Retinal Blood Flow Evaluation

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Key Words

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Abstract

Much of our basic knowledge of retinal blood flow regulation is based on data obtained from animal experiments through the use of invasive techniques. However, during the last decades, major developments in the field of optics and lasers have led to a variety of noninvasive techniques, which have been applied to the human eye for the investigation of retinal hemodynamics, and more specifically the regulation of retinal blood flow in response to a number of physiological and pharmacological stimuli. The Retinal Vessel Analyzer has markedly simplified the measurement of the diameter of retinal vessels, as well as the change in this diameter evoked by various physiological stimuli (dynamic measurements). Bidirectional laser Doppler velocimetry allows the measurement of absolute red blood cell centerline velocity, which, when combined with the diameter allows the calculation of retinal blood flow in the main retinal vessels. Laser Doppler flowmetry and laser speckle flowgraphy are techniques that

measure the velocities of blood in discrete areas of the retinal tissue microcirculation. Adding a scanning capability, a spatial map of velocities across the retinal tissue is obtained. The blue-field simulation technique allows the quantification of the velocity, number and velocity pulsatility of leukocytes moving in the retinal capillaries of the macular region. With color Doppler imaging, the peak systolic and end-diastolic values of blood velocity in the ophthalmic and central retinal artery are measured, from which a resistivity index is obtained. These techniques may help better understand the role of altered retinal blood flow and its regulation in the pathogenesis of retinal diseases of vascular origin.

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Introduction

The retinal circulation supplying the fundus of the eye can be observed using optical instruments that allow the recording of either the vessel diameter or the passage of dyes through the human retinal vascular system, from which the first quantitative measurements of retinal hemodynamics were derived [1].

Technological developments in the field of optics and lasers have since led to a variety of noninvasive tech-

niques, which have permitted the investigation of various parameters pertaining to human ocular hemodynamics and the response of these parameters to a number of physiological and pharmacological stimuli.

These techniques have provided information on human retinal circulatory physiology and have led to new, important information on the role of retinal blood flow in the pathogenesis of retinal diseases of vascular origin.

General Hemodynamic Considerations

Blood flow (*BF*) through a blood vessel depends upon the perfusion pressure (*PP*), i.e. the pressure that drives blood through the vessel, and the flow resistance (*R*) generated by the vessel. For an incompressible uniform viscous liquid (dynamic viscosity, η) flowing through a cylindrical tube (length *L*) with radius (*r*), *BF* is given by the Hagen-Poiseuille law: $BF = PP/R$, where $R = \eta L/2\pi r^4$. Many factors make it difficult to directly apply this law to a microvascular bed. These include the η dependence on local hematocrit, the changes in the velocity profile of the red blood cells (RBCs) and shear rate at branchings and junctions and others.

Another approach at characterizing *BF* through a system of blood vessels is based on Murray's law [2], which says that through each vessel of a circulatory system with optimal design (blood flowing with minimal loss of energy) $BF = k(r^3/\sqrt{\eta})$. The constant *k* depends upon *L* and *r* [3].

The mean ocular *PP* driving blood through the eye is the mean blood pressure in the ophthalmic artery minus the pressure in the veins leaving the eye. The venous pressure is close to the intraocular pressure (IOP) [4]. With the subject in sitting or standing position, mean ocular *PP* is about 2/3 of the mean brachial artery blood pressure (*ABP*), i.e.

$$PP = 2/3 [ABP_{diast} + 1/3 (ABP_{syst} - ABP_{diast})] - IOP.$$

The factor 2/3 stands for the drop in pressure between the heart and the ophthalmic artery. ABP_{diast} and ABP_{syst} are the brachial *ABP* during diastole and systole, respectively. It is understood that this expression for *PP* is based on a group average and therefore provides only an approximate value for a single individual. The value of η has been experimentally related to hematocrit at defined shear rates. It diminishes with increasing shear rate to become almost constant with further decreases of this rate [5]. An increase in viscosity (e.g. in cases of hyperglobulinemia, high hematocrit, leukemia, sickle cell anemia) substantially alters retinal *BF*, which may induce stasis in the veins and ultimately their occlusion [6].

The main resistance to *BF* is located in the arterioles with half of the resistance in vessels with a radius of 10–25 μm . As *R* is proportional to $1/r^4$, even a small change in *r* will have a considerable effect on *R*; *r* and consequently the diameter (*D*) of a vessel is modulated by the interaction of multiple systemic and local control mechanisms affecting the tone of the smooth muscle cells and perhaps the pericytes.

