ORIGINAL ARTICLES – Clinical science

Strict metabolic control and retinal blood flow in diabetes mellitus

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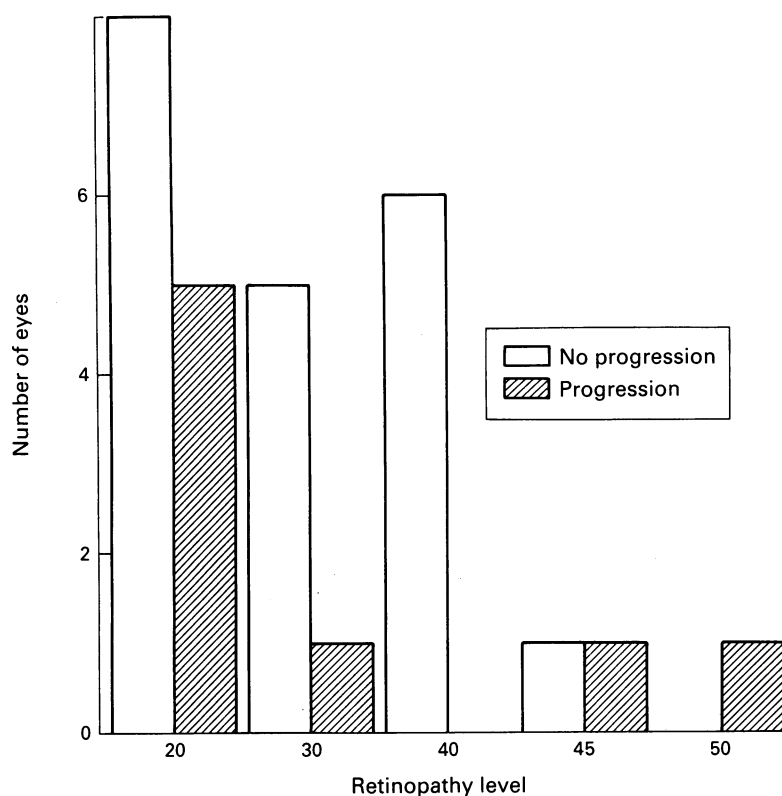19104, USA.

Accepted for publication
22 February 1994

Abstract

The effects of strict diabetic control on retinal haemodynamics were studied to elucidate whether such effects are associated with retinopathy changes. In 28 patients with poorly controlled insulin dependent diabetes mellitus and non-proliferative retinopathy, retinal haemodynamics were investigated at baseline, 5 days, 2 months, and 6 months after the institution of strict diabetic control using the bidirectional laser Doppler velocimetry technique and monochromatic fundus photography. Changes in retinal blood flow measured in a major retinal vein (Q) on the fifth day of strict diabetic control correlated significantly with changes in retinopathy level observed at the end of the 6 months of this study (rank correlation 0·65, p<0·01). On the fifth day of strict diabetic control, 16 out of 20 eyes that showed no progression (NP) of retinopathy at the end of the study had decreases in Q, whereas six out of eight eyes that showed progression (P) had increases in Q. The difference in these changes in Q between P and NP eyes was statistically significant (one way analysis of variance, p=0·001). No significant changes in Q were detected at 2 months or 6 months. Following the institution of strict diabetic control, no significant changes in time were detected in the regulatory response to 100% oxygen breathing characterised as the percentage decrease in Q at 4–6 minutes of oxygen breathing (analysis of variance, p=0·36). Changes in Q following institution of strict diabetic control are associated with progression of retinopathy. Measurements described in this study may help identify diabetic patients at risk of progression when their metabolic control is improved.

(Br J Ophthalmol 1994; 78: 598–604)

Progression of diabetic retinopathy is sometimes observed following the institution of strict diabetic glycaemic control.[1-7] The mechanism leading to progression of retinopathy under these circumstances is not clearly understood and probably is associated with haemodynamic changes in the retinal circulation.[8]

In an effort to investigate whether the institution of strict diabetic control has an effect on retinal circulatory parameters that could be associated with progression of retinopathy, we have performed a study in which a regimen of strict diabetic control was instituted and maintained for 6 months in a group of 28 poorly controlled patients with diabetes mellitus. Some of the preliminary results in the first 13 patients of this study have been published previously.[8]

Our current results show that patients whose retinopathy does not progress after the institution of strict diabetic control show decreases in retinal blood flow on the fifth day of strict diabetic control, whereas patients in whom retinopathy progresses have significantly different flow changes.

