Measurement of Retinal Vessel Diameters

6

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Core Messages

- Given that local vascular tone is an important regulator of blood flow, exact determination of vessel diameter is crucial. The development of new and sophisticated instruments allow now for the exact and non-invasive determination of vascular tone in vivo.

6.1 Introduction

Since the development of the first method for funduscopy by Hermann von Helmholtz in 1851, both clinicians and researchers were interested in a method for the assessment of retinal vessel diameters. It has very early been recognized that morphological and functional changes of retinal vessels do not only reflect ocular vascular pathologies. Moreover, changes of the retinal vascular system may also serve as an indicator for a couple of systemic vascular-related disorders and their associated risks factors. In this context, alterations of the vascular system may be seen in either morphological changes of the retinal vasculature or in alterations of the vascular function. Hence, given that, in the eye, noninvasive investigation of the microcirculation is possible, it provides a unique possibility for interdisciplinary vessel diagnostic in microcirculation. Using new optical technologies with high resolution down to the microscopic range, physiological vascular regulation mechanisms and alterations of the microcirculation can be investigated.

Beside the clinical use of methods to visualize the ocular fundus, the exact determination of retinal vessel size is in particular crucial for the measurement of retinal blood flow. Given that there is increasing evidence that blood flow alteration may contribute to the pathogenesis of several ocular diseases, the assessment of ocular blood flow has received more and more importance. Blood flow in the eye, as in every vascular bed, is mainly dependent on perfusion pressure and vascular resistance. The latter is regulated by the local vascular tone, in particularly, in small resistance vessels. Thus, the regulation of local blood flow is necessarily dependent on an intact regulation of vascular tone in the tissue. This underlines the importance of measuring precisely the retinal vessel size when attempting to assess blood flow in a specific vascular bed.

The regulation of vascular tone is based on a complex interaction between local, systemic, and

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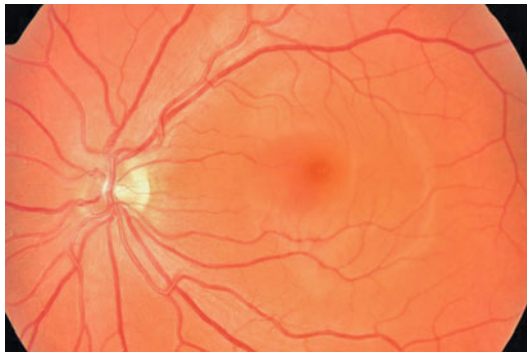

Fig. 6.1 Normal human fundus

neural components compensating for differing metabolic demands and changes in perfusion pressure. As a part of these regulation systems, it has been shown that the retina and the optic nerve head have the ability to adapt their blood flow to increasing metabolic demands caused by augmented neural activity. There is increasing evidence now that not only basal vascular tone is important to preserve physiological function but also the ability of the vascular system to adapt to changes in metabolic demands and that these mechanisms may be corrupted in several systemic and ocular diseases. The developments of sophisticated stimulation techniques allow now not only for the precise determination of vessel size but also for vascular and endothelial function. This may in future help for the early diagnosis and treatment of ocular and systemic vascular diseases.

6.1.1 Anatomy

The retinal vasculature is a classical end-artery system and supplies the inner layers of the neural retina. Observed ophthalmologically, it consists of a typically arranged network of vessel branches, entering the eye with the optic nerve head (Fig. 6.1). The central retinal artery is derived from the ophthalmic artery, which, in turn, is a branch of the internal carotid. Based on blood flow measurements, it has been calculated that the diameter of the central retinal artery is in the range from 134 to 208 μm in healthy subjects [8]. The central retinal artery enters the optic nerve approximately 10 mm behind the globe and divides near the lamina cribrosa into upper and lower main branches. Further divisions lead to the typical appearance of the retinal vasculature divided into four major branches, each supplying one quadrant of the neural retina. The retinal arteries are approximately 200 μm in diameter and, in an anatomical point of view, arterioles. The general structure of the retinal vessels is comparable to that of muscular arteries. Near the optic disc, the vessel wall consists of five to seven layers of smooth muscle cells, decreasing to two or three layers at the equator [23]. As a peculiarity, the retinal vessels lack any autonomic nerve supply.

Retinal blood flow is drained almost exclusively by the central retinal vein, which subsequently empties into the superior ophthalmic vein. Retinal veins are usually larger in diameter (up to 300 μm) and lack a well-developed smooth muscle covering. Calculations have revealed that total retinal blood flow in healthy subjects is in the range of 38–80 μl/min [8, 11] and may be considerably impaired under pathological conditions.

6.2 Vessel Diameter Measurements Based on Optical Images of Indirect Ophthalmoscopes

Already 1 year after the development of the ophthalmoscope by Hermann von Helmholtz, a first approach to measure retinal vessel diameters in vivo has been introduced by Ruete and Landolt. The measurement principle utilizes an optical image plane of the indirect ophthalmoscopy placing reference markers of well-defined size into it. The markers can be viewed in focus together with the vessels to gauge the vessel diameter by direct comparison. Subsequent modicifactions apply measurement oculars with measurement scales or moveable measurement markers like the Mikuni ocular [42].

Later on, the “Lobeck ocular” represented the first serious optical measurement device, based on an optical image splitting method and

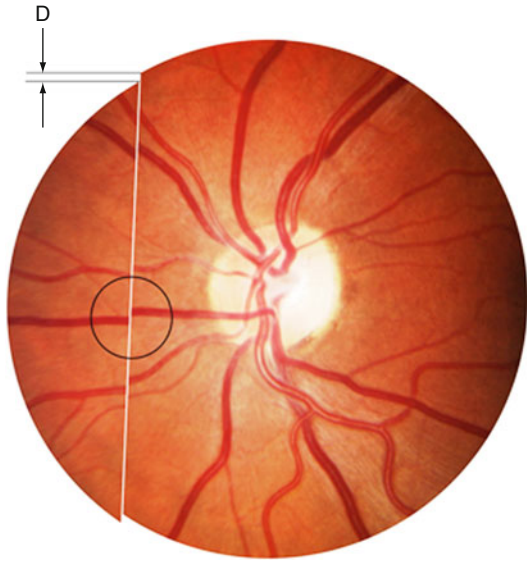

Fig. 6.2 Image-splitting principle (Modified from Vilser et al. [61])

developed by Lobeck [37] and manufactured at Carl Zeiss Jena during the 1930s. This principle was enhanced later to the use of a parallel plate micrometer [59] (Fig. 6.2).

The first electrooptical system for measuring retinal vessel diameters was presented by Delori [7]. In the image plane of an indirect ophthalmoscope, a light scanning system picks up brightness profiles perpendicular to the vessel course. The electronic signals representing these vessel cross sections are then analyzed in order to identify the vessel borders by the means of the half-height algorithm described in detail below. The specific purpose of this device was the measurement of oxygen saturation by use of different absorption profiles of oxy/desoxyhemoglobin including the diameter measurements. Another similar development was based on a modification of a commercially available photographic retinal camera (Carl Zeiss Jena, Germany) presented on the Leipzig fair in 1987 [58]. The device was equipped with a linear CCD sensor to gain brightness information necessary for diameter assessment.

However, diameter measurements in the live image of the fundus as visualized by indirect ophthalmoscopic devices are difficult, time consuming, and allow usually only for assessment one single-measurement location. An additional problem of these approaches is that the systems are very sensitive to eye movements. Thus, the reproducibility of the technique is strongly dependent on exact fixation abilities of the subject measured. The considerable numbers of disadvantages in combination with considerable error sources have limited the application of these early devices strictly to research purposes.

6.3 Vessel Diameter Measurements Based on Photographic and Digitally Stored Images

The development of retinal cameras and the advantage of the photographic or digital storage of fundus images with high temporal and spatial resolutions lead to improved measurement systems for vessel diameters and finally to the introduction of modern retinal vessel analysis. In particular, fundus photography for the first time allowed for the assessment of vessel diameters without the problems of a live examination. Acquired fundus images can be analyzed offline by trained persons, which allows for the anonymized analysis in double-masked clinical studies.

6.3.1 Basics for Measurements on Stored Images

Basically, all methods for measurement based on photographic and digital images follow a similar pattern: In a first step, the fundus image acquisition takes place, which is then followed by vessel diameter measurement based on the analysis of the recorded images.