Alterations in retinal vessel *D* have been linked to several vascular related pathologies, including systemic hypertension and diabetes, in large population-based studies. Abnormal retinal vascular regulation may also be identified through metabolic provocation, such as flicker stimulation of the retina. Thus, the ability to obtain exact measurements of *D* is of crucial importance to our understanding of retinal *BF* and its regulation during the evolution of retinal ischemic microangiopathies.

Noninvasive Techniques Used in Physiological and Clinical Research

In the last decades the development of noninvasive techniques has led to new and important information on retinal hemodynamics and *BF* regulation in the healthy and diseased human eye. These techniques include the measurements of the following retinal hemodynamic parameters: *D*, using photography or video recording and the newly developed Retinal Vessel Analyzer (RVA); mean circulation time (*MCT*) and arteriovenous passage time (*AVP*) of fluorescein through retinal segments by the dye dilution technique; velocity and number of leukocytes in the macular area by the blue-field simulation technique; velocity of fluorescent leukocytes and hyperfluorescent segments in retinal capillaries by video angiography; velocity and RBC flow in the main retinal vessels using bidirectional laser Doppler velocimetry (BLDV) combined with the measurement of *D*; blood flow in the tissue of the optic disk and peripapillary retina by laser Doppler flowmetry (LDF) and central retinal arterial blood velocity by color Doppler imaging.

Measurement of D of Retinal Vessels

D has been measured in the past from magnified fundus photographs using a caliper or by scanning across the vessels [7, 8]. In recent years, the RVA has markedly simplified this measurement [9, 10], allowing also the quasi-continuous recording of *D* changes evoked by various physiological maneuvers (dynamic measurements) (fig. 1).

In principle, the RVA assesses D by analyzing the brightness profile of the vessel. This assessment is based on the fact that the green light used for fundus illumination is mostly absorbed by the RBCs within the retinal vessels, whereas the environment mostly reflects this light. Thus, the RVA measures mainly the width of the RBC column within the selected vessels.

The interpretation of the vessel brightness map is hampered by the possible occurrence of several disturbances, such as shadowing structures or reflections on the vessel surface, which complicate the analysis. To overcome this problem, the RVA uses an adaptive algorithm based on variation in brightness, which compensates for reflections and other disturbances that occur during measurement.

With the RVA, a vessel is scanned 25 times/s within a rectangular window having a length of about 1.5 mm and a width slightly larger than the diameter of the vessel. After determination of the angle between the vessel and the direction of the window, D is calculated automatically. The RVA is particularly well suited for the evaluation of the temporal dynamic of D in response to various physiological stimuli, pharmacological and therapeutic agents (fig. 1). The technique requires dilatation of the pupil. Using vessels with a $D > 90 \mu\text{m}$, it is possible to obtain a precision in D of the order of $1 \mu\text{m}$. Temporal resolution of D recordings is about 40 ms.

Studies have reported that long- and short-term reproducibility of D measurements is slightly higher for retinal veins than for retinal arteries, probably reflecting a better optical resolution of veins (in comparable fundus locations, veins are larger than arteries) or differences in the absorption properties of arteries compared with veins [10].

The short-time coefficient of variation (CV) of D was assessed in a group of 9 healthy volunteers. The CVs of D for measurements taken 12 min apart have been reported to be 1.3 and 2.6% for retinal veins and retinal arteries, respectively. The CVs for the day-to-day variability of D were 4.4% for retinal veins and 5.2% for retinal arteries in the same group of volunteers [11].

D of retinal veins was assessed continuously for 5 min in 12 healthy volunteers and the measurements were repeated at the same vessel location after 2 h. This resulted in a short-term CV of D of 1.5% for retinal veins. The same study assessed long-term reproducibility comparing measurements at baseline and after 1 month. The reported CV of D was 2.8% for retinal veins [12]. CV of D , determined for short-term (less than 2 h) and long-term (2 weeks), was 1.5 and 2.8%, respectively [10].

Fig. 1. a The RVA device consists of a retinal camera, a charge-coupled device (CCD)-measuring camera for electronic online image acquisition, and a computer for system control analysis and archiving of the D data. In general, a vessel section of about 1.5 mm in length is scanned at a frequency of 25 images/s. **b** Time course variation of D of a retinal vein measured by the RVA over a given vessel length from P1 to P2. Vein constriction is due to inhalation of 100% oxygen by the subject.

