Quantification of Retinal Capillary Density and Flow Velocity in Patients With Essential Hypertension

Sebastian Wolf, Oliver Arend, Karin Schulte, Thomas H. Ittel, Martin Reim

Abstract Arterial hypertension is known to be an important risk factor for cerebral and cardiovascular disease. Previous studies in rats have demonstrated that changes in both capillary density and vessel diameter may contribute to increased vascular resistance in hypertension. In vivo studies of human subjects with essential hypertension revealed a reduction in the number of arterioles in the skin and conjunctiva; no other in vivo data are available from other tissues. By means of a new imaging technique, capillary density and capillary blood flow velocity can now be assessed in the human retina. We undertook the present investigation to determine whether patients with essential hypertension and only minor clinical retinal vascular alterations have decreased retinal capillary density and altered capillary flow velocity. Seventeen hypertensive patients with only minor retinal vascular alterations and 17 healthy volunteers matched for age were selected. All study participants underwent ophthalmologic examination and fluorescein angiographic studies by means of scanning laser ophthalmoscopy. Capillary density and capillary blood flow velocity in the perifoveal network were evaluated from the angiograms. The retinal microcirculation in the perifoveal capillary network of hypertensive patients showed significant alterations. Both the capillary density and capillary flow velocities were significantly reduced compared with the control group. For the first time alterations of capillary blood flow and capillary density in a vascular network very similar to that of the brain have been demonstrated in hypertensive patients in vivo. Further studies with this technique may help identify patients at high risk for cerebrovascular diseases. (Hypertension. 1994;23:464-467.)

Key Words • hypertension, essential • microcirculation • retinal vessels • blood flow velocity • retina

Essential hypertension is an important risk factor for the development of cerebral, coronary, and peripheral artery disease. Previous studies have demonstrated that changes in both capillary density4-5 and vessel diameter6-7 may contribute to increased vascular resistance in hypertension. In vivo studies in human subjects with essential hypertension revealed a reduction in the number of arterioles in the bulbar conjunctiva6-7 and of capillaries in the nailfold.8 However, these studies gave data on only the microvasculature of the skin and conjunctiva. Until now, examination of the retina allowed only for determination of vascular changes in arterioles. These retinal vascular alterations may provide a clue to the vascular status of various organs. Histopathologic studies have demonstrated similar changes in cerebral vessels in 90% of patients with retinal vascular alterations.9 Renal vascular changes have been found in approximately 64% of patients with retinal vascular alterations.9 However, only limited information on the microcirculation can be obtained by studying retinal arteriolar changes.10 Only studies including the capillary bed can give detailed information on the status of the microcirculation during hypertension. By means of new imaging techniques11 capillary density and capillary blood flow velocity can now be assessed in the human retina.12 We undertook the present investigation to determine whether patients with essential hypertension and only minor retinal vascular alterations have decreased retinal capillary density and altered capillary flow velocity.

Methods

Study Population

Sixty-five patients with essential hypertension who were followed at the outpatient department of the University Hospital Aachen were recruited for the study. Each patient had a well-established history of chronically elevated blood pressure (>145/95 mm Hg) without any apparent underlying cause and had been treated for at least 2 years with one or more antihypertensive agents. After ophthalmologic examination 17 patients with retinal vascular alterations of stage 1 of the Neubauer classification13 were included in the study. Stage 1 of the Neubauer classification represents the earliest fundus changes due to hypertension, with only minimal narrowing of the retinal arteries. Patients with diabetes mellitus or systolic hypertension were excluded from the study.

Diagnosis was established in all 17 hypertensive patients by complete clinical, laboratory, and radiographic investigations, including renal angiography. None had cardiac or renal failure. A population of 11 men and 6 women, matched with the patients for age, was selected as a control group. Histories, physical examination, electrocardiograms, and routine chemical analyses showed that the control subjects had no evidence of present or past hypertension, cardiovascular disease, or any other systemic condition and were taking no medication.

All participants gave written informed consent for all procedures. This study was approved by the local Review Board.
Protocol
All studies were performed in the morning. Participants underwent a postrefractive ophthalmologic examination to determine the best-corrected visual acuity, followed by slit-lamp biomicroscopy, Goldmann applanation tonometry, indirect and direct ophthalmoscopy, and color fundus photography. Retinal vascular alterations of systemic hypertension were classified according to Neubauer.13

One randomly selected eye from each patient and healthy volunteer underwent subsequent fluorescein angiographic studies. For the fluorescein angiography a 20-gauge microcatheter was inserted into the antecubital vein, and the patients were seated in front of a scanning laser ophthalmoscope (SLO-101, Rodenstock Instruments). Scanning laser ophthalmoscopy provides two different fields of view: a 40° field of view for an overview of the ocular fundus and a 20° field of view for a detailed analysis of the vascular bed.