These results, suggesting that early retinal blood flow changes may help identify patients at risk of retinopathy progression following the institution of strict diabetic control, are of particular relevance following the recent publication of the results of Diabetes Control and Complications Trial (DCCT) showing that strict metabolic control is associated with less progression of retinopathy.[7]

Materials and methods

Twenty eight patients with insulin dependent diabetes mellitus whose age ranged between 18 and 43 years (mean (SD) 31 (6) years) were included in this study (Table 1). Duration of diagnosed diabetes ranged between 5 and 28 years (mean 16 (7) years). Other patient characteristics are summarised in Table 1.

Before the beginning of the study, all patients had glycosylated haemoglobin (GHb) values >3 SD above the mean of non-diabetic subjects. Average GHb measured by affinity chromatography at the beginning of the study was 12·3% (2·2%) (upper limit of the normal range 8%). All patients had non-proliferative diabetic retinopathy (Fig 1).

Excluded from the study were patients who had (1) previous treatment with three or more daily injections of insulin or an insulin pump, (2) insulin resistance requiring a total of >2 U/kg

Table 1 Patient characteristics

Characteristics	Mean (SD)	Range
Age (years)	31 (6)	18–43
Disease duration (years)	16 (7)	5–28
Glycosylated haemoglobin (%)	12·3 (2·2)	9–16·7
Mean brachial pressure (mm Hg)	87 (10)	69–107
Intraocular pressure (mm Hg)	16 (3)	8–29
Perfusion pressure (mm Hg)	42 (7)	30–57

Figure 1 Distribution of baseline retinopathy level according to the Early Treatment of Diabetic Retinopathy Study[12] grading in eyes that showed progression or no progression of retinopathy at the end of our 6 month study.

body weight, (3) three or more documented episodes of diabetic ketoacidosis requiring hospitalisation, (4) history of systemic hypertension, (5) history of substance abuse, (6) obesity defined as body weight >130% of ideal body weight, (7) chronic disease requiring prescription medication that may be changed or discontinued during the study, (8) severe and recurrent hypoglycaemia, (9) topical ocular medications, (10) presence of intraocular pathology other than diabetic retinopathy, or (11) previous laser photocoagulation treatment.

All eyes studied had a best refracted visual acuity of 6/7·5 or better, and intraocular pressure equal to or smaller than 21.mm Hg, and a normal slit-lamp examination. After a detailed explanation of the study protocol, all subjects were asked to sign an appropriate consent form approved by the internal review board of our institution. This research followed the tenets of the Declaration of Helsinki.

BIDIRECTIONAL LASER DOPPLER VELOCIMETRY MEASUREMENTS

Pupils were dilated with a 1% solution of tropicamide and a 10% solution of phenylephrine hydrochloride. About 30 minutes later, a polaroid colour fundus photograph of the posterior fundus was obtained for localisation of the sites of bidirectional laser Doppler velocimetry (BLDV) measurements. BLDV measurements of the maximum or centreline erythrocyte velocity (V_{max}) were obtained in main superior and inferior temporal retinal veins. The location of the measurement site was marked on the Polaroid photograph for later reference. We chose to perform flow measurements from veins because the minimal flow pulsatility in these vessels permits a more accurate determination

of the average velocity.[9] Blood glucose was measured with an Accu-Check blood glucose monitor (Boehringer Mannheim, IN, USA) from finger capillary samples immediately after the BLDV measurements.

Fundus photographs were taken in monochromatic light at 570 nm. Intraocular pressure was measured by applanation tonometry, and brachial artery blood pressure was obtained by sphygmomanometry.

Volumetric blood flow rate (Q) was calculated as described previously[9][10] as

$$Q = V_{mean}\ \pi\ D^2/4$$

where mean blood velocity (V_{mean}) was calculated as $V_{mean} = CV_{max}$. The constant C equal to 1/1·6 was used, and the relation between V_{max} and V_{mean} was assumed to remain unchanged during the study. D represents the venous diameter at the site of BLDV measurement determined from projected photographic negatives. D corresponds to an average of the measurements obtained from six photographs.

All measurements of D were performed by one trained examiner, and all V_{max} determinations were done by another examiner. Each examiner was masked with regard to the results of the other examiner, status of diabetic control, and status of diabetic retinopathy.