Flicker Stimulation

Following the first demonstration that D of retinal vessels can be modulated by flickering the fundus illumination light [13] the use of the RVA has permitted the determination of the time course of the flicker-induced D response and of the effect of varying the flicker frequency on this response [14].

There is now compelling evidence from studies in animals and human subjects that visual stimulation with flickering light increases retinal vessel diameter, retinal blood flow and optic nerve head (ONH) blood flow in humans [15]. Therefore, stimulation with flicker light has been used as a physiological provocation to investigate the regulation of vascular tone. Further investigations in a large number of subjects might generate new insight into the reliability of flicker data. This is important for the design and planning of future studies.

Clinical Use

Measurements of D are important, not only for scientific purposes, but also because several large-scale epidemiological studies have consistently reported the existence of a correlation between systemic disease factors and retinal vessel D . In particular, increased systemic blood pressure is reflected in the generalized arterial vasoconstriction of retinal vessels [16]. Thus, there is increasing evidence that changes in the retinal vessel D not only carry information about the retinal circulation per se, but may also reflect systemic pathologies as such changes have been shown to predict risk for coronary heart disease, stroke and stroke mortality [17, 18]. Pooled data from the Beaver Dam Eye Study and the Blue Mountains Eye Study also showed that smaller arterial D and larger retinal venous D are associated with increased risk for stroke mortality [19]. These data clearly support the suggestion that retinal vessel D may serve as a predictor for events in other vascular beds, such as in the heart or brain.

It has been shown that changes in retinal vessel D observed during systemic pathologies may be influenced by treatment. In particular, D was found to be significantly reduced in diabetic patients who underwent laser treatment [20]. This finding has been interpreted as a sign for decreased blood flow due to better oxygenation of the photocoagulated tissue.

Dye Dilution Technique

The dye dilution technique allows the determination of MCT , i.e. the average time it takes for dye molecules, such a fluorescein injected into the circulation, to travel from the entry to the exit of a microvascular segment. The segment is assumed to have a single inflow (feeding artery) and a single outflow (draining vein). The passage of fluorescein through the artery and corresponding vein (arterial and venous dilution curves) is recorded by detecting photographically, photoelectrically, or by video the fluorescence intensity emitted by the dye, when the fundus is illuminated with light at wavelengths within the range of the excitation spectrum of fluorescein (blue). A barrier filter prevents excitation light from reaching the detection system. The dye dilution technique provides reliable data on MCT only if the vascular segment, the injected dye, the mode of recording of the dilution curves, and the correction for recirculation of the dye satisfy specific conditions [21–23]. In the application of this technique to the eye, particularly in cases of retinal vascular pathology (for instance, in proliferative retinopathy, diffusion of fluorescein from the retinal vessels may distort

the dilution curves), the measured MCT must be regarded as an approximation of true MCT [24].

Using scanning laser ophthalmoscopy, additional parameters from the fluorescence intensity time course have been determined, such as the mean velocity of the dye in a retinal artery, which has been calculated from the appearance time of the dye front at two sites along a vessel and the distance between the sites, and the AVP , which is the time difference between a reference point at the temporal retinal artery and the first appearance in a vein adjacent to the artery [25]. In contrast to MCT , AVP favors strongly the measurement of passage times of the dye through the shortest segment between an artery and a vein close to the papilla [24].

A study in monkeys, undertaken to determine the correlations between MCT and AVP and retinal BF , the latter measured by labeled microspheres, showed that these correlations were not statistically significant ($p > 0.05$) [26], even when the MCT was calculated using an impulse response technique [27]. Clearly, MCT and AVP data should not be interpreted in terms of BF .

Velocity of Leukocytes

The velocity of leukocytes (V_{leuk}) has been measured by means of scanning laser ophthalmoscopy after tagging the cells with various dyes and tracking their motion in the retinal capillaries, veins and arteries on video images [28–32].

