Retinal blood flow in type 1 diabetes patients with no or mild diabetic retinopathy during euglycemic clamp

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Running title: Retinal Blood Flow in Type 1 Diabetes

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Submitted 16 March 2010 and accepted 18 June 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Objective: To compare total retinal blood flow in diabetes patients with no or mild non-proliferative diabetic retinopathy and healthy control subjects and to investigate in patients whether there is a difference between retinal blood flow before morning insulin and under normoglycemic conditions using glucose clamp.

Research Design and Methods: 20 patients with type 1 diabetes with no or mild diabetic retinopathy were included in this open parallel group study. 20 healthy age- and sex-matched subjects were included as controls. Retinal blood flow was assessed by combining velocity measurements using laser Doppler velocimetry and diameter measurements using a commercially available Dynamic Vessel Analyzer. Measurements were performed before and during a euglycemic clamp.

Results: Total retinal blood flow was higher in diabetic patients (53 ± 16 µl/min) than in healthy subjects (43 ± 16 µl/min, \( P = 0.034 \) between groups). When plasma glucose in diabetes patients was reduced from 9.3 ± 1.7 mmol/l to 5.3 ± 0.5 (\( P < 0.001 \)) retinal blood flow decreased to 49 ± 15 µl/min (\( P = 0.0003 \) versus baseline). Total retinal blood flow during the glucose clamp was not significantly different from blood flow in normal control subjects (\( P = 0.161 \)).

Conclusions: Type 1 diabetes patients with no or only mild diabetic retinopathy have increased retinal blood flow before their morning insulin dosage. Blood flow is reduced towards normal during euglycemic conditions. Retinal blood flow may fluctuate significantly with fluctuating plasma glucose levels, which may contribute to the microvascular changes seen in diabetic retinopathy.

The mechanisms underlying the development of diabetic retinopathy are still not fully understood. Elevated glucose levels may be the initial factor leading to alterations of vessel architecture in the retina, perfusion abnormalities and progression of the disease. Early microstructural changes of retinal vessels preceding clinically visible retinopathy include thickening of the capillary basement membrane, pericyte loss, retinal capillary non-perfusion, and capillary loss (1). Capillary hypoperfusion is accompanied by the development of shunt vessels and decreased tissue oxygenation finally representing a stimulus for neovascularization in proliferative diabetic retinopathy (2).

Retinal blood flow in patients with diabetes has been investigated by various research groups and techniques in the last years, but the results are not conclusive for a variety of reasons. For example, using combined analysis of vessel diameters and bi-directional laser Doppler velocimetry in patients with type 1 diabetes it has been shown that total retinal blood flow and blood velocity are increased in early diabetic retinopathy (3). This was also seen even before the clinical onset of diabetic retinopathy (4). Other investigators have, however, reported that blood flow velocities in the retrobulbar central retinal artery (5) and in branch retinal arteries are reduced (6) or that blood flow is not different compared to healthy controls (7). Results from the various studies are often not directly comparable due to their different assessment methods in the various sections of the ocular vasculature and due to differences in the study population concerning the patient disease type, duration and stage. A detailed
overview of results from ocular blood flow studies in diabetes is available elsewhere (2). Looking at these partially inconsistent results of blood flow alterations in diabetes, it has to be noted that glucose and insulin plasma levels may have a considerable influence on retinal blood flow. Grunwald et al. reported that a pronounced reduction of plasma glucose levels in type 2 diabetes patients, induced by administration of insulin, leads to a significant reduction of retinal blood flow associated with a normalization of vessel reactivity in response to hyperoxia (8). Reduced retinal blood flow after glucose reduction was also found in patients with type 1 diabetes (9). In keeping with these results several studies identified a positive correlation between blood glucose levels and retinal blood flow parameters in diabetes patients (5,10). A recent study, however, reported unchanged ocular blood flow parameters after acute elevation of blood glucose in diabetes and healthy controls (11). It has to be noted that also in these interventional studies differences in methodology limit comparability of the results.