During each session, BLDV measurements and fundus photographs were obtained during room air breathing and 100% oxygen breathing. The regulatory response to 100% oxygen breathing (R_Q), characterised as the percentage change in Q at 4–6 minutes of oxygen breathing, was calculated using the formula

$$R_Q = 100\%\ (Q_{air} - Q_{o_2})/Q_{air}$$

Only one eye, chosen at random at the beginning of the study, was investigated. All retinal blood flow measurements were done on the same eye.

STUDY PROTOCOL

Patients who met our inclusionary criteria had a first session of baseline blood flow measurement. A second baseline blood flow measurement was performed 3–4 weeks later. Immediately after this measurement, patients were hospitalised, and a regimen of strict diabetic control of blood glucose similar to that used in the DCCT[7][11] was instituted under careful monitoring. Patients were asked to monitor blood glucose levels (finger capillary blood samples determined by an Accu-Check blood glucose monitor) four times daily before their meals and at bedtime. A fifth blood glucose check was performed at 3 am on selected days to safeguard against nocturnal hypoglycaemia. Patients self administered four daily insulin injections with the goal of achieving blood glucose levels within the following guidelines: preprandial, 3·9–6·7 mmol/litre; 2 h postprandial, <8·4 mmol/litre. Regular insulin was administered before meals and at bedtime. NPH insulin was also given at bedtime in an adequate dose to keep blood glucose within the normal range overnight. Patients met several times with a diabetes dietitian who adjusted their diet and provided in depth dietary education.

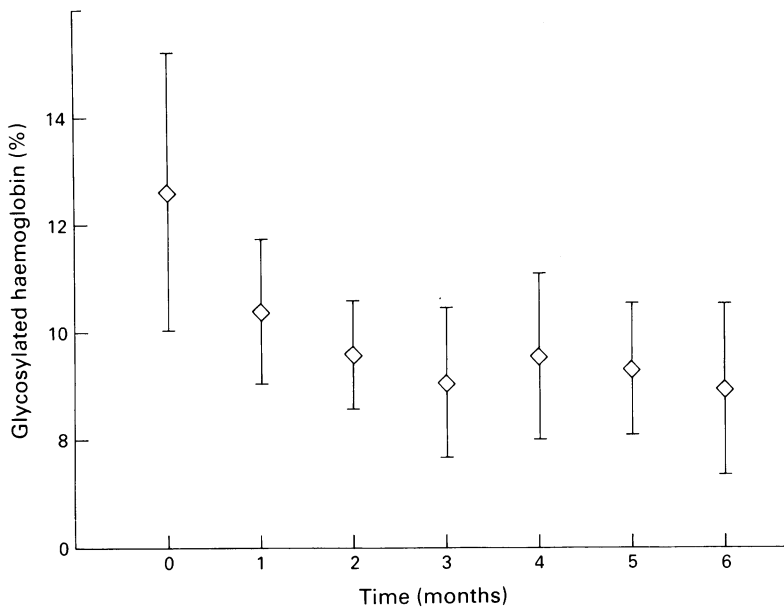After about 5 days in hospital, patients were