6.3.1.1 Measuring Principle

Figure 6.3 demonstrates the basic principle of measurement, on which most of the techniques currently available are based by use of retinal cameras. Imaging of the column of the red blood in the fundus image is dominated by light absorption. The brightness profile from the vessel cross section (vessel profile) is analyzed to detect the

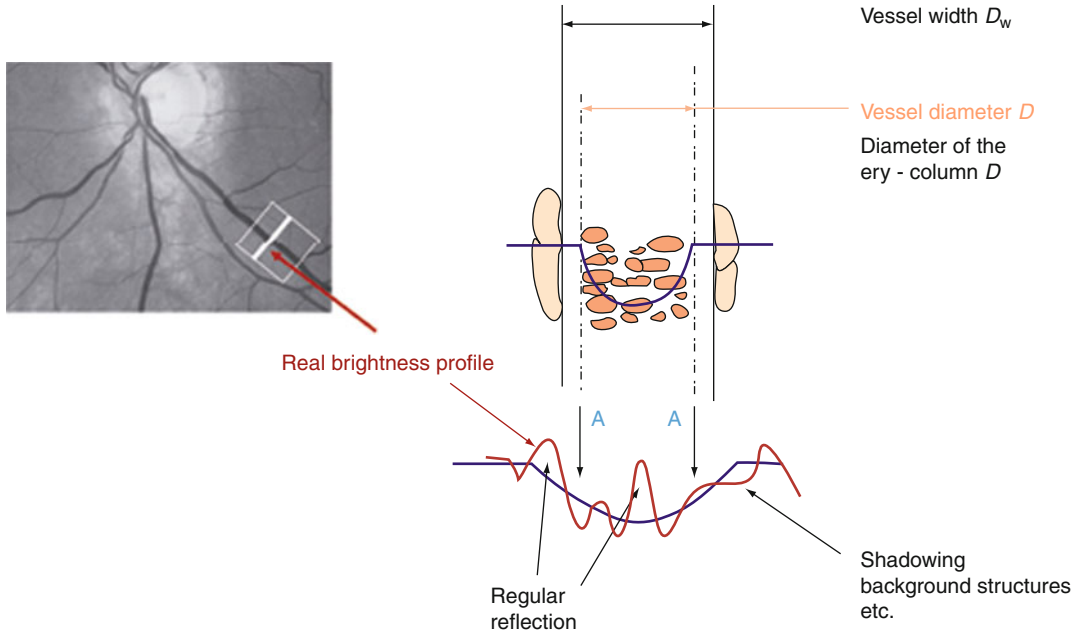

Fig. 6.3 Measuring principle

vessel edges and to define the measuring points inside these edges and therefore in the edges of the red blood cell column. The diameter of a vessel segment is estimated as distance between the measuring points on a perpendicular line to the flow direction of the vessel (Fig. 6.3).

The column of red blood cells is absorbing light backscattered from the retinal layers and results in an ideal brightness profile. However, in reality, that ideal profile is interfered with disturbances like local and global regular reflections and background structures. The optical properties of the eye, imaging system, and electronic image sensor influence the vessel profile by applying low-pass filtering and noise to the fundus image. The result is the real vessel profile, more or less disturbed like shown in the figure and changed by the optical magnification of the eye and optical device.

Imaging systems different from regular fundus cameras (e.g., laser scanning systems) usually introduce different imaging conditions, which make modifications of the measuring principle necessary. An exceptional position among the approaches holds the methods based on fluorescein angiography, which requires the application of a fluorescent dye. Given that fluorescein interfuses the whole vessel including the otherwise invisible plasma edge stream, the fluorescence pattern light represents the whole lumen, not only the width of the red blood cell column. This results in a different brightness profile, and a different diameter definition must be considered. Thus, the data assessed with fluorescein angiography are not entirely comparable with the data gained with other methods.

In the past, depending on the image acquisition and analysis technique, a variety of methods were developed and applied. The methodological properties of the different methods are determined both by the image acquisition system (retinal camera, laser scanner) and the measurement methods itself.

6.3.1.2 Definition of Retinal Vessel Diameter and Vessel Width

Although arbitrary, the terminology for defining vessel size is important. The vessel diameter is defined as the diameter of the red blood cell column and the vessel width as the diameter of the

column of a contrast agent at a particular point in time for a given local vessel segment.

Because the vessel diameter and width differ, it is necessary to clearly distinguish measuring results using contrast agents (i.e., width) from results obtained without the use of contrast agents (i.e., diameter). It is assumed that the thickness of the plasma zone is constant under a wide range of hemodynamic conditions, but the validity of this assumption needs to be verified. Another important assumption is that the measured vessel has a circular cross section. This seems a reasonable assumption for the pressure conditions near the optic nerve head, but whether this assumption holds true for all parts of the ocular vascular system has yet to be shown.

6.3.1.3 Vessel Edge Definition and Methods for Estimation of Measuring Marks in the Vessel Edges

For the correct determination of vessel diameters, a correct identification of vessel borders is crucial. As illustrated in Fig. 6.3, the vessel edges are not sharp brightness transitions from the vessel to the surrounding tissue, but rather they appear as gradual transitions from bright to dark. This edge brightness transition can be as much as 1/3 of the vessel diameter. This edge can be further obscured by the brightness profile of the whole image.

Defining the vessel edge requires proper placement of measuring marks at the edges of brightness transition. The ideal vessel edge definition (A-A) for the chosen definition of vessel diameter is shown in Fig. 6.2 and marks the beginning and the end of the red blood cell column. However, the position of the measuring marks determined by measuring algorithms automatically or manually by an examiner are often misplaced. This is a common cause of systematic and random errors in retinal vessel diameter measurements and the impetuous for the development of automatic algorithms immune to brightness disturbances and with high reproducibility. One main aim for the development of automatic algorithms is to be sufficiently robust to the brightness disturbances to get a high reproducibility [60].

In principle, two different methods for vessel border determination are available: a subjective and an (semi)automatic, objective one.

Subjective Edge Definition of Manual Measurement Directly in the Acquired Fundus Image

In the simplest approach, the grader has to adjust a measuring mark until it fits the vessel border according to his visual impression [22, 24]. Obviously, it is crucial to ensure a perpendicular cross sectional line between the marks. Alternatively, a circle can be drawn with the center on one vessel edge, and the radius has to be increased until the circle is tangent to the opposite vessel edge [24]. Manual measurements are time consuming and suffer from subjective systematic measurement errors, dependent on the individual perception of the edge position by the grader. As one consequence, there are different measurement results between graders (intraindividual variability of graders). A more subtle error is that graders tend to place the measuring marks towards the darker part of the profile. A manual assessment of the same vessel shown bright on a dark background will result in a wider diameter estimate than if the vessel is shown dark on a bright background. Such misperceptions introduce systematic errors between measurements in positive and negative images. Furthermore, vessels in fluorescein angiographic images are measured wider than in images without dye independent from difference caused by plasma zone and by steeper edge brightness transitions. Finally, it should be noted that examination conditions like different image brightness and contrast, ambient light, and different styles of measuring marks may introduce errors and difference between measuring results [59, 60].

Objective Edge Definition

To improve reproducibility by reducing a number of errors due to perception of the grader, some methods apply objective criterions for diameter assessment. Those methods can be classified into the following three groups:

1. *Half-height maximum*

   The measuring points for both edges are defined as the half height of the brightness of the surrounding tissue and the vessel center on each side of the vessel. This definition was used by densitometric and scanning techniques [5].
2. *Filter algorithms for automatic measurements*

   One group of algorithms for automatic measurements of vessel diameter are special filter kernels [34] to mark the measuring points in the vessel edges of the brightness profiles crossing the vessel.
3. *Model-based algorithms for automatic measurements*

   A modeled vessel profile is approximated to the acquired real brightness profile. Extracted model parameters from the best fitting represent the vessel diameter [39]. Models can also account for regular reflections and other systematic error sources. A principal drawback is the time need of such methods.

6.3.1.4 Problems and Measuring Errors

Errors can arise from the individual properties of the eye, the imaging system, and image processing. Depending on the measuring algorithms and examination conditions, there are different sources for systematic and random errors. Different examination or measuring conditions can turn systematic errors into random errors and vice versa.