V_{leuk} of leukocytes moving in perifoveal retinal capillaries (diameter 7–11 μm) measured by scanning laser ophthalmoscopy was reported to be 1.4 mm/s [31]. Using the blue-field simulation technique, which is based on the entoptic observation of one's own leukocytes moving in the macular area of the retina, it is possible to determine quantitatively the number (N_{leuk}), V_{leuk} , and V_{leuk} pulsatility of these particles [33]. With this technique, subjects are asked to compare and match the global motion of a field of computer-simulated leukocytes displayed on a video monitor to the global motion of their own leukocytes by adjusting with potentiometers N_{leuk} , V_{leuk} , and V_{leuk} pulsatility (variation of the speed during the cardiac cycle) of the simulated leukocytes [34]. Mean V_{leuk} obtained under physiological conditions by various investigators ranged from 0.23 to 1.9 $\mu\text{m/s}$ [35–39]. The motion of these leukocytes is pulsatile in phase with the heartbeat, with an average $V_{leuk, systole}/V_{leuk, diastole}$ of 2.8 [33].

Visual acuity must be better than 20/50 for a reliable measurement [40]. The ability of a subject to do the blue-field simulation test can be assessed by having subjects

Fig. 2. a Doppler effect. The frequency of laser light scattered by an RBC (moving at speed V_{max}) is shifted by an amount Δf_{max} compared to the frequency of the light incident (f_i) on the RBC. α_i and α_s are the angles between V_{max} and the directions of the incident and scattered light, respectively. λ is the wavelength of the incident light [with permission from ref. 42]. **b** Principle of bidirectional LDV (BLDV). Laser light scattered from the RBCs is detected along two directions: K_s^1 and K_s^2 (angle α between them). β is the angle between the plane of the vessel at the site of measurement and the direction of the velocity. V_{max} , the centerline velocity of the RBCs, is determined from the maximum shifts in the Doppler shift power spectrum obtained in each scattering direction and the geometrical scattering parameters. The measurement is independent from the direction of the incident light defined by K_{in} [with permission from ref. 43].

match the speed and number of two simulated leukocyte motions displayed on computer screens. Blue-field data have been confirmed by the objective scanning laser ophthalmoscope-adaptive optics imaging technique [41].

Bidirectional Laser Doppler Velocimetry

BLDV allows the measurement of *absolute* blood velocity. BLDV is based on the Doppler effect (fig. 2) [42]. Retinal *BF* (in $\mu\text{l}/\text{min}$) in the main retinal vessels is calculated from the centerline velocity (V_{max}) of RBCs [43].

$$V(d) = V_{max} \left[1 - \frac{|d - d_o|^K}{R_t^K} \right]$$

Fig. 3. Central velocity (V_{max}) of RBCs (proportional to cutoff frequency of Doppler shift power spectrum) in a retinal artery (diameter, $100 \mu\text{m}$). $V(d)$ is the velocity at distance (d) from the inner wall, d_o the position of the vessel center. K is 2.38 in the systolic, 1.94 in the diastolic phase [from ref. 47, with permission].

The measurement is independent of the direction of the incident light defined by K_{in} (fig. 2) [43, 44]. Combined with D measurements of these vessels mean *BF* is calculated as $\pi \times D^2 \times V_{mean}/4$. V_{mean} represents an average of the RBC velocities over the vessel cross section. For a parabolic velocity profile (see below), $V_{mean} = V_{max}/2$. The average RBC velocity during the heart cycle is obtained by integrating V_{mean} over this cycle.

In straight portions of the first-order retinal arteries and veins of the human eye, the velocity profile of RBCs does not differ significantly from the parabolic shape during both systole and diastole (fig. 4) [45–48]. Deviations from the parabolic profile increase, however, with the diminution of the diameter of the vessels and a correction factor in the formula given in figure 3 is needed to improve the fit [47, 49]. At retinal arterial branchings and venous junctions marked deviations from the parabolic profile are expected, as illustrated by measurements using the techniques of confocal scanning laser Doppler velocimetry (fig. 4) [47].

Averaged over the cardiac cycle, V_{max} ranges from approximately 7 to 35 mm/s in arteries with a D between 40 and $130 \mu\text{m}$ and from 5 to 25 mm/s in veins with a D between 60 and $180 \mu\text{m}$ [50–52]. Similar values were found in the primate retina with the targeted dye delivery technique [53, 54]. In normal human and primate monkey eyes, V_{max} increases linearly with D , as found in most vascular beds [50, 54].

