Graefe's Arch Clin Exp Ophthalmol
(2003) 241:181–186

CLINICAL INVESTIGATION

DOI 10.1007/s00417-002-0520-9

**José Ludovico**
**Rui Bernardes**
**Isabel Pires**
**João Figueira**
**Conceição Lobo**
**José Cunha-Vaz**

# Alterations of retinal capillary blood flow in preclinical retinopathy in subjects with type 2 diabetes

Received: 18 April 2002
Revised: 17 June 2002
Accepted: 18 June 2002
Published online: 7 February 2003


J. Ludovico · I. Pires · J. Figueira · C. Lobo
J. Cunha-Vaz (✉)
Department of Ophthalmology,
University Hospital of Coimbra, Portugal
e-mail: Cunhavaz@aibili.pt
Tel.: +351-239480100
Fax: +351-239480117

R. Bernardes · J. Cunha-Vaz
Center of New Technologies for Medicine,
AIBILI–Association for Innovation and
Biomedical Research on Light and Image,
Coimbra, Portugal

C. Lobo · J. Cunha-Vaz
Centre of Ophthalmology,
Institute of Biomedical Research
on Light and Image, Faculty of Medicine,
University of Coimbra,
3000-354 Coimbra, Portugal

J. Cunha-Vaz
AIBILI–Association for Innovation and
Biomedical Research on Light and Image,
Coimbra, Portugal

**Abstract** *Background:* To identify alterations of retinal capillary blood flow in the papillomacular area in preclinical diabetic retinopathy using the Heidelberg scanning laser Doppler flowmeter. *Methods:* Ten eyes from ten patients with type 2 diabetes and no lesions visible on fundus photography (level 10 of Wisconsin grading) and ten eyes from ten healthy subjects of similar age range were examined with the HRF. Intravisit reproducibility of retinal capillary blood flow measurements was assessed in normal subjects and in type 2 diabetic patients, comparing different measurement areas and different analysis procedures: (a) 10×10 pixel box with original software, (b) 10×10 pixel box with SLDF software, and (c) whole-scan analysis with SLDF software (automatic full-field perfusion image analysis). *Results:* Intravisit reproducibility for the whole-scan analysis in the papillomacular area was 3.52%, 4.81% and 4.60% for volume (VOL), flow (FLW) and velocity (VEL) respectively. Using this method, mean and SD values for retinal capillary blood-flow are 13.25±2.87, 214.58±55.30 and 0.74±0.17, for VOL, FLW and VEL for healthy eyes, comparing with 19.85±6.22, 360.87±158.70 and 1.20±0.48 in eyes with preclinical diabetic retinopathy ($P<0.010$, $P<0.019$ and $P<0.015$ respectively). *Conclusions:* The HRF shows acceptable reproducibility when using whole-scan analysis in the papillomacular area. Retinal capillary blood VOL, FLW and VEL were particularly increased in five of the ten diabetic eyes examined, with values over the mean + 2SD of the control population, suggesting that eyes showing increased retinal capillary blood flow may indicate risk of progression.

## Introduction

Diabetic retinopathy is still the main cause of loss of vision in people between 20 and 70 years of age in the western world [1]. Visual loss is attributed either to macular edema or to retinal neovascularization. Diabetic macular edema is a consequence of breakdown of the blood–retinal barrier(BRB) [12], whereas retinal neovascularization appears to be a direct result of vascular closure and ischemia [1].

Retinal ischemia due to vascular closure develops relatively early in the course of diabetic retinopathy and is attributed to lack of vascular autoregulation and microthrombosis formation. Retinal blood flow changes are considered to lead to the development of areas of poor perfusion, facilitating microthrombosis formation [2].

Retinal blood flow measurements in the early stages of diabetic retinal disease are needed to understand the role of alterations in retinal hemodynamics in the development of the retinopathy, and may eventually help in the management of the retinopathy.