Magnification Error

The magnification scale of the fundus image is strongly affected by the anatomical properties of the eye. As a nominal value, the magnification scale should be given for the Gullstrand normal eye adapted to the far which is well defined. Deviations from that model can be quantified as axial ametropia and refractive ametropia, changing the optical magnification [1, 36]. A process of biological self-correction may counterbalance the effects of the two forms of ametropia, resulting in an emmetropic eye. Both the ametropic and the emmetropic eye with an axial length different to the Gullstrand eye will induce magnification errors due to the optical system of the imaging device. The amount of error is influenced by the optical system of the fundus camera [60]. It is not sufficient to estimate merely total and axial ametropia of the system (eye) in order to correct magnification errors. Furthermore, deviations in corneal shape like astigmatism or keratocone may induce additional errors. The influence of various magnification-related errors can be minimized by the use of local or temporal relative values like the arteriovenous ratio.

Errors of Measurements in Angiographic Pictures

Typically, measurements are performed in photographic images (colored or black and white). Alternatively, it has been proposed to use fluorescein angiography to enhance the contrast of the vessel edge against its retinal background [22, 55]. However, the use of fluorescein results in the measurement of vessel width as discussed above. In general, measurements of vessel width in angiographic images face a lot of error sources which are difficult to control. The dye filling in arteries expands from the vessel center, whereas in the veins it remains heterogenous. Only over a short period of time the acquisition of images for vessel diameter measurement is possible. Most of the angiographic images lead to considerable measurement errors.

Additionally, fluorescein angiographic images often are overexposed in order to get good contrast and to display the capillary bed. This leads to problems in the identification of the vessel edges in the brightness profiles which feigns better reproducibility but induces additional errors. These fundamental drawbacks of fluorescein angiographic imaging methods remain independent of the methods for vessel diameter measurement applied.

6.3.1.5 Physiological Variability of Vessel Diameter

Variance of the measurement of retinal vessel diameters is not only caused by errors of the assessment technique itself. It has been shown

that retinal vessels exhibit changes in diameter during the cardiac cycle. Pulsation of the central retinal vein is visible on direct ophthalmoscopy in a large number of persons, and pulse-related changes in retinal vessel width can be measured by assessing diameter information at different phases of the cardiac cycle. Therefore, assessing retinal vessel diameters – regardless which technique was used – at random points in the pulse cycle may result in an unrecognized source of variation in the measurements of retinal vessel diameters between subjects and over time in the same individual. It has been shown that the arterial diameter can vary up to 3.5% and up to 4.8% in retinal veins of healthy subjects [6]. The same study described that the venular diameter was smallest in early systole, reaching a maximum in early diastole, whereas the arteriolar diameter peaked slightly earlier. Considering that the calculation of blood flow from vessel diameter and blood speed uses the square of the vessel diameter, the potential error introduced in such a calculation can be up to 9%, if this factor is not taken into account.

Several techniques have been proposed to overcome the pulse-depending changes. Dumsky and colleagues have introduced a technique which uses electrocardiographically synchronized fundus photography [9]. For this purpose, a dedicated electrocardiographic synchronization unit was built to trigger the camera at a preset time interval after the R wave of the ECG. Using six or more synchronized fundus photographs, the authors could detect diameter changes induced by exercise as small as 1.4% in a group of six volunteers. Knutson and colleagues showed that pulse synchronous digital images triggered by the means of a ear clip as triggering device can significantly reduce the variation of measurements [30].

Another cause of biological diameter variability is vasomotions and the blood pressure waves (Meyer waves) with periods near 10 and 20 s. In young people, the magnitudes are considerably higher than the pulsation magnitudes. These waves are different in arteries and veins and therefore cause changes in arteriovenous ratio. Newer instruments (DVA and RVA Imedos, Jena), which use the real time assessment of retinal vessel diameter, allow for the correlation of vessel diameter course to a wide number of biological signals such as the ECG. Alternatively, given that these systems are measuring retinal vessel size up to 25 times per second, retinal vessel diameters can simply be determined by averaging the measuring results over several seconds.

The brief overview should give an introduction into the methodology of vessel diameter measurements. It is by far not capable of reflecting the multitude of approaches and results worldwide regarding this topic.

6.3.2 Methods

Different imaging systems and different technical solutions to measure in the photographic or digital images with different edge definitions result in different methods, which are summarized in groups.

6.3.2.1 Optical Micrometric Measurements Based on Photographic Negatives

The optical micrometric determination of retinal vessel diameters was used in clinical studies for many years. Basically, the micrometric determination of the vessel diameter or width uses photographic image negatives of the fundus, viewed under a microscope. By the means of a micrometer ocular attached to the microscope and a known magnification of the instrument, the observer visually estimates the measuring points in the vessel edges (manual measurement).

Several improvements for the micrometric assessment have been proposed. In a technique also known as projection micrometry, a photographic fundus picture was taken with a fundus camera and then projected onto a screen with known magnification (up to 35 times). Vessel diameter measurement was performed manually using a caliper [4]. More sophisticated approaches utilized dedicated computer systems to translate the position of the caliper in measurable units.

As discussed already, common drawbacks of those manual methods are a variety of subjective errors and their extensive time need.

6.3.2.2 Microdensitometry Based on Photographic Negatives

In the so-called microdensitometric techniques, the vessel edges are defined based on a densitometric trace of the vessel images crossing the vessel. The result is similar to the brightness profile. The crucial point in the microdensitometric technique is the objective determination of the position of the measuring points in the vessel edges by means of the half-height diameter method enabling an objective measurement. The disadvantage of densitometry was again the considerable time need. So today, also this method is replaced by modern methods of digital image processing [5].

6.3.2.3 Measurements Based on Digital Images

During the changeover from photographic to electronic imaging, high-resolution image scanners were used to enable digital image processing on photographic fundus images. Nowadays, this technology has kept its relevance for the postprocessing of major studies from the past. Time need and errors from photographic and scanning processes are the drawbacks of this method.

The development and application of sensitive high-resolution image sensors like CCDs turned conventional photographic retinal cameras into digital imaging systems. This enables a direct computer processing of digital fundus images to assess vessel diameter in normal or vessel width in fluorescein angiographic images.

But even for the evaluation of digital fundus images, visual-measuring methods similar to the methods of optical micrometry were a common approach. The image is displayed on a monitor, and the user has to place the measuring marks (lines or circles) using a mouse [24]. The respective display magnification properties of the display monitor and the various subjective influences may have adverse impact on the measurement results.

Nowadays, vessel measurement is performed using more and more automated methods. Usually, the examiner has to select the measurement location by mouse click. There, the brightness profiles are acquired from the vessel cross sections and evaluated automatically using the methods discussed already. Sources of errors for these methods are the imaging conditions and effects of the optical devices and imaging sensors. Further developments of the digital systems for vessel diameter measurement lead to the introduction of vessel analysis.

6.4 Diameter Assessment for Blood Flow

Until now, volumetric blood flow in major retinal vessels cannot be measured directly. One of the most widely used approaches is to calculate blood flow from measured velocity and vessel diameter in the same vessel segment. One has, however, to note that this reflects blood flow in one single vessel. If one wants to determine total retinal blood flow, this procedure has to be carried out in each single vessel separately.

6.4.1 Assessment of Flow by Use of Doppler Technique (CLBF)

Another possibility to measure blood flow is the combination of Doppler techniques with measurements of vessel diameter in photographic fundus images [19]. The Canon laser Doppler blood flowmeter (CLDF, Canon, Tokyo, Japan) combines retinal blood velocity measurement according to the laser Doppler principle with a system for the measurement of retinal vessel diameters [10]. It is the first commercial device that is capable for measuring simultaneously vessel size and blood speed. This allows for the calculation of retinal blood flow in µl/min based on the Poiseuille principle in the selected vessel segments with high reproducibility [20] and can therefore provide an immediate measure of retinal blood flow in actual units of µl/min. Basically, the CLDF consists of a modified fundus camera, which is equipped with two lasers. The red blood cell speed is determined

by bidirectional laser Doppler velocimetry, which is described in detail elsewhere in this book. Briefly, Doppler-shifted light scattered from the flowing blood cells in the target vessel is detected simultaneously in two directions separated by a fixed angle. The Doppler shift in the backscattered laser light is a function of red blood cell velocity. A red 675-nm-diode laser is used for velocity measurement.

Retinal vessel diameters are determined automatically by computer analysis of a vessel cross section recorded by a CCD line scan sensor connected to the fundus camera system. For the determination of vessel diameter, the half height of the vessel brightness profile is used. Furthermore, the diameter measurements can be corrected for the refractive error of the eye. The instrument is equipped with an automatic vessel tracking system that maintains alignment of the laser beam on the target vessel during the measurement.