Retinal *BF* represents only about 4% of total ocular *BF* [55]. In humans, recent measurements of total retinal *BF* (40.8 – $52.9 \mu\text{l}/\text{min}$) using Fourier-Domain OCT (FD-OCT) [56] are in the range of values obtained by BLDV [50]. Retinal *BF* calculated from BLDV is larger in the temporal human retina than in the nasal region, supposedly due to the larger size (by 20–25%) and higher meta-

Fig. 4. Velocity profiles of RBCs in a retinal vein downstream of a venular junction. At a distance of about one (A) and two (B) vessel diameters from the junction, the velocity profiles are asymmetric. At 5 diameters downstream from the junction (C), the profile is symmetric, although still significantly blunted. Data collected in the systolic (closed circles) and diastolic (open circles) phase of the cardiac cycle. The continuous line (C) is the fit according to the equation given in figure 3, with $K = 2.38 \pm 0.5$ in the systolic and 1.94 ± 0.80 (95% CI) in the diastolic phase, respectively [from ref. 47, with permission].

bolic rate of the former. *BF* values in the superior and inferior hemispheres were found to be similar. In contrast, *BF* in the superior retinal hemisphere does not appear to differ from *BF* in the inferior hemisphere [50, 57–59]. This pertains also to *BF* in the superior and inferior parts of the macular region [60].

In normal subjects, *BF* differs between studies, with values between 30 and 46 $\mu\text{l}/\text{min}$ [50, 52, 56, 61, 62] and 65–80 $\mu\text{l}/\text{min}$ [57, 59, 63]. By comparison, the microsphere injection technique provided volumetric flow

rates of 25 and 34 $\mu\text{l}/\text{min}$ for the macaque monkey and $50 \pm 39 \mu\text{l}/\text{min}$ for the rhesus monkey [64, 65].

A stabilized retinal laser Doppler instrument adapted to a fundus camera achieved excellent reproducibility of results and *BF* measurements in units of microliters per minute, indicating that the instrument can be used for reliable comparison of blood flow characteristics at different retinal vascular sites in the same eye, at comparable sites in both eyes, and for comparison between patients and healthy control subjects [66].

Doppler FD-OCT

OCT provides high-resolution cross-sectional imaging and is commonly used in the diagnosis and management of retinal diseases. In addition to obtaining morphological images, OCT can also detect the Doppler shift of reflected light, which provides information on three-dimensional distribution of the axial velocity component of blood in retinal vessels [67, 68]. For Doppler FD-OCT [69, 70] light reflected by moving blood induces a Doppler frequency shift that is proportional to the velocity component parallel to the axis of the probing beam. This frequency shift introduces a phase shift in the spectral interference pattern that is captured by a line camera. The spectral information is converted into complex axial scans containing both amplitude and phase, using the fast Fourier transform. The phase differences between sequential axial scans at each pixel are calculated to determine the Doppler shift. In vivo flow measurements in branch retinal vessels have been reported using Doppler FD-OCT [71, 72].

Doppler FD-OCT combined with a double circular scan pattern around the optic disk to rapidly measure total retinal *BF* has been used in a group of normal human subjects [56]. Four pairs of circular scans that transected all retinal branch vessels were completed in 2 s. Total retinal *BF* was obtained by summing the flows in the branch veins. Total retinal *BF* could be measured in 8 of 10 subjects: mean (SD) = 45.6 (3.8) $\mu\text{l}/\text{min}$ (range 40.8–52.9 $\mu\text{l}/\text{min}$). The coefficient of variation for repeated measurements was 10.5%. These flow values are within the range previously established by laser Doppler velocimetry combined with *D* measurements [56].

Laser Doppler Flowmetry

In a tissue, such as the optic disk, *BF* can be measured by using LDF [73]. Two measurement modes have been implemented: In the first mode, a laser beam is focused onto the tissue and the light scattered from this tissue is detected to obtain the Doppler shift power spectrum re-

Fig. 5. Perfusion maps obtained from the ONH and temporal retina of a normal eye. From top to bottom: reflectance image, volume map, flow map, velocity map. The perfusion maps are color-coded; light colors show high perfusion values [from ref. 79, with permission].

sulting from the motion of RBCs. Applying the theory of Bonner and Nossal [74], the following flow parameters are derived from the Doppler shift power spectrum: *Vel*, the mean speed of the RBCs moving in the sampling volume, *Vol*, the number of moving RBCs and $BF = k \times Vel \times Vol$, the total flux of RBCs in this volume. *Vel* is expressed in Hertz, and *Vol* and *F* in arbitrary units; *k* is a constant of proportionality. With the second mode, scanning the laser beam across a two-dimensional area of the fundus provides an image of the RBC flux in the capillaries of the optic disk and peripapillary retina, as well as an intensity image of the perfused retinal vessels [75] (fig. 5).

