy) were eligible (Table 1). They differed from excluded volunteers by gender (*F* > *M*) and HbA_{1c} (lower) in both treatment groups and by age (older), adolescents (fewer), MBG (lower) and duration of diabetes (longer) in the intensive therapy group.

Analysis. The seven capillary specimens were refrigerated until they were brought to the clinical centres where they were frozen and then shipped on dry ice to the Central Biochemical Laboratory for analysis by a hexokinase method [6]. Quality assurance was provided by measuring optical density of each specimen to assess the volume of blood in the transport medi-

um. Quality control was expressed as CV and coefficient of reliability CR. Over the total duration of the study, the CV and CR for the total cohort of capillary glucose measurement were 8.5 % and 0.919 and of optical density were 4.16 % and 0.793, respectively.

Glycaemic Parameters. Using the 7-point capillary glucose profile, the following parameters were generated: (1) mean blood glucose (MBG): average of the 7 glucose values; (2) mean postprandial glucose: average of the 90 min post breakfast, lunch and supper glucose values; (3) mean preprandial glucose: average of the glucose values before breakfast, lunch and supper; (4) range of glucose: maximum minus minimum glucose values in the 7-point collection; (5) standard deviation of glucose: standard deviation of the 7-point glucose values;

(6) M-value [7]:

$$M = \frac{\sum}{N} \left| 10 \log \frac{B_6}{90} \right|^3 + W/20$$

This parameter is a logarithmic transformation of the deviation of glycaemia from an arbitrarily selected value (120 mg/dl in the original description; modified to 90 [8] to reflect true mean glycaemia in non-diabetic subjects) plus an amplitude correction factor ($W/20 = \text{maximum} - \text{minimum glucose in mg/dl} \div 20$); (7) mean amplitude of glycaemic excursion (MAGE) [9]: arithmetic mean of glucose nadir to peak (or vice versa) differences when each of these differences is greater than or equal to one standard deviation; (8) individual time points in the 7-point glucose collection: before breakfast, after breakfast, before lunch, after lunch, before supper, after supper and at bedtime.

Statistical analysis. Comparisons between groups were done using rank sum tests. Analyses which identified risk factors for the development of a 3-step change in retinopa-

thy used Cox regression analysis. Three versions of each glucose variable were considered for analysis, the value at baseline and two time dependent variables. The time dependent variables were continuously updated during follow-up. At each time (t) during follow-up, we computed the variable at t (current value) and the mean from baseline to t (updated mean).

The glucose risk factors tended to be those which measured the concentration of glucose exposure and those which measured the variability in glucose exposure. Because they were highly correlated, there was a need for a multivariate analysis to identify a subset of the most important risk factors. Multivariate assessment of risk factors was made using stepwise Cox regression analysis, stepping up, with $p < 0.05$ as the requirement for inclusion in the model. Standard SAS programs for fitting the Cox model were used. Polynomial regression models were used to assess departures from linear association. This approach, however, did not adequately describe the association between updated mean blood glucose (MBG) and the risk of retinopathy and was replaced by a cubic spline model with a join point at 8.3 mmol/l. Stepping down was done successively deleting the highest order terms until all remaining terms were significant at the 0.05 p value. Modelling of cubic splines was accomplished by using two sets of independent variables. The first was the cubic regression model coded in the usual way without consideration of a cutpoint. The second was a cubic regression model coding values as 0 when they took on values below the cutpoint and the actual value minus the cutpoint for values above the cutpoint. Cubic splines were found necessary only for the updated MBG. Log transformed HbA_{1c} was added to the final model and non-significant terms were successively deleted until all remaining terms were significant at a p value of 0.05.

Results

Within the study cohort the intensive treatment group had significantly ($p < 0.001$) lower values of each glycaemic parameter (mean blood glucose; mean postprandial blood glucose; mean preprandial blood glucose; range blood glucose; standard deviation blood glucose; MAGE; M-value; blood glucose before breakfast, 90 min after breakfast, before lunch, 90 min after lunch, before supper, 90 min after supper and at bedtime) and HbA_{1c} ($p < 0.001$) than the conventional treatment group during the total period of the study (Table 2).

Analyses of the associations between glycaemic variables and risk for sustained 3-step change in retinopathy were carried out for the conventional and intensive therapy groups combined since there were so few events in the intensive therapy group. In the univariate analysis, significant ($p < 0.01$) hazard ratios were noted for almost all glycaemic parameters for all three versions: baseline value, current value, and updated mean (see Methods) (Table 3). However, the magnitude of these associations was modest with the strongest associations found for the updated means during follow-up. The increase in risk per 10 unit change was less than 15% for each variable except standard deviation (25%).