6.5 Retinal Vessel Analysis

Given that several ocular and systemic vascular-related diseases are associated with concomitant changes in the microvasculature, the exact quantification of the retinal vessel size has gained more and more attention. Several lines of evidence indicate that changes in retinal vessel diameters reflect not only ocular diseases but also may serve as an early predictor for systemic diseases such as systemic hypertension or stroke. Along this line of thought, a lot of effort has been put into the investigation of structural alterations of retinal blood vessels and their association to ocular and systemic diseases.

6.5.1 Basics of Retinal Vessel Analysis

In principle, two different approaches for automatic vessel analysis are available (Fig. 6.4). The analysis of one singe image of the ocular fundus is usually referred to as static vessel analysis. Based on this technology, several parameters describing structural alterations of the retinal vascular system, such as generalized arterial narrowing, can be assessed. However, the limitation of this approach is that no information can be gained about vascular function. The further development of this technique led to the approach of dynamic vessel analysis. In principle, dynamic vessel analysis is based on the analysis of retinal vessel

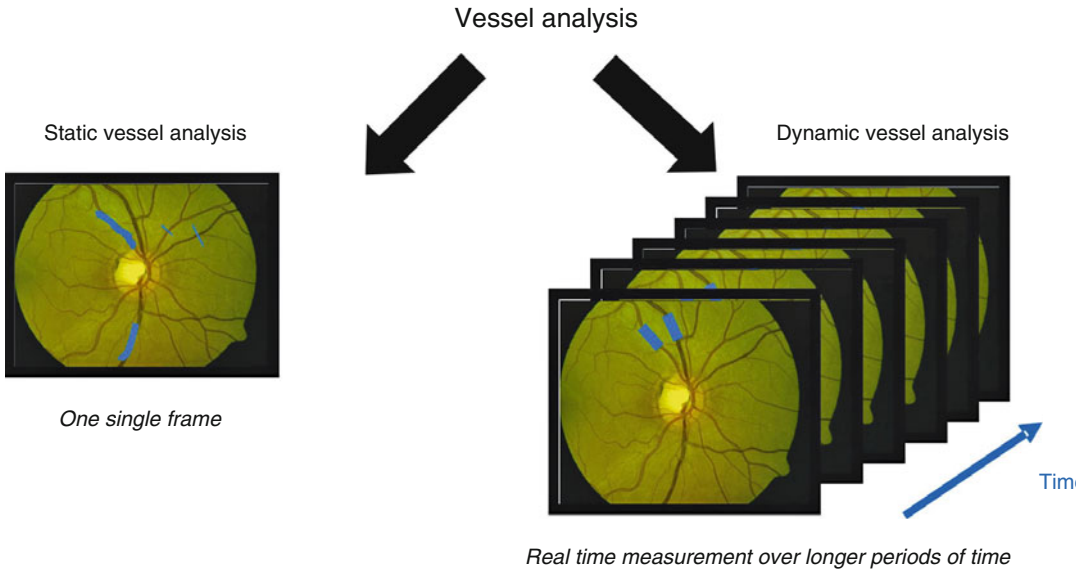

Fig. 6.4 Two approaches for retinal vessel analysis are available: static and dynamic vessel analysis

diameters in relation to time and location on the vessel. For this purpose, image sequences from seconds up to several minutes are analyzed, allowing for the investigation of time and location dependent vessel behavior. Additionally, as one of the advantages of this technique, the latter approach also allows for the use of different provocation methods such as flicker stimulation, squatting, or breathing tests, to assess vascular function in vivo.

The basic principles of diameter measurements have been described before in this section. Whereas for single measurements it may be sufficient to manually mark the chosen vessel segment, this approach is no longer feasible for the high number of measurements necessary for vessel analysis. To overcome this problem, sophisticated methods and algorithms of image processing with a high degree of automation have been introduced and are currently subject to research. Pursuing various approaches and different objectives, they share the following essential steps:

1. Identification of vessel segments (recognition of vessel segments inside of images)
2. Identification of connected segments or building vessel trees
3. Vessel classification (differentiation between arteries and veins)
4. Definition of reference points and recognition of eye movements
5. Correction of eye movements between images
6. Plausibility tests
7. Analysis of measured vessel diameter values and estimate of characteristic vessel parameters to describe vascular properties of vessel state and function such as the AVR

6.5.2 Static Vessel Analysis

Given that the eye is the unique site in the human body where a direct sight on the vessels is possible, special emphasis has been directed toward the early identification of morphological changes in several vascular-related diseases. In particular, it is known for a long time that changes in blood pressure, diabetes, or other diseases are reflected in morphologic changes of the ocular fundus. These changes include arteriolar narrowing, arteriovenous nicking, cotton wools spots, microaneurysm, blot hemorrhages, and others. Whereas morphologic changes such as microaneurysms and hemorrhages need to be analyzed by a trained specialist, the assessment of retinal vessel diameters can be done automatically by analyzing fundus images.

One of the first approaches to assess a disease risk factor by measuring the retinal vascular system was introduced by Quigley and colleagues [50]. For this purpose, the so-called pressure attenuation index (PAI) was defined. The PAI reflects the pressure loss along the arterial vessels, based on diameter measurements of retinal vessels. It was stated that low-end arterial pressures (high PAI values) may be a protective factor in diabetic retinopathy and that PAI may be use as risk indicator for the development and progression of the diabetic retinopathy.

Today, the most widely used approach to describe retinal vessels state is based on the observation that a decrease in arterial vessel caliber may reflect generalized arterial narrowing in other vascular beds and predict systemic and ocular diseases. Unfortunately, representative measurements of retinal vessel diameters are difficult to perform and complicated by several different factors [24]. First and most importantly, the angioarchitecture and the branching pattern of the retinal vessels differ considerably between subjects. This is of special importance because the total cross section of the arteriolar system increases with each bifurcation of the vessels leaving the optic nerve head. Thus, in order to achieve comparable and reliable results, the measurement procedures should also account for the specific branching pattern of the subject under study. Secondly, measurements of vessel size by the means of a fundus camera or fundus photography may be influenced by an improper focus or the individual refractive error of the subject under measurement.

To overcome these limitations, the arteriovenous ratio (AVR), a relative measure to assess arteriolar narrowing has been introduced. The AVR is currently the most widely used parameter for static vessel analysis and allows individual vessel

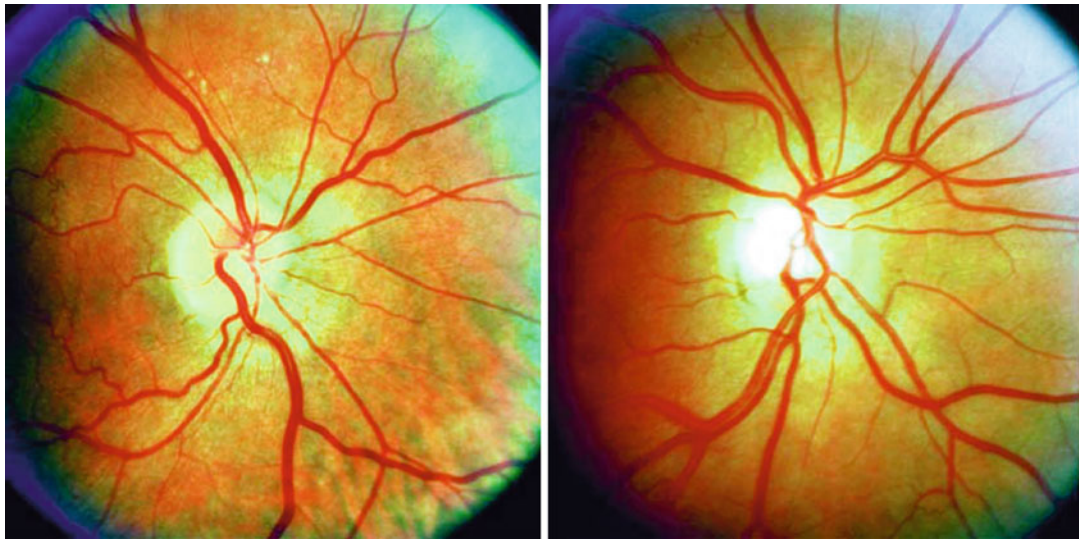

Fig. 6.5 Retinal photographs showing a fundus with generalized arteriolar narrowing with an AVR of 0.64 (*left*) and a fundus with normal retinal arterioles with an AVR of 1.08 (*right*) (Wong et al. [68])

diameters to be combined into summary indices reflecting the average arteriolar and venular diameters of the eye [24]. To assess the AVR, the quotient of the so-called central retinal arterial equivalent (CRAE) and central retinal venous equivalent (CREV) is calculated. The first approach to calculate the CRAE has been introduced by Parr and Spears [46, 47]. The formula used for CRAE calculation is based on theoretical models and includes factors such as the vessel width and the number of times the arteries have branched for the calculation of the CRAE. However, the studies by Parr and Spears are limited by the fact that only the CRAE has been used to assess arterial narrowing and the retinal veins have not been included in the analysis.