Alterations in retinal blood flow have been identified in the different stages of the progression of diabetic retinopathy. In patients with mild retinopathy and using a

two-point fluorophotometry technique we found an increase in retinal arteriolar velocity [4]. This finding was confirmed by Cuypers et al. [5] using laser Doppler flowmetry. Other authors have, however, registered a decrease in retinal blood flow. Sullivan and associates [18] found reduced blood flow when the glucose levels remained low and stable, increasing only in association with high glucose levels. In the more advanced stages of retinopathy, the results also conflict, with some authors reporting decreases in retinal blood flow [15, 17] and others reporting increases [3].

One of the major problems associated with these measurements is their technical complexity and variability.

Cuypers et al. [5] used the Heidelberg Retina Flowmeter (HRF; Heidelberg Engineering, Dossenheim, Germany) on a series of eyes from patients with either type 1 or type 2 diabetes. They included in the study patients with all grades of retinopathy. They considered the methodology to be reliable.

We examined with the HRF a series of eyes with preclinical retinopathy in subjects with type 2 diabetes and compared our findings with a healthy control population, after examining the reproducibility of the different software available and different examination methodologies.

## Materials and methods

### Subjects

Ten eyes from ten normal subjects (four men, six women) ranging between 40 and 60 years of age (mean 49.40 years, SD 5.77 years) and ten eyes from ten patients with type 2 diabetes (nine men, one woman) ranging between 52 and 64 years of age (mean 56.80 years, SD 3.28 years) were examined. The duration of their diabetes ranged from 5 to 7 years (mean 5.60 years, SD 0.66), and their glycosylated hemoglobin (HbA1C) value ranged from 0.060 to 0.072 (mean 0.0652, SD 0.0043). Blood pressure was at or below 150/85 mmHg. All subjects in the study underwent a complete ophthalmological examination, including slit-lamp biomicroscopy, measurement of intraocular pressure and indirect funduscopy. All patients were classified as level 10 in the Wisconsin grading.

The tenets of the Declaration of Helsinki were followed, and the approval of the institutional review board was obtained.

### Procedures

#### *HRF examination technique*

In each of the studies, a fixation point was provided at a distance of 3 m to prevent errors as a result of changes in accommodation between images and to help subjects ignore the sudden appearance of the scanning beam. After correct positioning of the camera by the operator, the subject fixated on the provided fixating point during the entire scan procedure.

Patients' eyes were not dilated, and the same operator performed all scans. The process was repeated three times for each patient.

#### *Data acquisition*

Before starting the acquisition process, two green lines on the operator screen mark the boundaries of the area from which flow data will be derived. After the laser beam has been centered on the area of interest, the focus is adjusted to produce maximal brightness within that area. With the laser position and focus optimized, sensitivity is set so that the brightest pixels within the area of interest are light yellow in color and not white, which would mean saturation. When acceptable alignment, focus and brightness have been achieved, the operator initiates the process.

After the scan has been completed, data are processed. This processing includes a Fourier transform to extract the individual frequency components of the collected light. For each pixel of the scan, a frequency power-spectrum is computed from the respective intensity time curve. On the frequency axis of the spectrum, each frequency represents a set of red blood cells with a given velocity, and the height of the spectrum at that frequency represents the number of red blood cells required to produce that intensity. Integrating the spectrum yields total red blood cells, called volume (VOL). The integration of height × frequency yields the "effective Doppler shift", called flow (FLW). The ratio between flow and volume is the mean red blood cell velocity (VEL).

#### *Reproducibility*

Reproducibility was expressed as the ratio between standard deviation (SD) and the mean of repetitive measurements. Three scans were performed within the macular area, avoiding large vessels, for each subject in a single visit.

Comparisons were made for reproducibility of VOL, FLW and VEL for the 20 eyes using three different methods (Table 1):

- Method A: 10×10 pixel box area with the current Heidelberg Engineering HRF software (version 1.02), in three different anatomical locations.