Figure 2   Average monthly glycosylated haemoglobin (GHb) levels in the 28 patients. Upper limit of normal range is 8%. Bars represent standard deviation.
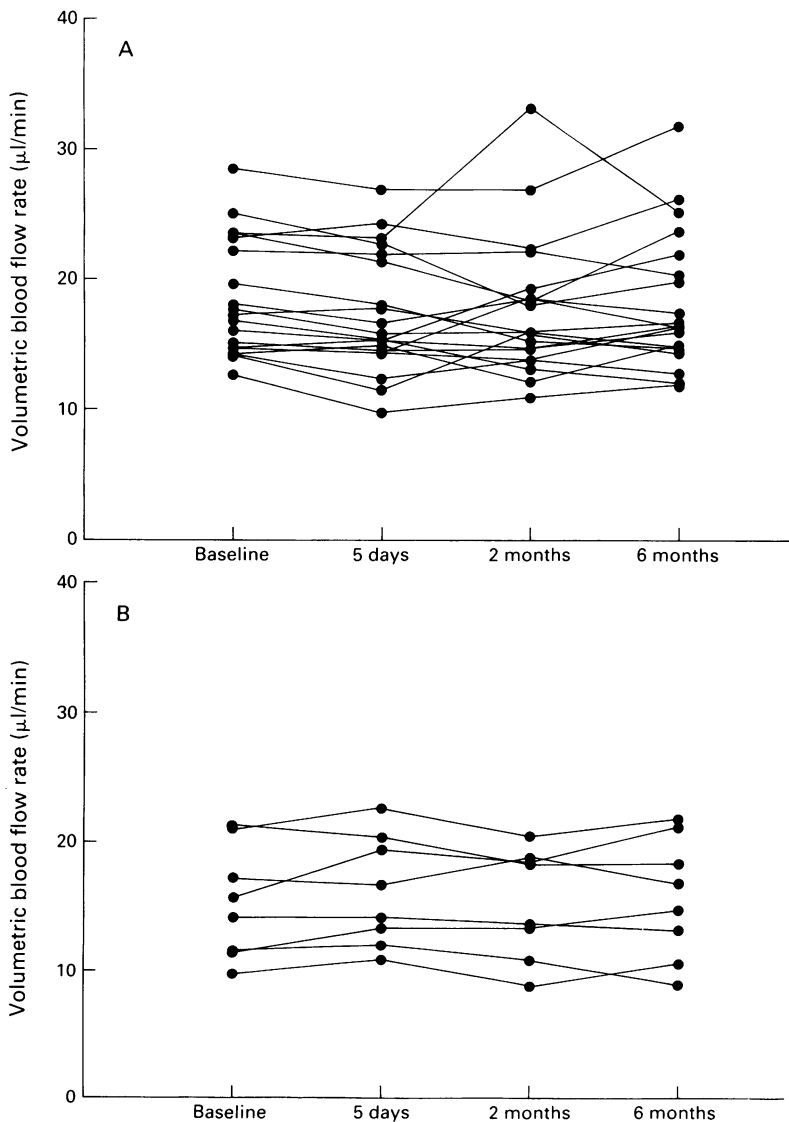

Figure 3   Volumetric blood flow rate (Q) in a major retinal vein at baseline and then 5 days, 2 months, and 6 months after institution of strict diabetic control. (A) Eyes that showed no progression of retinopathy (NP) at end of study. (B) Eyes that showed progression of retinopathy (P) at end of study.

released, and on the same day, a third session of blood flow measurement took place. Patients were asked to continue at home the regimen instituted during their time in hospital. Close regular telephone contact was maintained between patients, their treating physician, and the study nurse/coordinator.

The regimen of strict diabetic control was maintained for 6 months. Additional blood flow measurement sessions were held 2 and 6 months after the institution of this regimen.

Seven standard field stereo colour fundus photographs and fluorescein angiograms were obtained according to the Early Treatment of Diabetic Retinopathy Study (ETDRS)[12] protocol at baseline and at the end of the 6 months of the study. Assessment of retinopathy was performed in a masked fashion at the Fundus Photographic Reading Center of the University of Wisconsin. An overall retinopathy level according to the ETDRS grading protocol was assigned to the study eye of each patient at baseline and at the end of the study.

Mean brachial artery blood pressure (BP_m) was calculated as $BP_d + \frac{1}{3}(BP_s - BP_d)$, where BP_s and BP_d are the brachial artery systolic and diastolic pressures. Perfusion pressure (PP) was calculated as $\frac{2}{3} BP_m - IOP$, where IOP is the intraocular pressure.

One way analysis of variance and covariance for repeated measures, paired and unpaired Student's t test, and correlation and rank correlation analyses were used in the evaluation of the results. The Wilk-Shapiro test was used to assess the normal distribution of the results: $p < 0.05$ was considered statistically significant.
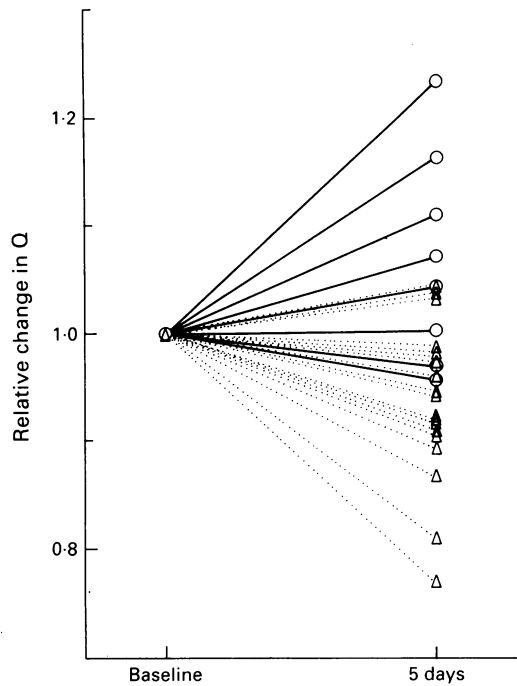
Results

At the end of the 6 month study, eight study eyes showed progression of retinopathy (P) by one or more retinopathy levels (Fig 1). Twenty study eyes had no progression of retinopathy (NP). Among them, four actually showed a decrease of one retinopathy level.