Later, extending the approach from Parr and Spears, a corresponding formulas for the calculation of CREV have been developed by Hubbard and colleagues [24]. As a further improvement, the AVR has been included calculated as a quotient of the CRAE and CREV. The authors suggest that the AVR is a more appropriate measure to reflect arterial narrowing because it also includes information of retinal veins [24]. The main advantage of this approach is that the AVR, as a relative factor, is presumed to be largely unaffected by the arteriolar branching pattern in a certain individual, magnification of the fundus photo due to refractive errors, or the broadening of the vessel diameter due to the opacity of the ocular medias [24]. An AVR of 1 indicates that, on average, arteriolar diameters are the same as venular diameter in that eye, whereas a smaller AVR indicates arteriolar narrowing (Fig. 6.5). However, this approach is based on the assumption that the diameter of retinal veins remains relatively constant despite other influencing factors such as blood pressure. Whether this assumption holds true in all cases will be discussed later in this chapter.

Further modifications leading to revised formulas for calculation of CRAE and CREV have been proposed by other authors. For example, Knudtson and colleagues have pointed out that the above mentioned formulas for the calculation of the CRAE and the CREV are not completely independent from number of vessel measured [31]. Consequently, the authors have developed new formulas which also account for differences in branching of the vessels included [31].

To guarantee comparable results, several prerequisites for the calculation of the AVR have to be fulfilled. Most importantly, the image used for the analysis has to be taken centered on the optic disc (Fig. 6.6). Given that, as stated above, the

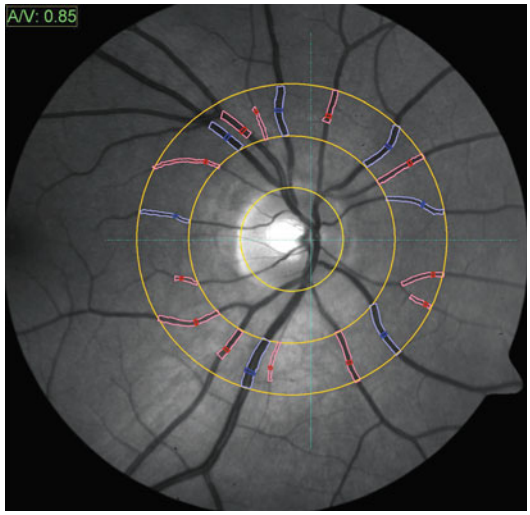

Fig. 6.6 Fundus image with marked measuring circular area and measured vessel diameters (Software VM2/ Imedos GmbH Jena/Germany)

individual branching pattern plays a crucial role for the assessment of the AVR, only vessels coursing through a specified area surrounding the optic disc are taken into consideration. At their first descriptions of the static vessel analysis [24], digitized photographic images and a manual diameter measurement had been used. The technique was then improved by the development of automatic algorithms [67].

Newer technologies are based on the analysis of digitally taken fundus pictures and specially developed software programs for the automatic detection and classification of the vessels and the concomitant diameter measurement (*X1-IVAN-software of the Wisconsin University*). However, up to now, the software is still dependent on the input of an experienced user to confirm the measurements or to correct false or missing vessel detections, wrong classifications, and obvious measurement errors. While the range of use of the "IVAN" software (University of Wisconsin) is strictly limited to public research, Imedos GmbH (Jena, Germany) introduced a commercially available software "Vesselmap" and an integrated device system for static vessel analysis "static vessel analyzer" (SVA) (Fig. 6.6). Other software solutions are provided by Thalia and Medivision. The most recent development in static vessel analysis is a follow-up tool (Imedos) capable of performing automatic individual progression observations. Relative changes of arterial and venous vessel diameters are determined related to a reference examination. In this way, a consideration of central equivalents for individual diagnosis becomes possible.

Today, the standardized assessment of the AVR based on arterial and venous central equivalents got widely accepted and became a gold standard in epidemiologic studies. However, examination protocols, measuring restrictions, procedures, conditions as well as used formulas, and retina cameras must be considered when attempting to compare results of different studies.

6.5.3 Results and Limits of Static Vessel Analysis

The importance of retinal vessel diameter in the diagnosis of systemic vascular-related diseases was recognized already in the 1930s, especially the relevance of the ratio between corresponding artery and vein [37]. Unfortunately, a number of interesting scientific study results from Lobeck and others related to diabetes, arterial hypertension, and nephrological diseases and other fell into oblivion [38]. However, the observation that patients suffering from systemic hypertension shows decreased retinal arterial diameters, which has been used as a diagnostic criterion and a grading scale for hypertensive retinopathy for many years [56]. For a long time, this decrease in retinal arterial diameter was explained as a counter regulatory response of the retinal arteries to increased perfusion pressure caused by the elevated systemic blood pressure. Since it is now possible to exactly quantify the changes in vessel size, these observations have been further investigated by large epidemiological studies.

One of the fist studies investigating the association between retinal vessel size and systemic blood pressure in a large epidemiological design was the Arteriosclerosis Risk in Communities Study (ARIC). Including more than 11,000

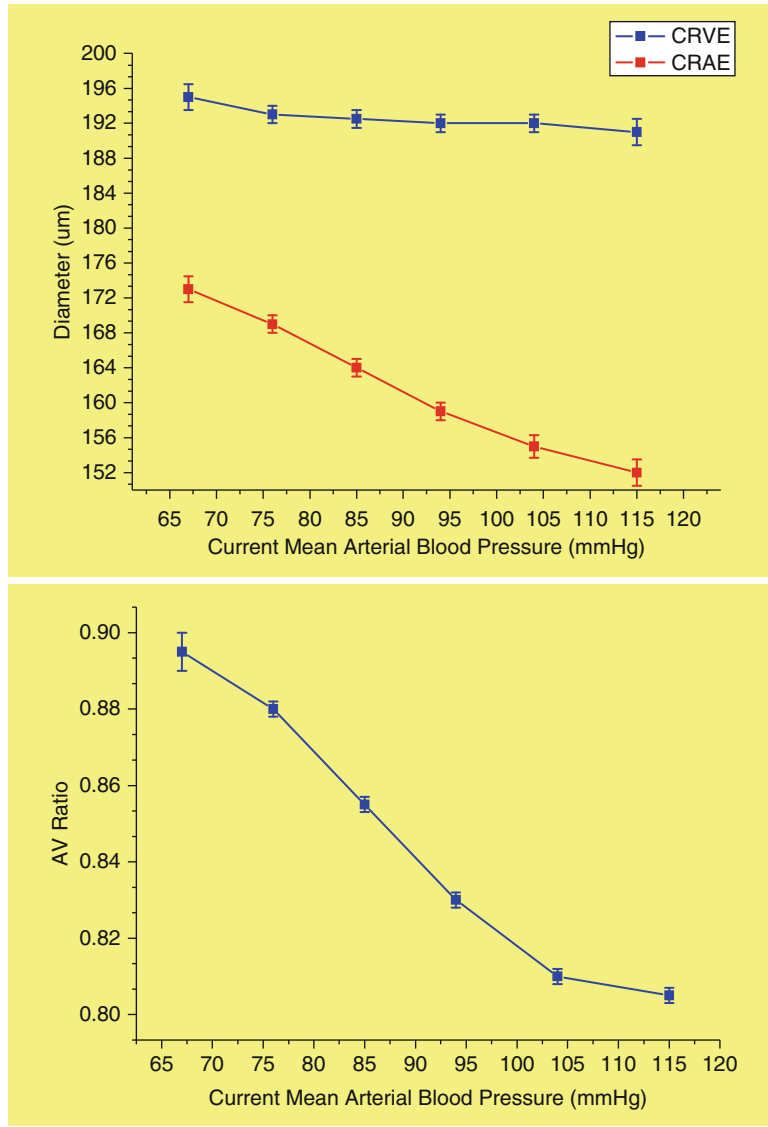

Fig. 6.7 Average central retinal artery equivalent (CRAE) and venous equivalent (CRVE) by mean arterial blood pressure and AV ratio versus mean arterial pressure (Data are derived from the Atherosclerosis Risk in Communities Study ($n = 9{,}040$). Both figures are modified from Hubbard et al. [24])

participants in four examination centers, retinal vascular abnormities were documented using fundus photography. The AVR was then calculated based on digitized pictures of the fundus photographs. The data of the ARIC study indicate that the AVR was strongly associated with current blood pressure in both women and men (Fig. 6.7) [57].