**Table 1** Reproducibility of the different methods used for capillary blood flow measurement with the Heidelberg Retina Flowmeter

| Group | | Reproducibility (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Method A (HRF – 10×10 box) | | | Method B (SLDF – 10×10 box) | | | Method C (SLDF – whole area) | | |
| | | VOL | FLW | VEL | VOL | FLW | VEL | VOL | FLW | VEL |
| Normals | Mean | 8.29 | 11.05 | 10.04 | 11.47 | 21.81 | 21.47 | 2.81 | 3.74 | 3.67 |
| | SD | 3.75 | 4.42 | 3.87 | 4.18 | 11.21 | 11.77 | 1.94 | 2.09 | 1.79 |
| Diabetes | Mean | 8.02 | 10.96 | 10.40 | 10.39 | 25.03 | 24.50 | 4.23 | 5.87 | 5.54 |
| | SD | 3.49 | 3.28 | 3.07 | 6.80 | 15.71 | 15.45 | 3.51 | 3.87 | 3.22 |
| All | Mean | 8.16 | 11.00 | 10.22 | 10.93 | 23.42 | 22.99 | 3.52 | 4.81 | 4.60 |
| | SD | 3.62 | 3.90 | 3.50 | 5.67 | 13.74 | 13.82 | 2.92 | 3.29 | 2.77 |

- Method B: 10×10 pixel box area with SLDF Tool Analysis supplied by Heidelberg Engineering (version 3.4.0), in three different anatomical locations.
- Method C: whole scan area (256×64 pixels) with SLDF Tool Analysis supplied by Heidelberg Engineering (version 3.4.0).

For methods A and B a clear anatomical landmark common for the three scans and to the two methods was selected. From this location a given number of pixels was counted both in x- and y-directions to ensure that the same area was considered for the different scans (same patient).

Methods B and C make use of SLDF Tool Analysis. With this software the resulting perfusion image was processed with respect to: (1) underexposed and overexposed pixels, (2) saccades and (3) the retinal vascular tree. Capillaries and vessels of the retinal vascular tree were identified automatically by pattern analysis. Retinal vessels with a diameter greater than 30 μm, underexposed or overexposed areas, and saccades were automatically excluded.

For the whole-scan analysis, the automatic full-field perfusion image analyzer procedure [13] was used and total mean flow, total mean volume, total mean velocity, standard deviation, cumulative distribution curve of flow and capillary pulsation index were automatically calculated. Heartbeat-associated pulsation of capillary blood flow was estimated by plotting the mean capillary flow of each horizontal line against time.

Reproducibility for each eye when using three scans and analysis in three different locations during each scan was considered as the mean of the local reproducibilities established for each one of the three locations.

*Comparison between the normal population and diabetic patients*

Using the data collected for reproducibility as explained in the previous section, mean values for the whole scan area, using the automated full-field perfusion image analysis (256×64 pixels) obtained with SLDF Tool Analysis (version 3.4.0) for VOL, FLW and VEL, were used to compare the findings in normal and diabetic patients (Table 3). The statistic analysis was performed using the *t*-test for VOL, given its normal distribution, and the non-parametric Mann–Whitney test for FLW and VEL with SPSS software, version 11 for Windows.

**Table 2** Retinal capillary blood volume (VOL), flow (FLW) and velocity (VEL) in healthy control (*N*) and diabetic (*D*) individuals (mean values, method C)