The situation is further complicated by the vasodilator effects of insulin in the eye (12) and by the additive effects of insulin and glucose on ocular blood flow (13). Taken together these results indicate that findings from blood flow studies in diabetes patients have to be interpreted with caution, because fluctuations in blood glucose and insulin levels in diabetes patients may affect blood flow considerably and need to be taken into account to assess blood flow changes due to diabetes correctly. The present study addressed this problem by investigating retinal blood flow in diabetes during normalized glucose levels using the glucose clamp technique. The hypothesis that retinal blood flow is altered in patients with type 1 diabetes with no or mild diabetic retinopathy at elevated compared to physiologic plasma levels was tested.

RESEARCH DESIGN AND METHODS

Subjects. The study was started after the approval of the study protocol by the competent authorities and the positive judgement of the responsible Ethics Committee. It was performed in adherence to the guidelines of the Declaration of Helsinki and Good Clinical Practice guidelines. 20 patients with type 1 diabetes with no or mild non-proliferative retinopathy were included in this open parallel group study. The patients were classified according to the criteria established in the early Early Treatment Diabetic Retinopathy Study (14) using 7 standard field color fundus photographs. As a control group 20 age and sex matched healthy subjects were included. All subjects passed a screening examination including physical examination, blood pressure measurement and ophthalmic examination. Exclusion criteria were an age < 19 years, ametropia > 6 dpt, best corrected visual acuity < 0.8 presence of ocular pathologies other than diabetic retinopathy in diabetes patients, previous laser photocoagulation treatment, HbA1C > 8% and systemic hypertension (defined as either systolic blood pressure > 145 mmHg or diastolic blood pressure > 90 mmHg, or a diagnosis of systemic hypertension in the medical history). Other clinically relevant illness prior to the study, pregnancy or lactation also excluded participation in the study as well as drug intake in the healthy subjects in the 3 weeks before the study.

Experimental paradigm. All participants received one drop of 0.5% tropicamide (Mydriaticum “Agepha”, Agepha, Vienna, Austria) to achieve pupil dilation. Measurements of all outcome variables were done in the morning between 8 a.m. and 9 a.m. before morning insulin dose. After a resting period of 20 minutes to achieve hemodynamic stabilization, baseline measurements of retinal
blood flow parameters were performed in the right eye. Thereafter all measurements were repeated during normoglycemic conditions. To achieve glycemic control in diabetes patients, the glucose clamp technique was applied as described earlier (15). For this purpose two indwelling intravenous cannulas were inserted into antecubital veins for simultaneous monitoring of glucose plasma concentrations via venous blood sampling on one arm and for insulin and glucose administration on the other arm. Diabetes patients received insulin (Insulin "Lilly" Huminsulin Normal, Lilly, Fegersheim, France) intravenously over 60 minutes with a continuous dose of 6 pmol/kg/min. Simultaneously, 20% glucose solution (Glucose 20% Leopold Infusionsflaschen, Leopold Pharma, Linz, Austria) was administrated intravenously at a rate necessary to maintain blood glucose levels at 5.6 ± 1.1 mg/dl. Blood glucose levels were measured from venous blood samples every 5 minutes using a Beckman Glucose Analyzer (Beckman Coulter, Inc., Brea, CA). 30 minutes after start of the infusion blood flow measurements were repeated. Blood pressure, pulse rate and ECG were monitored throughout the trial.

Assessment of retinal blood flow.
Measurement of retinal blood flow velocity was performed with a fundus camera based laser Doppler velocimeter (LDV-5000, Oculix Inc., Arbaz, Switzerland). All major retinal veins around the optic nerve head were investigated to achieve measurement of total retinal blood flow. The principle of LDV is based on the optical Doppler Effect. Laser light of a single mode laser diode with a wavelength of 670 nm is scattered and reflected by moving erythrocytes leading to a broadened and shifted frequency spectrum. This frequency shift is proportional to the blood flow velocity in the retinal vessel whereupon the maximum Doppler shift corresponds to the centerline erythrocyte velocity (Vmax). In bidirectional LDV two Doppler shift power spectra are recorded simultaneously for two directions of the scattered light which enables absolute velocity measurements (16). From Vmax mean blood velocity in retinal vessels (Vmean) may be calculated as \( V_{\text{mean}} = \frac{V_{\text{max}}}{2} \). Vessel diameters at all measurement sites were obtained with the Dynamic Vessel Analyzer (DVA; IMEDOS Systems, Jena, Germany). The DVA assesses retinal vessel diameters (D) by analyzing the brightness profile of the vessel using a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany), a high-resolution digital video camera and a personal computer with analyzing software (17). Images are recorded, digitized and analyzed in real-time with a frequency of 25 Hz. After selection of the measurement location the DVA is able to track the vessels during eye movements within the measurement window. The system provides excellent reproducibility and sensitivity (18).