Further evidence from the Beaver Dam Eye Study, a population-based prospective cohort study including 2,450 subjects, reported that narrowed retinal arterioles are associated with long-term risk of systemic hypertension. In particular, subjects with smaller retinal arteriolar diameters were more likely to develop hypertension than people with larger arteriolar diameters, independent of other known risk factors for hypertension [68]. For the analysis, the AVR was categorized into quarters, the first quarter representing the most pronounced arterial narrowing and the fourth quarter representing the reverence value. It was observed that, after adjustment for age and sex, subjects with a low AVR (first quarter) have a threefold higher risk of developing systemic

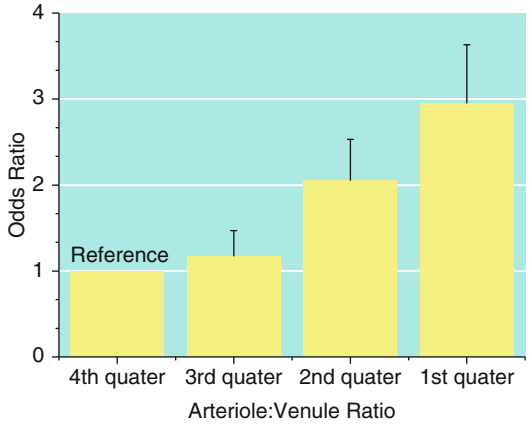

Fig. 6.8 Odds ratio for incident hypertension in relation to retinal arteriole-venule ratio (Wong et al. [68])

hypertension (odds ratio 2.95) compared to those with the highest AVR (Fig. 6.8). Based on these results, the authors suggested that structural alterations of the microvasculature may be linked to the development of hypertension and that arterial narrowing may precede the development of systemic hypertension.

The Blue Mountains Eye Study, a large population-based cohort study including more than 3,600 subjects, revealed that a low AVR is associated with increasing age, which was interpreted as a generalized arterial narrowing in the elderly [33]. These results are in keeping with the data from other large epidemiologic studies, such as data from the ARIC study or the Cardiovascular Health Study [64, 66] (CHS) also indicating for decreasing retinal arterial diameters with increasing age. Furthermore, a low AVR was found to be associated with other cardiovascular risk factors, such as cigarette smoking or systemic blood pressure.

Several lines of evidence indicate that retinal vessel diameters may also reflect systemic pathological changes in the body. In particular, data of the ARIC study show that nonspecific inflammatory markers, such as increased white blood cell and fibrinogen levels, are associated with a smaller AVR, indicating a for generalized arterial narrowing, independent from other known risk factors [29]. In contrast, the Rotterdam study showed increased white blood cell count lead to a particularly vasodilatation in retinal veins, whereas retinal arteries dilate only in a lesser extent [25]. These findings are consistent with the data of the Beaver Dam Eye Study, also indicating a relationship between increased inflammation markers and venular arterial diameters [28]. However, the results of the latter studies need to be interpreted with caution, given that no information is available whether the subjects under study had acute infections or some subgroups took any anti-inflammatory medication. Furthermore, given that the above mentioned studies were cross-sectional, temporal sequence of inflammatory-induced endothelial dysfunction and venular diameter cannot be determined. Data of an interventional study in healthy subjects showed that after experimental induction of inflammation by the means of administration of low dose of *Escherichia coli* endotoxin, retinal veins significantly dilated, paralleled by an increase of white blood cell count [32].

Interestingly, data from recent experiments indicate that retinal vessel diameters may carry also information about other vascular beds. It has been shown that narrowing of retinal vessels is associated with lower myocardial blood flow and perfusion reserve in asymptomatic subjects [63]. The authors from this study conclude that retinal arteriolar narrowing may serve as a marker of coronary microvascular disease. Along these line of thought, a couple of studies have shown that changes in retinal vessel diameters can predict the risk of coronary heart disease, stroke, and stroke mortality [64–66]. Pooled data from the Beaver Dam Eye Study and the Blue Mountains Eye Study showed that smaller arterial diameters and larger retinal venous diameters are associated with an increased risk of stroke mortality [62]. These data clearly support the idea that retinal vessel diameters may serve as a predictor for event in other vascular beds such as the heart or the brain.

With regard to ocular diseases, it has been hypothesized that generalized arterial narrowing can predict the development of open-angle glaucoma. Given that – as outlined in detail in another chapter of this book – reduced blood flow may be involved in the pathogenesis of glaucoma, much emphasis has been put into

the investigation of vessel diameters and glaucoma. Using manual diameter measurements of projected retinal images, it was reported that eyes with open-angle glaucoma have significantly reduced peripapillary retinal vessel diameters compared with a healthy, age-matched control group [27]. Further studies revealed a general narrowing of retinal vessel diameters in more than 50% of patients with early stage glaucoma, whereas these alterations were only visible in 15% of normal eyes [51]. However, the interpretation of those studies is hampered by the fact that the studies were neither population based nor adequately adjusted for blood pressure. Furthermore, subjective methods for the assessment of retinal vessel size have been used. Using an automatic technique based on the analysis of digitized fundus images, the Blue Mountains Eye Study examined the relationship between retinal vessel diameter and open-angle glaucoma. The result of this study indicate that generalized arterial narrowing is significantly associated with optic nerve head damage in patients with open-angle glaucoma, independent of age, gender, smoking, or blood pressure [43]. Whether these changes in diameter reflect an ischemic process leading to the optic nerve head damage, or occur secondary to the neuron loss caused by the disease, is however unclear. The Rotterdam Study failed to show a predictive value of retinal baseline diameter to changes of the optic disc [26]. The authors of the study conclude that the Rotterdam Study does not provide evidence for a retinal vascular role in the pathogenesis of open-angle glaucoma. One needs, however, to consider that retinal vessel diameter may not be an adequate parameter of retinal perfusion status in glaucoma.

Recent analysis, however, have changed the view of the AVR as the optimal measure for generalized arteriolar narrowing. The calculation of the AVR is mainly based on the assumption that the venous diameter remains relatively constant in response to blood pressure, age, or other factors. The above mentioned results indicate that this assumption may not hold true for every cases [35]. In particular, the fact that retinal veins dilate in response to increase inflammatory markers indicate that venules may carry different information than arteries and should consequently be analyzed separately. Consequently, as an alternative, it has been proposed to analyze retinal arterial and venous diameters separately.

Furthermore, static vessel analysis allows for the stratification of microvascular risk factors based on measurement of stationary vessel diameters. However, a further limitation of static vessel analysis is that these measured parameters allow only for a very limited conclusion about function alterations of the vasculature. Given that several physiological variations such as vasomotor changes or alterations in ocular perfusion pressure will interfere with the measured values, an exact determination of vessel function is dependent on the measurement of additional parameters as it is done in dynamic vessel analysis.

6.5.4 Results and Limits of Dynamic Vessel Analysis

In contrast to this static vessel analysis, reflecting a snapshot of retinal vessel diameter, carrying information on vascular tone, the so-called dynamic vessel analysis has been introduced to assess alterations of vascular function. For the dynamic vessel analysis, images of the fundus are recorded over a longer period of time ranging from a few seconds up to several minutes. During the measurement, period provocation tests, i.e., stimulation of the eye with flickering light, exercise, or breathing gases with variable mixtures of O_2, CO_2, and others, are used to assess vascular function. As compared to static vessel analysis, dynamic vessel analysis can provide several advantages. First and most importantly, changes of retinal vessel diameter can be measured as a function of time and position along the vessel.