| Individual | VOL | FLW | VEL |
|---|---|---|---|
| N 1 | 14.82 | 220.82 | 0.76 |
| N 2 | 17.46 | 292.92 | 0.95 |
| N 3 | 13.71 | 200.77 | 0.69 |
| N 4 | 10.81 | 178.52 | 0.63 |
| N 5 | 9.62 | 146.41 | 0.52 |
| N 6 | 15.07 | 288.84 | 0.96 |
| N 7 | 8.85 | 152.28 | 0.53 |
| N 8 | 15.05 | 217.05 | 0.75 |
| N 9 | 16.44 | 290.00 | 0.98 |
| N 10 | 10.63 | 158.20 | 0.58 |
| Mean (N) | 13.25 | 214.58 | 0.74 |
| SD (N) | 2.87 | 55.30 | 0.17 |
| D 1 | 14.24 | 212.96 | 0.73 |
| D 2 | 15.66 | 282.06 | 0.95 |
| D 3 | 22.48 | 404.48 | 1.36 |
| D 4 | 33.64 | 765.13 | 2.40 |
| D 5 | 21.79 | 319.63 | 1.07 |
| D 6 | 15.88 | 259.61 | 0.88 |
| D 7 | 26.83 | 478.91 | 1.54 |
| D 8 | 20.25 | 406.40 | 1.37 |
| D 9 | 13.96 | 263.57 | 0.91 |
| D 10 | 13.76 | 215.90 | 0.75 |
| Mean (D) | 19.85* | 360.87** | 1.20*** |
| SD (D) | 6.22 | 158.70 | 0.48 |

* $P<0.010$; ** $P<0.019$; *** $P<0.015$

## Results

### Reproducibility

Reproducibility was expressed as the ratio between standard deviation (SD) and the mean of three measurements. The data from the normal volunteers and diabetic patients are presented in Table 1.

Using the 10×10 pixel box measurement window with HRF software version 1.02 (method A), the overall reproducibility was: (a) 8.16% (SD 3.62) for VOL, (b) 11.00% (SD 3.90) for FLW and (c)10.22% (SD 3.50) for VEL.

When using the SLDF software version 3.0.4. with the same window area the overall reproducibility was: (a)10.93% (SD 5.67) for VOL, (b)23.42% (SD 13.74) for FLW and (c) 22.99% (SD 13.82) for VEL.

Analyzing the whole scan with the automated full field perfusion image analysis the reproducibility was: (a) 3.52% (SD 2.92) for VOL, (b) 4.81% (SD 3.29) for FLW, and (c) 4.60% (SD 2.77) for VEL. In this group, the reproducibility for normal volunteers was: (a) 2.81% (SD 1.94) for VOL, (b) 3.74% (SD 2.09) for FLW and (c) 3.67% (SD 1.79) for VEL and for patients with type 2 diabetes: (a) 4.23% (SD 3.51) for VOL, (b) 5.87% (SD 3.87) for FLW, and (c) 5.54% (SD 3.22) for VEL.

### Normal vs. diabetes type 2 patients

Using the whole scan with automated full-field perfusion image analysis, the most reproducible method, the normal population showed mean values of: (a) 13.25 (SD 2.87) for VOL, (b) 214.58 (SD 55.30) for FLW and (c) 0.74 (SD 0.17) for VEL, whereas the diabetic population showed mean values of: (a) 19.85 (SD 6.22) for VOL, (b) 360.87 (SD 158.70) for FLW and (c) 1.20 (SD 0.58) for VEL (Table 2).

Statistically significant differences in VOL, FLW and VEL were found between diabetic and normal subjects. The mean values of the diabetic population were in each case higher than those for the normal population, with *P* of 0.010, 0.019 and 0.015 for VOL, FLW and VEL, respectively. These statistically significant differences are mainly due to five eyes (nos. 3, 4, 5, 7, 8) with four of these eyes (3, 4, 7, 8) showing values for VOL, FLW and VEL above the mean + 2SD for the normal control eyes. The fifth eye (no. 5) showed a marked increase in

**Table 3** Metabolic characterization of diabetic patients at time of examination. *GLU* Blood glucose (mg/dl), *HbA1C* hemoglobin A1C (%), *T Chol* total cholesterol (mg/dl), *HDL* high density lipoproteins (mg/dl), *LDL* low density lipoproteins (mg/dl), *Trigl* triglycerides (mg/dl), *BMI* body mass index (kg/m$^2$)