Blood flow (Q) was measured in all retinal veins \( i \) that were entering the optic nerve head as \( Q_i = V_{\text{mean},i} \cdot D_i^2 \pi / 4 \) (16). Total retinal blood flow was obtained using the equation

\[
Q = \sum_{i=1}^{n} V_{\text{mean},i} \cdot D_i^2 \pi / 4.
\]

The number of veins that were used for calculation of total retinal blood flow varied from 4 to 5 depending on the individual retinal angioarchitecture. In addition, the total venous diameter was calculated as the sum of all \( D_i \)s as well as the mean vessel diameter and mean venous blood velocity.

Measurement of systemic hemodynamics.
Systolic, diastolic and mean arterial blood pressures (SBP, DBP, MAP) were measured repeatedly before and after blood flow measurements on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded.
by the same unit from a finger pulse oxymetric device.

**Statistical analysis.** Data are presented as means ± standard deviation (SD). To assess differences between the diabetes patients compared to the data of healthy controls an unpaired t-test was performed. Repeated measures ANOVA was applied to data of the diabetes group to compare values before and during glucose clamp. A $P$ value < 0.05 was considered as the level of significance. To control for associations between blood pressure and retinal blood flow a Pearson product-moment correlation coefficient was calculated.

**RESULTS**

The baseline characteristics of both groups are presented in Table 1. All diabetes patients either presented with no (n = 17) or mild (n = 3) diabetic retinopathy with a mean disease duration of 9 ± 3 years. There were no significant differences in age and pulse rate and observed between the two groups. SBP, DBP and MAP were higher in diabetes patients. Baseline plasma levels of glucose and HbA1c were elevated in diabetes patients whereas normoglycemia was found in healthy subjects. As expected insulin and c-peptide values were significantly diminished in the diabetes patients compared to the healthy subjects. Total retinal blood flow in diabetes patients was increased at baseline compared to controls (53 ± 16 µl/min vs. 43 ± 16 µl/min, $P = 0.035$, unpaired t-test). There was no significant correlation observed between blood pressure and retinal blood flow ($r = 0.150$, $p = 0.355$). Retinal vein diameters were slightly larger in diabetes patients than in healthy controls, but the difference was only of borderline significance ($p = 0.054$). Similarly, total and mean retinal vein diameter as well as mean venous blood velocity tended to be increased in patients with diabetes, but this effect also did not reach the level of significance.

Parameters in diabetes patients during euglycemic clamp are also presented in Table 1. Blood glucose levels of diabetes patients were reduced from 9.3 ± 1.7 mmol/l to 5.3 ± 0.5 mmol/l ($P < 0.001$, ANOVA), whereas insulin was increased during clamp. Total volumetric blood flow was reduced from 53 ± 16 µl/min to 49 ± 15 µl/min ($P = 0.0003$, ANOVA). At the same time mean venous blood velocity was also significantly reduced. By contrast total and mean retinal venous diameters as well as SBP, DBP, MAP and pulse rate did not change during the glucose clamp. Although retinal blood flow tended to be higher in patients with diabetes during the euglycemic clamp compared to the values of the healthy control subjects, this difference was no longer significant ($P = 0.161$, unpaired t-test).

**DISCUSSION**

Using various techniques to study retinal blood flow in patients with diabetes different conclusions have been drawn whether blood flow is increased or decreased (2-10, 19-20). The present study indicates that retinal blood flow in diabetes patients with no or mild retinopathy is slightly elevated before the patients take their insulin, but is reduced towards normal when blood glucose levels are normalized.