Laser Speckle Flowgraphy

The temporal variations of laser speckle resulting from the interference of laser waves scattered by the tissue can be used to determine the velocity of RBCs in the ONH, retinal and choroidal circulations [76, 77]. This laser speckle flowgraphy and the LDF approaches are different ways of looking at the same phenomenon. Both techniques measure at a single point in the tissue. In both

cases, adding scanning provides a map of the spatial velocity and flux [78, 79].

One important point when applying these laser-based techniques is that the measured flux depends on the scattering and optical absorption properties of the tissue. Therefore, direct comparison between flux values from different eyes may not be valid due to variations in the scattering properties resulting from differences in tissue structure and composition [80, 81]. Furthermore, for a valid comparison between flux values obtained at different times in the same eye by dynamic and scanning LDF and laser speckle flowgraphy, the tissue must be assumed to maintain the same scattering properties over time, which may not be the case during the development of various pathologies.

Color Doppler Imaging

Color Doppler imaging for retrobulbar vessels is an ultrasound technique that combines B-scan imaging of tissue structure, color representation of blood flow based on Doppler-shifted frequencies, and pulse-Doppler measurements of blood velocities. The flow parameters measured are the peak-systolic velocity (*PSV*), the end-diastolic velocity (*EDV*), and the resistivity index $RI = (PSV - EDV)/PSV$ [82]. The application of color Doppler imaging to ophthalmology has provided data on central retinal arterial *PSV* and *EDV* [83]. A nonexhaustive review of the literature between 1991 and 2006 reveals that the average *PSV* and *EDV* values in normal subjects range from about 6 to 20 and 1.7 to 10 cm/s, respectively [24, 84].

Modifications of Retinal Blood Flow in Ischemic Microangiopathies

Branch Retinal Vein Occlusion

Branch retinal vein occlusion (BRVO) most commonly occurs at an arteriovenous crossing where arteries and veins are bound together in a common adventitia. As a consequence, arteriosclerotic changes can disturb *BF* in the venule, damage the endothelial cells and leading to thrombus formation [85, 86]. Clinical and angiographic findings have confirmed disturbances of venous *BF* and, exceptionally, reveal complete flow interruption. The importance of measuring retinal *BF* in retinal venous occlusions as an indicator of the severity of microcirculatory disorders has been discussed. In particular, blood velocity appears to provide an important criterion for the assessment of the stasis conditions and the diameter of the vessels offers essential information on local regulative

processes [87–89]. Decreased [90] or retrograde [91] *BF* into the arterioles, as well as blood flowing from the venules into the capillaries [92], were observed in monkey and cat models of BRVO, respectively. In humans, pulsatile venular outflow and reverse *BF* in arterioles during diastole have been observed with videoangiography [93]. Scanning LDF has documented a decreased *BF* in retinal capillary areas affected by BRVO [94].

In experimental BRVO, a decline of preretinal nitric oxide by 75% down to 25% of its baseline value was observed at 2 h post-occlusion. It occurred simultaneously with a decrease of the arteriolar *D* in the affected territory [95]. Such arteriolar constriction could be reversed by addition of nitric oxide donors [96], confirming that BRVO impairs the retinal nitric oxide-producing metabolic process responsible for the maintenance of the vascular tone [97].

Endothelium-driven myogenic vasoconstriction, related to the increased pressure within an occluded venule and the resulting increase in transmural pressure in the arterioles irrigating areas affected by BRVO [98], represents an additional mechanism involved in the arteriolar vasoconstriction. The resulting arteriolar vasoconstriction is probably mediated by the endothelium through interactions between ET-1 and ETA receptor. In patients with ischemic BRVO, ET-1 plasma concentrations are increased. This situation may aggravate vasoconstriction of the retinal arterioles, thus exacerbating ischemia [99, 100]. A significant increase in retinal arteriolar *D* was demonstrated after juxta-arteriolar endothelin A receptor inhibitor BQ-123 microinjection in healthy and in acute BRVO minipig retinas. The results suggest a role for endothelin-1 in maintaining retinal basal arteriolar tone. Reversing the BRVO-related vasoconstriction by juxta-arteriolar BQ-123 microinjection could bring a new perspective to the management of BRVO [101].