| Patient | Age (years) | Sex | Duration (years) | GLU | HbA1C | Creat | T Chol | HDL | LDL | Trigl | BMI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D 1 | 53 | M | 5 | 109 | 6.1 | 1.0 | 273 | 46 | 154 | 340 | 22.4 |
| D 2 | 55 | M | 5 | 121 | 6.4 | 0.9 | 288 | 47 | 165 | 353 | 23.0 |
| D 3 | 64 | M | 6 | 133 | 6.4 | 0.9 | 247 | 45 | 139 | 161 | 27.0 |
| D 4 | 58 | M | 5 | 130 | 6.2 | 0.9 | 249 | 45 | 142 | 180 | 22.0 |
| D 5 | 55 | M | 6 | 169 | 7.2 | 0.9 | 196 | 40 | 99 | 280 | 38.2 |
| D 6 | 56 | M | 7 | 112 | 6.2 | 0.9 | 243 | 45 | 136 | 260 | 23.0 |
| D 7 | 52 | M | 6 | 149 | 7.0 | 0.9 | 255 | 53 | 116 | 212 | 23.5 |
| D 8 | 57 | F | 5 | 255 | 6.0 | 0.8 | 260 | 55 | 154 | 105 | 27.6 |
| D 9 | 59 | M | 5 | 168 | 6.5 | 0.9 | 193 | 34 | 112 | 121 | 28.0 |
| D 10 | 59 | M | 6 | 268 | 7.2 | 0.8 | 230 | 53 | 134 | 118 | 27.4 |

all parameters, although only the VOL value was raised above the mean + 2SD of the normal control eyes. The eyes that showed these marked increases in capillary blood flow did not show any consistent correlation between the capillary blood flow alterations and the systemic metabolic parameters evaluated (Table 3).

## Discussion

Studies on retinal blood flow in diabetes have given conflicting results, apparently due to the complexity and variability of the measurement methods available for clinical use.

We have, in this study, used the HRF, which is based on scanning laser Doppler methodologies and is technically restricted to the performance of reliable measurements of blood flow in small vessels of the retinal capillary superficial layers. Firstly, we compared protocols and available software used by different authors in previous studies [5,13, 16, 17] to examine their reproducibility. Three different protocols were compared in a series of healthy eyes and in eyes with preclinical retinopathy from subjects with type 2 diabetes. Retinal capillary blood flow measurements performed in the papillomacular area, using whole-scan analysis and the automated full-field perfusion image analysis proposed by Michelson et al. [14], showed very good reproducibility in both healthy and diabetic eyes (Table 1). The reproducibility of the method in diabetic patients is lower than in normal volunteers, but the differences are acceptable, with mean reproducibility remaining below 6%. This methodology involves automatic subtraction of large vessels and takes into account heartbeat-associated pulsation, artificial movements and local variations in brightness of the fundus.

When comparing the retinal capillary blood flow measurements obtained from the papillomacular area with the HRF using the automated full field perfusion image analysis method in diabetic eyes with preclinical retinopathy and in healthy control eyes, there was an overall statistical significant increase in retinal blood flow in the diabetic eyes. However, when analyzing the results obtained in each eye it became clear that this increase in retinal blood flow varied markedly between them. Five of the ten diabetic eyes showed clearly abnormal increases in retinal capillary blood flow, i.e. with four of them presenting values higher than the mean + 2SD of the values registered for all parameters (volume, flow and velocity) in the normal control group. The other five diabetic eyes showed values within the normal range.

These findings may have particular relevance. They may explain the conflicting reports in the literature and indicate that changes in retinal capillary blood flow are an early alteration in the diabetic retina, but do not occur to the same degree or at the same time in every retina. They may develop as a result of other retinal alterations, and may be of particular value in indicating the eyes that are at risk for progression of retinopathy.

In this study, the increases in retinal capillary blood flow registered in five of the ten diabetic eyes with preclinical retinopathy did not show any clear correlation with level of metabolic control (HgA1C), blood glucose values on the day of the examination, duration of the disease, blood pressure levels or other systemic variables. This is in agreement with the observations of Cuypers et al. [5].

There are several possible explanations for the increase in capillary blood flow in diabetes [11]. An increase in red blood cell capillary flow could indicate shunting, an increase in capillary diameter, or capillary recruitment.