The degree of improvement in glycaemic control obtained in the study is shown in Figure 2 which depicts average monthly GHb levels for the 28 subjects of our study. Although these averages were not within the normal range, the overall improvement in glycaemic control obtained in our study was very similar to that attained in the DCCT study.[11]

Measurements of Q in one major temporal vein at the first baseline session and then 5 days, 2 months, and 6 months after the institution of strict diabetic control are shown in Figure 3A for NP eyes and in Figure 3B for P eyes. A one way analysis of variance for repeated measurements showed no significant trend in time ($p = 0.89$) nor differences between NP and P eyes ($p = 0.26$). Similar results were observed for measurements of a second retinal vein in each eye. Baseline Q values were not correlated with level of retinopathy ($p = 0.15$).

Relative changes in Q from baseline were calculated for each subject. On the fifth day of strict diabetic control, 16 out of the 20 eyes that showed no progression of retinopathy 6 months later had decreases in Q. On the other hand, six

Figure 4 Relative changes in volumetric blood flow rate (Q) from baseline measured in each subject about 5 days after institution of strict diabetic control. Broken lines are eyes that showed no progression of retinopathy at end of study; solid lines are eyes that showed progression of retinopathy at end of study.

Figure 5 Relation between changes in volumetric blood flow rate from baseline observed on fifth day of strict diabetic control and change in retinopathy level observed in study eye 6 months later. Rank correlation 0·68, p<0·01.
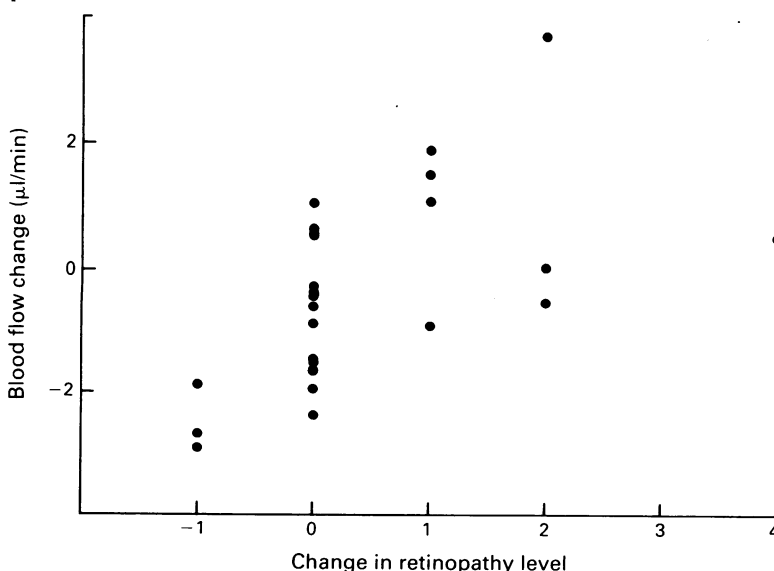

out of eight eyes that showed progression had increases in Q on the fifth day (Fig 4). A one way analysis of variance for the data shown in Figure 4, using P and NP as a grouping factor, showed a statistically significant difference in the changes in Q over time between the two groups (p= 0·001). NP eyes had a significant average decrease in Q of 1·0 (1·2) μl/min (paired t test, p=0·0012). In P eyes, the average increase in Q of 0·9 (1·5) μl/min was not statistically significant (p=0·13).

Further strengthening the relation between retinal blood flow changes and retinopathy changes is the fact that the magnitude of the changes in Q on the fifth day of strict diabetic control correlated significantly with the amount of change in retinopathy at the end of the 6 months of the study (rank correlation 0·65, p<0·01, Fig 5). No other significant differences in the changes in Q from baseline between NP and P eyes were observed at 2 or 6 months.