Diabetic Microangiopathy

The hypothesis that altered retinal *BF* plays a role in the development of diabetic retinopathy (DR) was made more than 25 years ago [102]. It has since led to numerous investigations of *BF* in the various tissues of the eye in this disease aimed at getting some insight into the progression of DR. At present this knowledge is rather controversial since some of the observations appear to be contradictory [103, 104]. Discrepancies in the findings may be due to differences in the type of patients, i.e. type I versus type II diabetes, controlled versus poorly controlled diabetes, improvement in the degree of glucose control over the years, short versus long duration of the disease, and

presence or absence of other concomitant diseases, such as systemic hypertension [105]. Increased *D* of the retinal arterioles and veins is seen early in the disease [105, 106]. On the other hand, retinal *BF* seems to be unaffected in eyes with well-regulated diabetes until more severe retinopathy develops [106–108]. In patients with proliferative DR, retinal hemodynamics seem to depend on the specific pathologic features. For example, reduced retinal *BF* and vessel staining seem to be associated with severe capillary nonperfusion [109].

Dysregulation of Retinal BF in Diabetes

Numerous studies have shown that damage to the vascular wall along with impaired rheological properties of blood may affect the ability of the diabetic retina to regulate its *BF*.

Hypoxia

In patients with nonproliferative DR, isocapnic hypoxia induces a significant increase in V_{leuk} in the perfoveal circulation, whereas *D* of retinal vessels remains unchanged. Patients with proliferative DR show a nonsignificant change in both parameters, indicating that *BF* regulation in response to a hypoxic challenge is blunted in proliferative DR, consequent of the hypoxic retinal conditions. After laser treatment inducing restoration of the retinal oxygenation, V_{leuk} and vessel *D* significantly increase in response to hypoxia [110].

Hyperoxia

Early studies have revealed a blunted hyperoxia-induced vasoconstriction of the vessels branching from the central retinal artery in diabetic patients [111]. Hyperoxia induces a retinal *BF* decrease by 61% in healthy individuals, 53% in diabetic patients without overt DR, 38% in patients with background DR, and 24% in patients with proliferative DR [112]. Most diabetic patients have a blunted retinal *BF* response to increases in oxygen concentration in inhaled air, which has been attributed to the presence of chronic retinal hypoxia [105, 107]. Alternatively, this blunted response could result from an increased production of endothelin-1 [113], a mediator of the hyperoxia-induced retinal *BF* response. The constriction of the vessel *D* is attenuated in patients with proliferative DR [114]. In patients with proliferative DR, pan-retinal photocoagulation almost restored the hyperoxia-induced *BF* response to normal values as this stimulus resulted in a 54% decrease in retinal *BF* particularly in those who also showed regression of neovascularization [115].

Smoking significantly alters the reactivity of retinal *BF* to hyperoxia and almost abolishes the autoregulatory vasoconstriction in diabetic patients [116]. Similarly, eyes with either nonproliferative or proliferative DR showed, during hyperoxia, a significantly decreased V_{leuk} in the perifoveal capillaries. After photocoagulation, the decrease in V_{leuk} observed during hyperoxia was no longer significant, suggesting a reduced response in retinal capillary *BF* [110].

Glycemia

Glycemia may affect retinal *BF* [104]. *AVP* is positively correlated with hemoglobin A_{1c} in adults with type I and type II diabetes [117, 118]. According to a number of studies, retinal *BF* markedly increases when blood glucose is acutely elevated [119–122], as a result of an increase in retinal oxygen consumption during hyperglycemia [123]. Additional finding failed to confirm any changes of *BF* velocity in macular capillaries and retinal vessel *D* following glucose infusion, neither in healthy controls nor in diabetic patients [124] nor during the postprandial phase [108, 125]. Strict diabetic control by intensified insulin therapy is ambiguous, since soon after its introduction, a number of patients showed marked progression of DR [126–128]. A blue-field study demonstrated that leukocyte flux, calculated as the product of $V_{leuk} \times N_{leuk}$, was significantly bigger in eyes that showed progression of retinopathy than in eyes that did not [129]. This suggests a relationship between progression of DR and the failure of retinal *BF* to decrease within days after institution of strict diabetic control [129–131]. The ocular hyperperfusion following the onset of intensified insulin therapy is inversely correlated with the plasma concentration of ET-1 in type I diabetes, suggesting that ET-1 levels affect ocular perfusion [132].