The flow of red blood cells through retinal capillaries is modulated by intravascular and extravascular factors. The intravascular pressure gradient between the precapillary arteriole and the postcapillary venule is considered to be the most important regulator of capillary flow. In this

study, all patients with type 2 diabetes had similar and acceptable levels of blood pressure. On the other hand, an increase in capillary diameter is considered to have a relatively small effect on the pressure gradient and, therefore, results in a small decrease in capillary flow [10]. Shunting phenomena or capillary recruitment are the most likely candidates to explain the marked increase in capillary red blood cell flow observed in five of the ten diabetic eyes.

It is thought that under normal physiological conditions most retinal capillaries are perfused by both plasma and red blood cells. Fluorescein angiographic studies indicate that retinal capillaries are continuously perfused. However, the fluorescein method does not distinguish between flow of plasma alone and flow of plasma and red cells together.

It is accepted that in the brain, in a small fraction of capillaries, red blood cell perfusion may stop for brief periods – not longer than a few seconds – indicating some degree of intermittence in red blood cell flow in the capillaries.

Whether capillaries open and close at rest, and during adaptation of capillary blood flow to changing metabolic needs, is still a matter of controversy. Functional "thoroughfare channels" or preferential capillaries with high resting flow have been proposed to play a central role in the microcirculation of the brain, surrounded by other capillaries, characterized by slow resting flow, which could be recruited when the tissue blood supply is challenged [7].

Finally, another possibility is that in the retinal capillaries plasma flow is continuous, but red blood cells travel through only a proportion of all capillaries at all times. In this case, capillary recruitment would be a natural response either to increased metabolic demands by the retinal tissue in diabetes [8] or to a situation of relative hypoxia as proposed for the diabetic retina [6].

An important contribution to clarifying these questions may be given by the application of the analytical procedure for measurement of intercapillary distances developed by Michelson et al. [14].

It is interesting to mention here that one of the earliest abnormalities considered to be observed in the diabetic retina on clinical examination is an increased visibility of the superficial retinal capillary network.

It now needs to be clarified which eyes are at risk of progression to retinopathy: the eyes which show increased capillary blood flow, thus apparently creating conditions for more rapid and progressive damage of the capillary walls, or the eyes that do not show this autoregulatory response. It remains a possibility that the near normal values for capillary blood volume, capillary blood flow and capillary blood velocity registered in five of the ten diabetic eyes indicate failure of their autoregulatory response.

In this pilot study, there were no apparent correlations between capillary blood flow alteration and the metabolic alteration of the diabetic patients at the time of the examination. This observation suggests that the capillary blood flow alterations registered are not acute and transitory, but may indicate a more permanent status of the retinal circulation.

Laser Doppler flowmetry of the retinal capillary circulation using whole-scan automated full-field perfusion image analysis is, in normal healthy individuals and diabetic patients with preclinical retinopathy, a reproducible method of examination. It offers direct information on the retinal capillary blood flow that cannot be obtained by other available methods used for the study of retinal blood flow, such as color Doppler imaging [9] or fluorescein angiography dye-dilution curves [18], which analyze information obtained from the larger retinal vessels.

Our observations indicate that in some diabetic eyes, even before the development of visible retinopathy, there is (probably due to local factors) a marked increase in retinal capillary blood flow, with the maximal utilization of the retinal capillary net, whereas others do not show this circulatory response. It now remains to be seen which one of these two groups of eyes is more vulnerable, and at risk of rapid development and progression of retinopathy.

A prospective follow-up study of diabetic eyes such as the ones included in this study, to identify the onset of morphological changes detected by funduscopy and fundus photography, is clearly the next step. It is considered important to verify which eyes develop ophthalmoscopically visible retinopathy first: eyes showing increased capillary blood flow or eyes with near-normal values in spite of their diabetes.