Table 2. Glycaemic parameters for study cohort during DCCT

	Conventional therapy	Intensive therapy ^a
<i>n</i>	296	269
Mean blood glucose, mmol/l	13.0 ± 2.5	8.4 ± 1.2
Mean postprandial blood glucose, mmol/l	14.4 ± 2.7	9.4 ± 1.4
Mean preprandial blood glucose, mmol/l	11.7 ± 2.4	7.7 ± 1.3
Range blood glucose, mmol/l	12.2 ± 2.2	9.5 ± 1.7
Standard deviation blood glucose	4.4 ± 0.8	3.5 ± 0.7
M-value	111 ± 43	44 ± 17
MAGE, mmol/l	9.4 ± 1.8	7.4 ± 1.4
Before breakfast blood glucose, mmol/l	11.4 ± 2.5	8.3 ± 1.6
90 min after breakfast blood glucose, mmol/l	15.5 ± 3.1	10.8 ± 2.1
Before lunch blood glucose, mmol/l	11.0 ± 2.9	7.0 ± 1.5
90 min after lunch blood glucose, mmol/l	13.8 ± 2.8	8.6 ± 1.6
Before supper blood glucose, mmol/l	12.6 ± 3.1	7.7 ± 1.7
90 min after supper blood glucose, mmol/l	13.9 ± 3.4	8.8 ± 1.6
Bedtime blood glucose, mmol/l	12.6 ± 3.4	8.0 ± 1.6
HbA _{1c} (%)	9.0 ± 1.3	7.0 ± 0.7

^a $p \leq 0.001$ comparing intensive and conventional therapies for each glucose variable

In the multivariate analysis, the primary determinants for risk of a 3-step change in retinopathy were updated MBG ($p < 0.001$) and baseline MAGE ($p < 0.005$). No other variables added significantly to the final model. The association between updated MBG and risk for retinopathy was nonlinear (Fig. 1). No association with updated MBG was observed for values below 8.3 mmol/l. In order to gain further insights into the possibility of an association, we made additional analyses including only values of updated MBG of less than 8.3 mmol/l. We computed the difference between the updated MBG value for the person developing retinopathy and the mean among all the MBG values at that time point (restricted to updated MBG < 8.3 mmol/l). If risk increases with increasing MBG, one would expect these differences to be positive.

The results (Table 4) indicate that the mean difference (0.1 ± 0.7) did not differ significantly from 0 ($p = 0.321$). Nevertheless, despite the large numbers of subjects at each event time in the range of updated MBG of less than 8.3 mmol/l, there were only ten retinal events, so that the effective sample size is ten. Because of this small sample size, we cannot confidently conclude that no gradient of risk exists among updated MBG values less than 8.3 mmol/l. Beyond 8.3 mmol/l the risk increased with increasing updated MBG with approximately a 15-fold increase in risk at updated MBG of 16.6 mmol/l relative to updated

Fig. 1. The relation between updated MBG and risk of progression of retinopathy relative to updated MBG of 8.3 mmol/l was calculated by a cubic spline model. Although no difference was found at updated MBG < 8.3 mmol/l vs at 8.3 mmol/l, the power was insufficient to make this determination. Therefore, the rate in this range has not been plotted

MBG at 8.3 mmol/l. We checked the robustness of our findings with other join points with similar results. The association with MAGE was linear: for every 0.6 unit increase in MAGE, there was a 4% increase in risk for 3-step change in retinopathy. When HbA_{1c} was added to the model, updated MBG continued to be a significant risk factor ($p < 0.006$) but MAGE was not $p = 0.06$.

Our results show that an increase in updated MBG from 8.3 mmol/l to 11.1 mmol/l increases the risk by approximately fourfold (Fig. 1). By comparison, a 2.8 mmol/l increase in baseline MBG in the univariate analysis is estimated to increase risk by approximately 33%, thus illustrating the importance of a more comprehensive, and albeit more complicated, cumulative longitudinal assessment of exposure.

The updated mean HbA_{1c} was a highly significant risk factor $p < 0.001$ (Fig. 2). Furthermore, for the

Fig. 2. There is a curvilinear relation between risk for progression of retinopathy and percent increase in updated mean HbA_{1c} (Cox regression)

same updated mean HbA_{1c}, persons with a positive difference (baseline minus time in study) had a significantly ($p < 0.001$) increased risk for 3-step change in retinopathy.

Discussion

The relation between the risk of progression of retinopathy and measures of glycaemic exposure can be framed by several questions.

Are there components of the 7-point glucose profile or measurements of glucose variability derived from this profile which either supersede or complement the correlation between mean glycaemia and the risk for progression of retinopathy? Each glucose parameter of the 7-point profile and derivatives (e.g., mean postprandial glucose, mean preprandial glucose and measures of glucose variability; e.g., range, standard deviation, M-value and MAGE) were improved by intensive insulin therapy and correlated

Table 3. Univariate analysis. Hazard ratios of significant parameters per 10 unit change

Parameter	Baseline	At follow-up time	Updated mean during follow-up
Mean blood glucose	1.06	1.06	1.14
M-value	1.06	1.05	1.12
MAGE	1.04	NS	1.10
Range blood glucose	1.04	NS	1.09
Standard deviation blood glucose	1.14	NS	1.25
Mean postprandial blood glucose	1.05	1.05	1.12
Mean preprandial blood glucose	1.05	1.06	1.14
Before breakfast blood glucose	1.04	1.05	1.12
After breakfast blood glucose	1.02	1.03	1.10
Before lunch blood glucose	1.03	1.04	1.10
After lunch blood glucose	1.03	1.04	1.10
Before supper blood glucose	1.03	1.03	1.09
After supper blood glucose	1.03	1.04	1.11
Bedtime blood glucose	1.04	1.03	1.11