6.5.4.1 Stimulation with Flicker Light

It is known for a long time that retinal vessels have the ability to adapt to changes in ocular

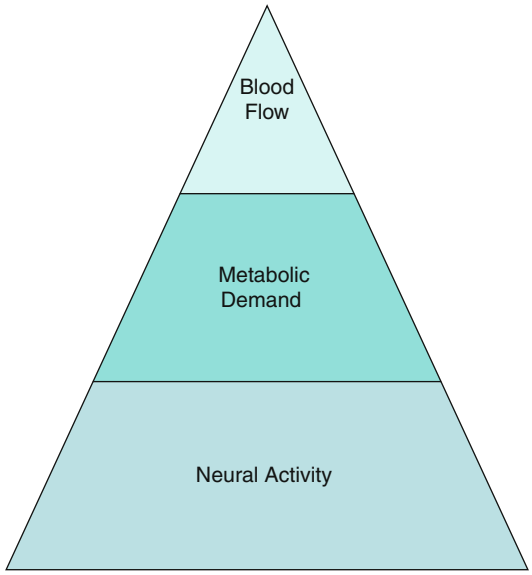

Fig. 6.9 Concept of neurovascular coupling. Increased neural activity leads to increased metabolic demand and in turn to augmented blood flow

perfusion pressure, which is commonly referred to as autoregulation. Whereas the phenomenon of blood flow autoregulation has been investigated in several vascular beds including the eye, it has recently become clear that retinal vessels can also adapt to changes in metabolic demands. Experimental evidence for a coupling between increased metabolism and increased blood flow in the eye was first suggested by the findings that glucose metabolism was enhanced in the retinal ganglion cells by flickering light. According to the current concept of neurovascular coupling, increased neural activity during stimulation with flicker light leads to an increased ganglion cell activity and to augmented metabolic demand in the retina (Fig. 6.9). This subsequently leads to retinal vasodilatation and increased blood flow. Whereas a detailed review of the current view of neurovascular coupling and its possible mediators has been published recently [53], this article will focus on the effect of flicker stimulation on retinal vessel diameters.

Initially, the effect of flicker light on the retinal circulation was investigated using photographs of the human eye fundus taken in red-free light [12]. These photos were synchronized with the cardiac pulse and taken after 1 min of flicker stimulation. After digitalization of the pictures, the diameters of straight segments of retinal vessels were analyzed before and during illumination with flickering light. The authors report a significant increase in retinal vessel diameters due to the stimulation with flickering light.

Subsequent investigations of the flicker-induced retinal vessel diameter response were conducted with an automatic tool for the retinal vessel analysis (RVA, Imedos, Jena, Germany) [44, 49]. Initially, flicker stimulation was achieved using light from a xenon arc lamp that was chopped with a rotating sector disc [49] or by light flashes [15] introduced into the illumination pathway of the fundus camera by a fiber optics. Using an optical filter system to spectrally differentiate the flicker light and the fundus illumination, the flicker was superposed on the continuous fundus illumination needed to measure the vessel diameter. Later, to increase the stimulus contrast, the flicker light was generated by electronic chopping of the fundus illumination at a frequency of 12.5 Hz [44].

Although the exact mechanism of flicker-light-induced vasodilatation is still a matter of controversy, it has been used as a provocation test for the ability of retinal vessels to adapt to different metabolic situations in health and disease. A variety of studies have shown that flicker-induced vasodilatation is altered in ocular and systemic diseases.

It has been shown that flicker light responses are diminished in patients with early stage glaucoma. In this study, 31 patients with early stage glaucoma and 31 age- and sex-matched healthy volunteers were included [16]. To avoid any vascular effects of glaucoma medication, a washout period was scheduled for all patients. Compared with age-matched control group, the study provides evidence that flicker responses are diminished in patients with early stage glaucoma, independently of the glaucoma medication administered. Interestingly, the reduced flicker response was only observed in retinal veins, not in retinal arteries. A further remarkable observation of this study is that flicker responses are already diminished already in

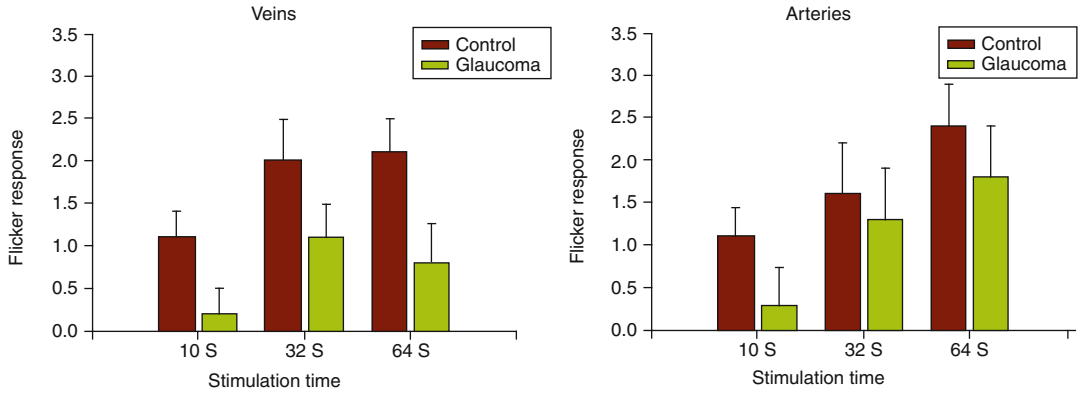

Fig. 6.10 Flicker-light-induced vasodilatation of retinal arteries and retinal veins in percent change from baseline in patients with open-angle glaucoma and a healthy control group (Modified from Garhofer et al. [16])

patients with early stage glaucoma, having only moderate glaucomatous changes. Whether the decreased flicker response in patients with glaucoma can be attributed to a vascular dysregulation or to a reduction of neural activity caused by ganglion cell loss, as it appears in glaucoma, has yet to be clarified.

The latter results were later confirmed by the work of Riva and colleagues. The authors report that flicker-evoked response measured at the optic-disc rim is reduced in ocular hypertension and early glaucoma [54]. These studies are of particular interest because it has been shown that a short-time increase of IOP by the means of an episcleral suction cup does not alter flicker-induced vasodilatation in young, healthy volunteers [14]. These results indicate that factors other than increased intraocular pressure must be responsible for the missing flicker-induced vasodilatation.

Given that vasospasm, defined as inappropriate constriction of insufficient vasodilatation, is a major risk factor for developing glaucoma, Gugleta and colleague have investigated flicker-induced vasodilatation in young, healthy women with vasospastic syndrome [21]. The authors show that in subjects with vasospasm as identified by nail-fold capillaroscopy, the maximum dilatory response to flicker stimulation was significantly reduced compared to the control group. Whether this reduced response is related to a higher risk for developing glaucoma or other vascular-related diseases has to be investigated.

Decreased flicker responses were also observed in patients with early stage diabetic retinopathy. Patients suffering from diabetes show decreased flicker-induced vasodilatation in retinal arteries compared to a healthy control group [17] (Fig. 6.10). However, no significant difference between the diabetes group and the control group was detected in retinal veins. Whether this is simply related to the smaller percentage change in retinal veins during flicker stimulation or to another mechanism has yet to be investigated. The results of this study are keeping with evidence from a recent study that showed also diminished flicker response in retinal vessel from diabetic patients in a larger group of diabetic patients [41]. Furthermore, the present study demonstrates that the vasodilatation of retinal arteries and veins under the flickering light decreases continuously with increasing stages of diabetic retinopathy. This has led to the suggestion that the effect of flicker-evoked vasodilatation could be used as an early screening tool to detect vascular dysregulation in patients with glaucoma [41].

Along this line of thought, Nagel and colleagues investigated the effect of age, systemic blood pressure, and baseline retinal vessel diameters on flicker-induced vasodilatation [44]. This is of special interest because it is known that the retinal vessels constrict with increasing blood pressure. The finding of the study indicates that flicker-induced vasodilatation is significantly

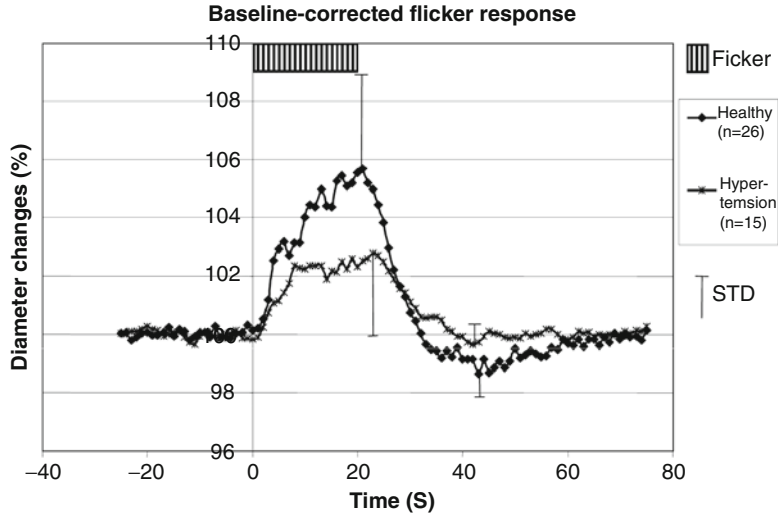

Fig. 6.11 Mean flicker response curve in healthy subjects and untreated hypertensive subjects (Nagel et al. [44])