BF Response to Changes of Ocular Perfusion Pressure and to Flicker Stimulation

The ability of the retinal circulation to respond to changes in ocular *PP* is altered in diabetes [133], regardless of whether the pressure is decreased through an increase in IOP, by treatment with tyramine [134] or by systemic sympathetic stimulation induced by isometric exercise [135–137] (fig. 6). This alteration is further accentuated by hyperglycemia [134] and more prevalent in patients with autonomic dysfunction than in those with an intact autonomic nervous system [138]. The myogenic constriction of the arterial wall in human retinal arterioles is significantly improved as a rise in blood pressure of 19.5 ± 9.9 mm Hg during improved metabolic control

Fig. 6. Measurements of the myogenic response of retinal arterioles using the RVA during isometric exercise in subjects with different stages of DR. Group I: no DR, group II: mild/moderate DR, group III: moderate/severe nonproliferative DR with laser treatment, and group IV: proliferative DR with laser treatment. A similar blood pressure (BP) rise was achieved in all subjects. In response to this BP rise, group I demonstrated a vasoconstriction of $-7.6\% (\pm 4.1)$. In groups III and IV, respectively, an abnormal vasodilation of $+0.44\% (\pm 5.9)$ and of $+3.2\% (\pm 5.9)$ was found [from ref. 137, with permission].

is associated with a significantly improved arterial vasoconstriction from -3.2 ± 2.9 to $-5.9 \pm 2.7\%$ [137].

The response of *D* of retinal vessels to diffuse luminance flicker is blunted in insulin-dependent diabetic patients in comparison to healthy controls [139, 140] (fig. 7), either due to a vascular abnormality (endothelial dysfunction or loss of pericytes) and/or decreased neural activity response resulting from selective abnormalities of Müller glial cell function [141]. These cells probably play an important role in the coupling between retinal neural activity and *BF* [15]. The blunting occurs already in patients without DR and increases with the stage of DR [142].

Dysregulation of Retinal BF in Glaucoma

The role of vascular disturbances in the pathogenesis of glaucoma remains controversial, but most experts agree that elevated IOP alone does not explain the whole spectrum of individuals with open-angle glaucoma. Apart from IOP, a variety of other systemic and ocular risk factors for glaucoma have been identified, such as heart disease [143], low systolic blood pressure and low ocular perfusion pressure [144]. A large number of stud-

Fig. 7. Flicker-induced *D* responses of retinal arteries are abnormally reduced in patients with insulin-dependent diabetes mellitus with no or mild nonproliferative retinopathy. Box (mean, 25 and 75% CI) and whisker (5 and 95% CI) plot of *D* responses (percentage changes) as recorded after 16, 32, and 64 s of flicker stimulation [from ref. 139, with permission].

ies, using different clinical techniques, have demonstrated reduced ONH *BF* in eyes with glaucoma when compared to normal eyes. Whether this is secondary to loss of neural tissue or a causative factor, however, is not known. As *BF* through the retina and the ONH is efficiently autoregulated, moderate increments in IOP would have no or little effect on *BF* through these tissues. The

situation may be different in the presence of deficient ocular *BF*, and *BF* autoregulation could then be affected even by small increments in IOP. An impaired autoregulation of the retinal circulation was indeed found in eyes with glaucoma with the blue-field simulation technique [145]. This investigation determined the maximum, acutely increased IOP above resting IOP at which V_{leuk} is maintained constant by autoregulation. This maximum IOP was 25 ± 1.5 mm Hg (± 1 SD) for patients with primary open-angle glaucoma and 30 ± 3.6 mm Hg in normal subjects. Studies with other techniques have also suggested that autoregulation of retinal and/or ONH *BF* is reduced in eyes with glaucoma. Thus retinal venous *D* response to short-term elevations in IOP was found to be altered [9] and changes in rim perfusion after a therapeutic IOP reduction [146] suggest that autoregulation may be defective in eyes with glaucoma while intact in ocular hypertension. Also the increase in ONH *BF* [147] and *D* of retinal veins [148] in response to flicker were found to be significantly diminished in glaucoma patients as compared with healthy volunteers, both results being indicative of an impairment of neurally mediated vasoreactivity.

Disclosure Statement

Both authors confirm to disclose any sponsorship or funding arrangements related to their research and any possible conflicts of interest.

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