The average baseline regulatory response of the retinal circulation to hyperoxia (R_Q) for all 28

subjects studied was −49% (SD 10%). Average R_Q at baseline, fifth day, 2, and 6 months were −51% (9%), −47% (10%), −48% (12%), −48% (9%), respectively, in NP eyes and −45% (10%), −43% (11%), −42% (7%), −44% (13%) in P eyes. An analysis of variance for repeated measures showed no effect in time of strict diabetic control on R_Q (p=0·16). Although average R_Q tended to be smaller and more abnormal in P eyes than in NP eyes, the difference was not statistically significant (p=0·37).

No significant correlation was observed between Q measurements and blood glucose determinations obtained immediately following retinal blood flow measurements. Also, no such correlation was detected between Q or R_Q and GHb. On the fifth day, however, strong, positive correlations were observed between average blood glucose levels present during the 4 days preceding the fifth day blood flow measurement session and both the absolute change in Q from baseline (Fig 6, r=0·54, p=0·0028) and the percentage change in Q from baseline (r=0·59, p=0·001). Patients with higher average glucose tended to have increased flow and those with less high average glucose had decreased flow. No such significant association was detected at 2 months or 6 months.

An additional, interesting, significant positive correlation was observed between average blood glucose during the 4 days preceding the fifth day measurement session and the change in retinopathy level seen at the end of the 6 months of the study (rank correlation 0·56, p<0·01). As expected, patients with higher glucose levels tended to have more progression than those with less high blood glucose levels. No similar correlations were detected at baseline, 2 months, or 6 months.

No significant differences in average age, duration of diabetes and baseline GHb, IOP, BP_s, BP_d, or BP_m were observed between P and NP eyes (Table 2).

Although still within the normal range, average perfusion pressure at baseline was significantly higher in P eyes (46·4 (5·6) mm Hg) than in NP eyes (40·4 (6·4) mm Hg, p=0·03). Perfusion pressure changes in NP and P eyes during the study, however, did not correlate with the Q changes observed at the fifth day of strict diabetic control.

No significant differences were detected between P and NP eyes in the degree of improvement in glycaemia, as represented in each patient by the largest drop in GHb during the study, nor in the lowest GHb achieved.

An additional, interesting significant negative correlation was also seen between red blood cell count and Q (r=−0·49, p=0·04; linear regression equation is Q=45·7−6·RBC count). Patients with lower red blood cell count tended to have higher Q and vice versa.

Discussion

The results of this study show that changes in Q occur soon after the institution of strict diabetic control. This effect is most probably due to metabolic imbalances that follow a sudden improvement in glycaemic control. These
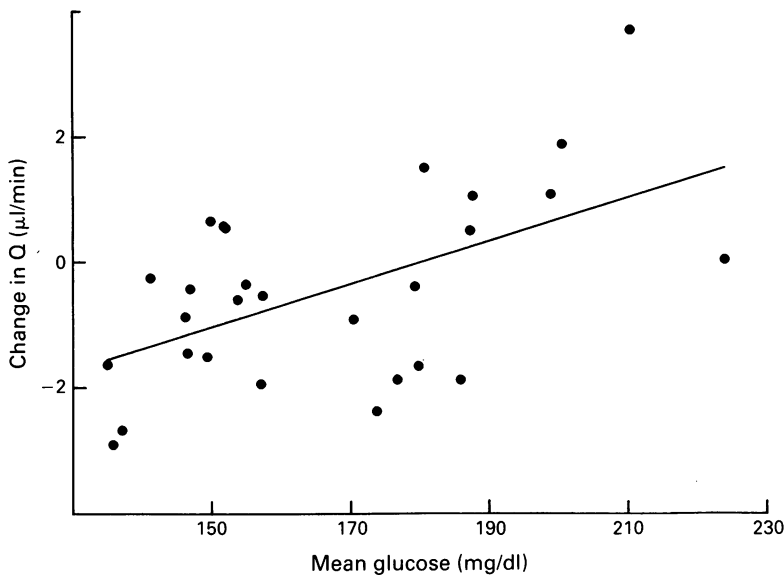
Figure 6 Correlation between mean blood glucose level (BG) during the 4 days preceding the fifth day blood flow measurement session (mean of four daily glucose determinations obtained on 4 consecutive days using the Accu-Check blood glucose monitor) and the absolute change in volumetric blood flow rate (Q) from baseline measured on the fifth day. Correlation coefficient, r= 0·64, p=0·0029. Regression line $\Delta Q = -8·43 + 0·048\,BG$.

changes in Q, which were detected on the fifth day of strict diabetic control (the first time that measurements were performed), were not present at 2 months and at 6 months suggesting that the metabolic imbalance leading to Q changes was of relatively short duration.