As compared to previous studies addressing the question of retinal blood flow in diabetes, the present approach offers several methodological advantages. Most importantly only few studies have measured total retinal blood flow (3,4). The technique applied in this study, combining velocity data with diameter data is currently the only approach to study this in humans. As such our data are in good agreement with a previous study indicating increased retinal blood flow in diabetes before the onset of diabetic retinopathy (4). As compared to this previous study, however, the method used for the measurement of retinal venous diameters in
the present study provides a much better resolution. In the present study a tendency towards increased retinal venous diameter in diabetes was found, although this effect did not reach the level of significance, most likely due to the relatively small sample size. This is in keeping with a number of large scale studies clearly indicating that patients with diabetes have larger retinal arteriolar and venial calibers and that increased retinal vessel diameters are associated with diabetic retinopathy (21,22).

As mentioned above one advantage of this study using LDV and DVA is that total volumetric retinal blood flow rate was assessed. Most results from previous studies presented results of a single vessel only (7,11,20). The main point to be considered in this approach is that due to the large variability in the retinal angioarchitecture between subjects a conclusion from a single vessel on the entire retinal circulation cannot necessarily be drawn.

The present study focused on patients with type 1 diabetes only. Whether our results can be extrapolated to patients with type 2 diabetes is unclear, because this disease might represent other retinal vascular characteristics due to higher age and concomitant diseases like hypertension and hyperlipidemia. In addition, we focused on a homogenous group of patients with relatively good glycemic control and no vasoactive medications to reduce the influence of these confounding factors on our results. Blood pressure in the diabetes patients was found to be higher compared to the healthy control group, but did not reach abnormal levels. There was no correlation found between blood pressure and retinal blood flow. In a previous study in 200 young, healthy subjects we could show that there is a weak association between retinal blood flow and blood pressure (23). The sample size of the present study may likely be too small to determine a correlation of such dimension. A considerable influence of the observed blood pressure values on ocular blood flow seems, however, unlikely.

Only very few studies employed glucose clamps to control for blood glucose when measuring retinal perfusion in diabetes. In keeping with a study employing fluorescein angiography to study retinal blood velocities in early type 1 diabetes (9) our study indicates that normalizing blood glucose leads to a reduction of retinal blood velocities. This is compatible with a variety of studies in diabetes indicating that glucose increases blood flow in the ocular vasculature (5,10). In healthy subjects hyperglycemic euinsulinemic clamp leads to an increase in ocular blood flow parameters (13). A reduction of elevated blood glucose by administration of insulin reduces retinal blood flow (8) in keeping with the results of the present study. One study, however, found no change in retinal blood flow after an acute glucose load (11), although such experiments did not control for plasma insulin, which has vasoactive properties in the ocular vasculature as well. We have previously shown that insulin increases ocular blood flow (12,13). In the present study insulin reached levels highly above normal to achieve normoglycemia in diabetes patients. However, retinal blood flow was found decreased compared to baseline and retinal vein diameter was not changed during glucose clamp. Therefore it seems likely that possible vasoactive effects of insulin were exceeded by the blood flow changes due to glucose reduction. Besides the effects of glucose and insulin, altered tissue oxygenation due to perfusion changes during the experiment could also have an effect on blood flow in the diabetic retina (4).

Measurement of blood velocities in all vessels entering the optic nerve head using LDV is a time consuming procedure and requires a high degree of subject cooperation. As such this procedure can neither be applied in clinical routine nor in large scale epidemiological studies. Only recently, however, retinal blood
velocity measurements in human eyes based on optical coherence tomography were introduced (24,25). With the further development of this technology it may well be that instruments become available commercially that allow for measurement of total retinal blood flow in a fast and reliable way.

In conclusion the present study indicates that patients with early type 1 diabetes have increased retinal blood flow that is reduced towards normal under euglycemic conditions. Hence, retinal blood flow may fluctuate significantly with fluctuating plasma glucose levels. This may well contribute to the microvascular damage seen early in diabetic retinopathy.

**Author Contributions:** B.P. wrote/reviewed/edited manuscript, E.P. researched data, G.G. reviewed/edited manuscript, contributed to discussion, M.B. contributed to discussion, A.K. reviewed/edited manuscript, contributed to discussion, L.S. wrote/reviewed/edited manuscript, contributed to discussion.