## References


1. Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL 3rd, Klein R (1998) Diabetic retinopathy (technical review). Diabetes Care 21:143–156
2. Boeri D, Maiello M, Lorenzi M (2001) Increased prevalence of microthromboses in retinal capillaries of diabetic individuals. Diabetes 50:1432–1439
3. Bursell SE, Clermont A, Kinsley B, Simonson, DC, Aiello LM, Wolpert HA (1996) Retinal blood flow changes in patients with insulin-dependent Diabetes mellitus and no diabetic retinopathy – a video fluorescein angiography study. Invest Ophthalmol Vis Sci 37:886–897
4. Cunha-Vaz JG, Fonseca JR, Abreu F de, Lima JJP (1978) Studies on retinal blood flow. II. Diabetic retinopathy. Arch Ophthalmol 96:809–811
5. Cuypers MH, Kasanardjo JS, Polak BC (2000) Retinal blood flow changes in diabetic retinopathy measured with the Heidelberg scanning laser Doppler flowmeter. Graefes Clin Arch Exp Ophthalmol 238:935–941
6. Ditzel J, Standl E (1975) The problem of tissue oxygenation in diabetes mellitus. Acta Med Scand [Suppl] 578, 49–58

7. Hasegawa T, Ravens JR, Toole JF (1967) Precapillary arteriovenous anastomoses. “Thoroughfare channel” in the brain. Arch Neurol 16:217–224
8. Kageman L, Harris A, Chung HS, Costa VP, Garzozi H (1999) Basics and limitations of color Doppler imaging. In: Pillmat LE, Harris A, Anderson DR, Greve EL (eds) Current concepts on ocular blood flow in glaucoma. Kugler, The Hague, pp 103–110
9. Kageman L, Harris A, Chung HS, Evans D, Buck S, Martin B (1998) Heidelberg retinal flowmetry: factors affecting blood flow measurement. Br J Ophthalmol, 82:131–136
10. Keen H. Chlouverakis C (1965) Metabolic factors in diabetic retinopathy. In: Graymore CN (ed) Biochemistry of the retina. Academic Press, New York, p123
11. Knuese DE, Feher G, Hudetz AG (1994) Automated measurement of fluorescently labeled erythrocyte flux in cerebrocortical capillaries. Microvasc Res 47:392–400.
12. Kohner EM, Hamilton AM, Saunders SJ, Sutcliffe BA, Bulpitt CJ (1975) The retinal blood flow in diabetes. Diabetologica 27:48–52
13. Lobo C, Bernardes R, Faria de Abreu J, Cunha-Vaz J (2001) One-year follow-up of blood-retinal barrier and retinal thickness alterations in type 2 mild nonproliferative diabetic retinopathy. Arch Ophthalmol 119:1469–1474
14. Michelson G, Welzenbach J, Pal I, Harazny J (1998) Automatic full field analysis of perfusion images gained by scanning laser Doppler flowmetry. Br J Ophthalmol 82: 1294–1300
15. Michelson G, Welzenbach J, Pal I, Harazny J (2001) Functional imaging of the retinal microvasculature by scanning laser Doppler flowmetry. International Ophthalmol 23:327–33
16. Nicolela MT, Hnik P, Schulzer M, Drance SM (1997) Reproducibility of retinal and optic nerve head blood flow measurements with scanning laser Doppler flowmetry. J Glaucoma 6: 157–64
17. Strenn K, Menapace R, Rainer G, Findl O, Wolzt M, Schmetterer L (1997) Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in $PO_2$. Br J Ophthalmol 81: 360–364
18. Sullivan PM, Davies GE, Caldwell G, Morris AC, Kohner EM (1990) Retinal blood flow during hyperglycemia: a laser Doppler velocimetry study. Invest Ophthalmol Vis Sci 31:2041–2045
19. Wolf S, Arend O, Sponsel WE, Schulte K, Cantor LB, Reim M (1993) Retinal hemodynamics using scanning laser ophthalmoscopy and hemorheology in chronic open-angle glaucoma. Ophthalmology 100:1561–1566