Alterations of Q observed in NP eyes on the fifth day of strict diabetic control were significantly different from those of P eyes. Out of 20 NP eyes, 16 had decreases in Q, and therefore, as a group, the average Q decreased significantly. On the other hand, six out of eight P eyes showed increases in Q. Although the average increase in Q was not significant for the group of P eyes, this increase was significantly different from the decrease in Q observed in NP eyes.

Further strengthening the hypothesis that early changes in flow may be associated with changes in retinopathy is the fact that the magnitude of the change in Q seen on the fifth day correlated significantly with the amount of change in retinopathy level observed at the end of the study (Fig 4). Although these results demonstrate an association between blood flow changes soon after the institution of strict diabetic control and retinopathy changes, they do not prove a causal relation between these two parameters.

A question arises as to how changes in Q, which were detected on the fifth day but were not present later in the study, could be related to changes in retinopathy observed 6 months later.

Table 2 Characteristics of patients in which study eyes showed no progression (NP) or progression (P) of retinopathy. (Standard deviation in parentheses)

	NP	P	p Value
Age (years)	30·8 (6·3)	31·8 (6·1)	0·70
Duration of diabetes (years)	16·6 (7·2)	13·5 (4·1)	0·26
GHb, baseline (%)	12·1 (2·3)	12·5 (2·2)	0·71
GHb, largest drop (%)	4·1 (2·2)	4·4 (1·9)	0·75
Lowest GHb (%)	8·0 (1·0)	8·1 (1·4)	0·84
BP_{syst}, baseline (mm Hg)	118 (14)	126 (15)	0·21
BP_{diast}, baseline	68 (9)	75 (7)	0·08
BP_{mean}, baseline	85 (10)	92 (8)	0·09
IOP, baseline	16 (3)	15 (3)	0·27
PP, baseline	40 (6)	46 (6)	0·03

GHb=glycosylated haemoglobin; BP_{syst}=systolic blood pressure; BP_{diast}=diastolic blood pressure; BP_{mean}=mean blood pressure; IOP=intraocular pressure; PP=perfusion pressure.

It is important to note that although changes in retinopathy were assessed at 6 months, the actual progression may have occurred much earlier, perhaps closer to the institution of strict diabetic control and the changes in Q.

During the development of our experimental protocol we had to choose the timing of the measurement sessions following the institution of strict diabetic control. We arbitrarily chose to perform the first session at about 5 days because at that time patients were discharged from the hospital and their glycaemia was expected to have improved. Although we detected Q changes on the fifth day, it is plausible that these changes could be present before or after the fifth day. Additional studies are needed in order to collect more information regarding the exact timing and duration of these Q changes.

The results of our study show significant decreases in Q on the fifth day of strict diabetic control in NP patients. Decreases in Q have also been reported in diabetic patients following an acute normalisation of blood glucose levels by insulin administration.[10] This regulatory decrease in Q, which was apparent in patients with short disease duration and tended to disappear in patients with longer disease duration,[10] suggested that the disease progression produced an impairment of the retinal vascular regulatory response to fluctuations in blood glucose.

Tight regulation of retinal blood flow in response to the metabolic changes that occur in the retina when blood glucose levels are suddenly decreased is probably an important factor in the maintenance of normal retinal function. A decoupling between retinal flow and metabolic changes could be detrimental to the retina and thus lead to progression of retinopathy. Possibly, the lack of a regulatory decrease in Q seen in our study in patients that showed progression of retinopathy could be causally related to the progression of retinopathy.

Another retinal vascular regulatory response that has been extensively studied in diabetes is the effect of changes in respiratory oxygen concentration on retinal blood flow. The retina is very sensitive to increases in arterial oxygenation. In the normal retina, 100% oxygen breathing for about 5 minutes produces a reduction in Q of about 60%.[13 14] This response is abnormal in diabetic patients, decreases with the progression of retinopathy[13] and improves following panretinal photocoagulation, particularly in patients that show regression of proliferation after treatment.[14] Grunwald et al have hypothesised that this response to 100% oxygen breathing may provide an index of the degree of inner retinal hypoxia in the diabetic retina.[14 15]

At baseline, the average percentage decrease in Q during 100% oxygen breathing (R_Q) was −49% (SD 10%), a value that is significantly lower and more abnormal than the decrease of −61% (8%) reported previously with the same technique[14] in 19 age matched normal subjects (non-paired Student's t test, p<0·001), suggesting that the oxygenation of the inner retina may be abnormal in these patients. Although P eyes tended to have smaller average R_Q than NP eyes, the difference was not statistically significant, and therefore, we cannot conclude that P eyes

have more abnormal regulatory responses than NP eyes. Institution of strict diabetic control also did not seem to produce any significant effect on R_Q.