**Disclosure:** No conflict of interest exists for any author

**REFERENCES**


Table 1 - Characteristics of participating patients and matched healthy subjects and parameter changes in diabetes patients during euglycemic clamp.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 20)</th>
<th></th>
<th>Diabetes patients (n = 20)</th>
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<tbody>
<tr>
<td></td>
<td>P (between groups)</td>
<td></td>
<td>Before clamp</td>
<td></td>
<td></td>
<td>During clamp</td>
<td></td>
<td></td>
<td>P (change from baseline)</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>33 ± 7</td>
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<td>34 ± 6</td>
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<td>-</td>
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<tr>
<td>Sex (male/female)</td>
<td>12 / 8</td>
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<td>12 / 8</td>
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<td></td>
<td>-</td>
<td></td>
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<td>-</td>
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<tr>
<td>A1C (%)</td>
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<td></td>
<td>6.9 ± 0.4</td>
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<td>-</td>
<td></td>
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<tr>
<td>Blood Glucose (mmol/l)</td>
<td>4.5 ± 0.5</td>
<td><strong>&lt; 0.001</strong></td>
<td>9.3 ± 1.7 *</td>
<td></td>
<td></td>
<td>5.3 ± 0.5 †</td>
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<td></td>
<td><strong>&lt; 0.001</strong></td>
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<tr>
<td>Insulin (pmol/l)</td>
<td>319 ± 49</td>
<td><strong>&lt; 0.001</strong></td>
<td>104 ± 41 *</td>
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<td>1155 ± 278 †</td>
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<td></td>
<td><strong>&lt; 0.001</strong></td>
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<tr>
<td>c-Peptide (nmol/l)</td>
<td>2.4 ± 0.4</td>
<td><strong>&lt; 0.001</strong></td>
<td>0.002 ± 0.002 *</td>
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<td>-</td>
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<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>119 ± 10</td>
<td><strong>0.024</strong></td>
<td>126 ± 7 *</td>
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<td></td>
<td>127 ± 7</td>
<td></td>
<td></td>
<td>0.447</td>
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<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>61 ± 8</td>
<td><strong>&lt; 0.001</strong></td>
<td>72 ± 7 *</td>
<td></td>
<td></td>
<td>72 ± 7</td>
<td></td>
<td></td>
<td>0.899</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>81 ± 8</td>
<td><strong>&lt; 0.001</strong></td>
<td>90 ± 6 *</td>
<td></td>
<td></td>
<td>90 ± 6</td>
<td></td>
<td></td>
<td>0.651</td>
<td></td>
</tr>
<tr>
<td>Pulse Rate (beats/min)</td>
<td>62 ± 9</td>
<td>0.077</td>
<td>67 ± 7</td>
<td></td>
<td></td>
<td>66 ± 8</td>
<td></td>
<td></td>
<td>0.091</td>
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</tr>
<tr>
<td>Mean Retinal Vein Diameter (µm)</td>
<td>124 ± 10</td>
<td>0.054</td>
<td>131 ± 12</td>
<td></td>
<td></td>
<td>132 ± 12</td>
<td></td>
<td></td>
<td>0.152</td>
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</tr>
<tr>
<td>Total Retinal Vein Diameter (µm)</td>
<td>537 ± 56</td>
<td>0.062</td>
<td>573 ± 60</td>
<td></td>
<td></td>
<td>578 ± 69</td>
<td></td>
<td></td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td>Mean Venous Blood Velocity (cm/s)</td>
<td>1.21 ± 0.14</td>
<td>0.106</td>
<td>1.30 ± 0.19</td>
<td></td>
<td></td>
<td>1.17 ± 0.17 †</td>
<td></td>
<td></td>
<td><strong>&lt; 0.001</strong></td>
<td></td>
</tr>
<tr>
<td>Total Volumetric Blood Flow (µl/min)</td>
<td>43 ± 12</td>
<td><strong>0.035</strong></td>
<td>53 ± 16 *</td>
<td></td>
<td></td>
<td>49 ± 15</td>
<td></td>
<td></td>
<td><strong>0.0003</strong></td>
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</table>

Data except for sex are presented as means ± SD. * significant difference between the two groups at baseline (unpaired t-test), † significant changes during glucose clamp in diabetes patients (repeated measures ANOVA), a p-value < 0.05 was considered the level of significance.