Table 4. Risk of retinopathy at updated MBG ≤ 8.3 mmol/l at time of event

Quarterly visit	Number of patients at risk	Updated MBG mmol/l individual	Updated MBG mmol/l cohort	SD cohort	Delta
2	198	7.9	6.9	1.0	0.9
2	198	7.3	6.9	1.0	0.3
2	198	7.7	6.9	1.0	0.8
6	179	6.6	7.0	0.9	-0.5
6	179	5.9	7.0	0.9	-1.1
10	175	7.5	7.2	0.7	0.3
12	175	8.0	7.3	0.7	0.8
20	143	7.2	7.4	0.6	-0.2
20	143	7.4	7.4	0.6	0
26	69	7.1	7.4	0.7	-0.2

Differences between updated MBG for each individual who sustained a retinopathic event and updated MBG for the cohort of patients with updated MBG < 8.3 mmol/l were

0.1 ± 0.6 , which was not different from zero ($p = 0.321$), thus failing to indicate a decline in rate of events below updated MBG < 8.3 mmol/l

significantly with the risk for progression of retinopathy. However, from the multivariate analysis, the primary determinant of risk for progression was the updated mean blood glucose. This finding indicates that there is no single nyctohemeral time point for glucose determination even when measured repeatedly, which will predict risk for progression of retinopathy better than an estimate of mean glycaemia such as that generated from a 7-point profile. Furthermore, mean postprandial glycaemia evinced hazard ratios similar to mean preprandial glucose and slightly less than mean blood glucose. This observation should not be interpreted as minimizing the importance of controlling postprandial glycaemia since postprandial glycaemia is an important contributor to mean blood glucose.

Is there a threshold of mean blood glucose for the risk of progression of retinopathy? The issue of a threshold of glycaemic exposure has generated controversy. This study which used Cox proportional hazard model and the original DCCT report [1] which used Poisson regression observed a curvilinear association between HbA_{1c} and the gradient in risk for progression of retinopathy. In response to a report of an HbA_{1c} threshold of 8% for the development of microalbuminuria in a retrospective analysis, [10] the DCCT found the gradient in risk to be similar above and below this HbA_{1c} concentration. [11] In the Kumamoto study claims for a threshold for HbA_{1c} of 6.5%, for fasting blood glucose of 6.1 mmol/l and for postprandial blood glucose of 10.0 mmol/l were made for risk of microvascular disease.

For updated mean blood glucose, the primary glycaemic determinant for risk of the progression of retinopathy the association was nonlinear: risk progressively increased above 8.3 mmol/l and no association was observed below this value. The latter could reflect the fact that only a small number ($n = 10$) of subjects with events had an updated mean blood glucose of less than 8.3 mmol/l at the time of the event.

Thus, our data are insufficient to determine if the risk for progression of retinopathy at an updated mean blood glucose of less than 8.3 mmol/l differs from the risk at 8.3 mmol/l. Therefore we cannot conclude that updated mean blood glucose has a threshold. It should be pointed out that retinopathic events, although few, did occur at an updated mean glucose of less than 8.3 mmol/l.

Do glucose measurements add any further information to the risk for retinopathy than does HbA_{1c} ? Our data indicate that updated mean blood glucose indeed does add to HbA_{1c} . Glucose measurements therefore serve not only as a guide to insulin adjustments, but provide an independent assessment of risk for retinopathy.

We interpret our findings within the context of the limitations of the study. Measurement of glucose at 7 time points in a 24 h period and conducting such determinations every 3 months, although done over 4 or more years, provide a limited assessment of glycaemic behaviour. Fewer than half of the original DCCT cohort qualified for glucose analyses because of incompleteness of capillary glucose profile collections. Those that did qualify were in better glycaemic control at baseline and were more likely to adhere to protocol requirements as attested to by their fidelity in providing the complete 7-point capillary glucose profiles. However, a check on the robustness of the final models using the larger data set from the original DCCT cohort provided similar results; specifically, each variable remained statistically significant, the sign for each coefficient was in the same direction and the magnitude was 2SD, or less, of the original coefficient.

The observation that the updated mean blood glucose was the primary glycaemic risk factor among many measures of glucose for the progression of retinopathy is not surprising and is consistent with the association noted for HbA_{1c} and retinopathy observed in this study and in the original DCCT report [1]. It cannot be concluded that an updated MBG of

8.3 mmol/l represents a threshold for the development of retinopathy. There is no question that the risk for retinopathy increases above this level. However, retinopathic events also occurred below this level; whether the rate declined or remained the same as at the concentration of 8.3 mmol/l could not be determined exactly.

In conclusion, this study confirms the relation between the glycaemic surrogate, HbA_{1c} and risk for retinopathy in showing a similar association between mean glycaemia and risk for retinopathy. Neither specific nyctohemeral time points nor parameters of glucose variability provide any additional risk assessment information beyond that provided by mean capillary glucose.

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