The effects of institution of strict diabetic control on Q and R_Q shown by this study are somewhat different from those observed in more acute experiments. Grunwald et al[10] reported that in diabetic patients during severe hyperglycaemia there is an increased Q and decreased R_Q. An insulin induced acute normalisation of blood glucose resulted in a return of Q towards normal and an improvement in R_Q. Ernest et al[16] showed that acutely induced hyperglycaemia also resulted in decreased retinovascular regulation in dogs.

The acute large changes in glucose studied in these two investigations, however, may produce different effects on the retinal circulation than those obtained in our current study in which blood glucose was affected in a more chronic fashion. It is also possible that some of the haemodynamic changes accompanying acute fluctuations of blood glucose may be short term effects that disappear under more long term improvement of glycaemia.

Our results also showed a significant correlation between the changes in Q from baseline found on the fifth day of strict diabetic control and the average blood glucose levels present during the four preceding days (Fig 6). Patients with higher average glucose tended to have increased Q and those with less high glucose had decreased Q. Surprisingly, however, no significant correlation was found with blood glucose level present at the time of Q measurement, suggesting that the overall glucose levels present during the preceding days may have a stronger influence on Q than the glucose level present at time of Q determination. In other words, the cumulative effect of increased glucose levels present during the earlier days could have an effect on retinal blood flow.

A single glucose measurement obtained at the time of Q measurement, which can vary widely according to the time elapsed from the last meal and insulin injection and the type and amount of food ingested, provides a poor index of the overall glycaemic condition and this may be a reason why no significant correlation was detected in our study between this parameter and Q. On the other hand, if changes in Q are associated with the overall glycaemic condition present in the preceding days, then a question arises as to why no significant correlation was found between changes in Q observed on the fifth day of strict diabetic control and GHb levels. GHb provides information about glycaemic conditions present 2 to 3 months before the test is performed.[17] Since on the fifth day of strict diabetic control glycaemia had been improved for only a few days, the GHb measured at that time would not accurately represent the glycaemic conditions present during the last few days.

Interestingly, we also found a significant rank correlation between average glucose level during the 4 days preceding the fifth day measurement session and the amount of change in retinopathy level at the end of the study. No other correlation

between blood glucose or GHb at any time during the study and amount of change in retinopathy were observed, suggesting again that events occurring during the first days of strict diabetic control are important in the development of retinopathy progression.

Our results also show that both changes in Q measured on the fifth day of strict diabetic control and the average glucose levels present during the preceding 4 days were significantly correlated with the change in retinopathy observed at the end of the study. Because of the small number of patients studied we cannot assess independently these two associations and conclude whether one is more important than the other.

Although still within the normal range, P patients in our study had significantly higher perfusion pressure than NP patients (Table 2). Increased perfusion pressure above normal has been associated with progression of diabetic retinopathy,[18] a finding that supports the hypothesis that increased intraluminal capillary pressure may play a role in the development of diabetic vasculopathy.[19 20] Our results show that even within the normal range, higher perfusion pressure is associated with progression of retinopathy.[18 21-23]

Changes in perfusion pressure, however, could not explain the differences in Q changes observed on the fifth day of strict diabetic control between NP and P eyes because our results did not show any significant correlations between Q changes and perfusion pressure changes.

Our results also demonstrate a significantly negative correlation between red blood cell count and Q. Patients with smaller erythrocyte counts may need higher Q rates to deliver and remove gases from the retina. Some of the variability in Q observed in diabetic patients in previous studies may be caused in part by differences in red blood cell counts.

Diabetic retinopathy is a slow, chronic process. Because of this, it is very difficult to study the factors that influence the progression of the disease. The transient deterioration of retinopathy that occurs in some patients following the institution of strict diabetic control may provide a short term model for the events that produce progression of retinopathy. Similar changes in Q could also be related to the chronic progression of retinopathy seen in diabetic patients over the years.

In summary, our results suggest that patients who show progression of retinopathy have some typical retinal haemodynamic characteristics. Measurements of retinal blood flow after institution of strict diabetic control may help identify patients at risk of progression of retinopathy.

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