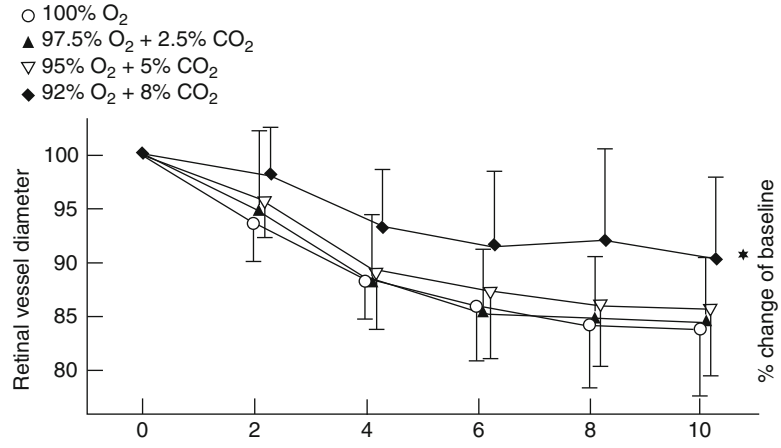

Fig. 6.12 Retinal vessel diameter during breathing of different mixture of O_2 and CO_2. Modified from Luksch et al. [40]

diminished with increasing blood pressure, whereas baseline retinal diameter had no influence on flicker responses (Fig. 6.11). Surprisingly, no significant correlation was observed between flicker response and age. This was attributed by the authors to the small sample size of the study.

6.5.4.2 Other Provocation Tests

Other provocation tests than flicker stimulation have also been used to assess vascular function in vivo. Although a detailed description of all methods and results are beyond the scope of this chapter, some examples for other provocation methods will be given.

It is known that increasing the tissue pO_2 by means of inhalation of 100% oxygen induces a pronounced vasoconstrictor effect in retinal vessels [52] (Fig. 6.12). Given that oxygen is nontoxic to adults and widely available in the clinical setting, a couple of studies have used oxygen to test vascular function. It has been reported that in diabetic patients, the vasoconstrictor response decreases with increasing stage of the disease and improves after panretinal photocoagulation [18], indicating for an impaired vascular regulation in the diabetic eye.

Another common approach to test vascular function is to change ocular perfusion pressure. Given that ocular perfusion pressure is determined by intraocular pressure and blood pressure, this can either be done by changing blood pressure – pharmacologically or by the means of

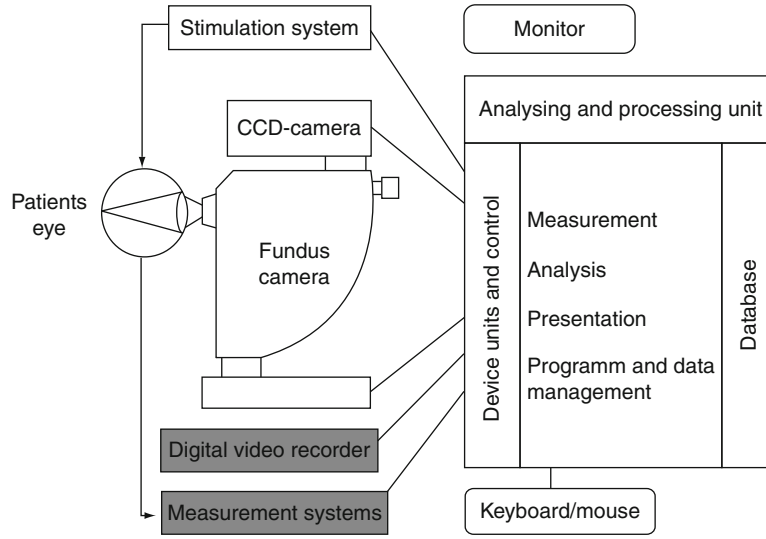

Fig. 6.13 Device unit of dynamic vessel analyzer (DVA, Imedos GmbH Jena, Germany)

isometric exercise – or by changing intraocular pressure. It is known that arteries constrict with increasing systemic blood pressure, reflecting an autoregulatory response to maintain perfusion pressure [2]. It has, however, been shown that this vascular answer is impaired under high blood glucose levels, indicating for a vascular dysregulation [3]. Additionally, evidence has been provided that the diameter response of retinal vessels to increased blood pressure is reduced in patients with type 2 diabetes [13].

In addition, regulation of vessel diameter can be tested by modifying intraocular pressure (IOP). Based on the observation that regulation of vascular tone is altered in patients with glaucoma, several experiments have focused on investigating diameter changes of retinal vessel in response to altered IOP. Nagel and colleagues have used the episcleral suction cup technique to induce an increase in IOP. Subsequently, retinal arterial and venous diameters were measured in healthy subjects, patients with open-angle glaucoma, and patients with ocular hypertension. It was observed that the change in retinal vessel diameter induced by short-time increase of IOP was significantly different among the three investigated groups [45]. Interestingly, short-time increase of intraocular pressure again by the means of a suction cup does not modify the response of retinal vessel diameter to flicker stimulation [14]. The latter study reveals that the response of retinal vessel diameters to flicker stimulation is maintained up to an IOP of 43 mmHg. This indicates that even at high IOPs, blood flow is responding to neural stimulation caused by flickering light. Accordingly, based on this data, it appears that a reduced flicker response as seen in patients with glaucoma in previous studies [16, 17] is not obligatory a direct consequence of increased IOP. However, one needs to be careful to directly apply the data of the present study to the results observed in glaucoma patients, because long-term changes in IOP were not mimicked in the present study. Because changes in IOP as induced by the suction cup technique is uncomfortable for the subject and time consuming, this approach is limited to research purposes and is currently not used in clinical practice.

6.5.5 Systems Available for Dynamic Vessel Analysis

There are currently 3 commercial systems available for dynamic vessel analysis: RVA (Retinal Vessel Analyzer), DVA (Dynamic Vessel Analyzer plus), and DVA-light (all Imedos GmbH in Jena, Germany). The schematic setup of the DVA is shown in Fig. 6.13.

The DVA is a modified fundus imaging system with the capability for dynamic and static vessel analysis. The main component of the vessel analysis system is an optical device for illumination and imaging of the fundus. For that purpose the DVA currently utilizes a fundus camera. The optical image from the fundus camera is received by a special CCD-camera attached to the system. The resulting digitized image (or image sequence) is further analyzed by the processing unit. This unit integrates all aspects of the measurement and analysis and controls the fundus camera as well as additional hardware.

For diameter measurements in the static and dynamic vessel analysis, the DVA applies dedicated adaptive filter algorithms, able to support both the actual measurement and vessel detection. The algorithms used are largely independent of changes in contrast and brightness. The spatial resolution in terms of the size of segments along the vessel is up to 12 µm, spatial resolution in the direction of measurement (perpendicular to the vessel) is better than 1 µm, and temporal resolution is 40ms.

By default the DVA is equipped with a device for flicker stimulation, with an option to connect further measurement systems (i.e. ECG, blood pressure). Flicker stimulation is achieved by an electro-optical shutter module inserted into the optical path of the fundus camera. The resulting flicker stimulus has a frequency of 12.5 Hz and a contrast ratio of about 25:1. Flicker stimulation can be used with a standardized protocol to examine vessel function.

Based on a standard or high performance imaging system (Visualis), standalone versions of dynamic vessel analysis (Retinal Vessel Analyzer, RVA) and static vessel analysis (SVA) are possible. DVA, RVA and DVAlight combine high reproducibility with a high temporal and spatial resolution [48].

6.6 Further Perspectives

The fast technical development of new instruments and software for the automatic and highly reproducible assessment of retinal vessel diameters has brought a new and interesting perspective for the early diagnosis of vascular related diseases. In particular, the observation that changes in retinal vessel size can reflect ocular pathologies and predict future disease progression underlines the importance of this new field of research. Dynamic vessel analysis further broadens the potential applications These techniques are noninvasive and easy to perform with high reproducibility and accuracy. Thus, retinal vessel analysis may be of interest for both ophthalmology and internal medicine and may help in the early identification of high risk patients and the early diagnosis of vascular related diseases.

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