After a good fixation was obtained and the 20° field of the scanning laser ophthalmoscope was focused on the macular region, 2.5 mL sodium fluorescein (10%) with a 10-mL saline flush was injected into the antecubital vein. The dye passage through the retinal vessels was recorded by means of a digital recorder.14 In the digital recordings of the angiograms with 20° field of view, the complete perifoveal capillary network and segments of low and high fluorescence moving through the perifoveal network can be observed.

For the analysis of capillary density and capillary flow velocity, the following parameters were evaluated off-line from the digital recordings: (1) mean perifoveal intercapillary area and coefficient of variation of perifoveal intercapillary areas and (2) mean perifoveal capillary flow velocity and coefficient of variation of perifoveal capillary flow velocity.

The mean perifoveal intercapillary area in the perimacular network was calculated with digital imaging processing. Within a circle of 5° around the fovea, 100 different areas surrounded by capillaries were randomly chosen. The borders of intercapillary areas were marked interactively by drawing around the surrounding capillaries with the cursor in the digital image. The area described by the cursor was calculated to assess the homogeneity in size of the perifoveal intercapillary areas.

Perifoveal capillary flow velocity was assessed in the perimacular region by tracking the movement of a hypofluorescent front through a capillary. The mean perifoveal capillary flow velocity was calculated from 150 single flow velocity measurements carried out in different capillaries during approximately 5 seconds. The coefficients of variation of each subject for all velocity measurements were calculated. This parameter characterizes the homogeneity of the perifoveal capillary flow velocity. Dynamic and morphological data were corrected for the refractive error.15 All angiograms were evaluated in masked fashion with no clinical data available.

All blood pressures and heart rates were recorded by automatic sphygmomanometry (Dinamap, Vital Daten Monitor 1846 Sx, Critikon) immediately before each angiogram. Mean arterial blood pressure was calculated by adding one third of the pulse pressure to the diastolic pressure.

Statistical Analysis
The Kolmogorov-Smirnov test was used to determine normal distributions. Nonparametric (Mann-Whitney U test) or parametric tests (unpaired Student's t test), as appropriate, were used to assess the significance of the results. All calculated probability values are two-tailed; values less than .05 were considered to indicate significance. Group data are reported as mean±SD unless otherwise indicated.

### Results

#### Population Characteristics

The characteristics of the healthy control subjects and patients with hypertension were similar (Table 1). The systolic blood pressure in the hypertensive patients ranged from 110 to 160 mm Hg and the diastolic blood pressure from 60 to 100 mm Hg. All eyes under study had a visual acuity of 20/20 or better and an intraocular pressure of less than 20 mm Hg. Besides minimal narrowing of the retinal arteries, all ophthalmologic examinations were within a normal range. Antihypertensive medication received by the patients is specified in Table 2.

#### Angiographic Data

The Figure shows the data of the perifoveal intercapillary areas in the two groups. The perifoveal intercapillary areas were increased by approximately 50% in the hypertensive patients compared with the control group (5591±838 versus 3742±391 µm², P<.01, Student's t test). The coefficient of variation of perifoveal intercapillary areas was comparable in the two groups: 37±5 in the hypertensive patients and 34±8 in the normotensive control group (P=NS). Capillary blood flow velocity was significantly reduced in the hypertensive group compared with the control group (2.23±0.33 versus 2.87±0.53 mm/s, P<.01, Student's t test). The coefficient of variation for the capillary blood flow velocity showed no significant difference between the groups (hypertensive patients, 19±7; control group, 17±7%; P>.05, Student's t test).

### Table 1. Characteristics of Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Subjects</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>11/6</td>
<td>14/3</td>
</tr>
<tr>
<td>Age, y</td>
<td>36±9</td>
<td>40±13</td>
</tr>
<tr>
<td>Resting heart rate, bpm</td>
<td>77±13</td>
<td>73±11</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.7±9.5</td>
<td>72.6±15.9</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>93.7±6.1</td>
<td>98.1±14.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>127±10</td>
<td>134±15</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77±7</td>
<td>80±15</td>
</tr>
<tr>
<td>History of hypertension, y</td>
<td>...</td>
<td>5.9±7.0</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute. Values are mean±SD.

### Table 2. Antihypertensive Medications of Study Population

<table>
<thead>
<tr>
<th>Medication</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Adrenergic blocker</td>
<td>7</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>7</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>1</td>
</tr>
<tr>
<td>β-Adrenergic blocker, ACE inhibitor, diuretic</td>
<td>1</td>
</tr>
<tr>
<td>β-Adrenergic blocker, calcium channel blocker, diuretic</td>
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</tbody>
</table>

ACE indicates angiotensin-converting enzyme.
Scatterplot shows perifoveal capillary density in patients with essential hypertension and control subjects (5591 ± 838 vs 3742 ± 391 μm², P < .01, Student's t test). Circles represent perifoveal intercapillary area for a single subject. Horizontal lines indicate mean values for each group.

Discussion

Since the development of the ophthalmoscope, the retinal vascular bed can be directly examined in vivo. Retinal vascular changes in hypertensive retinopathy have provided a useful guide to physicians for the care of hypertensive patients. The development of fluorescein angiography has allowed the dynamics of retinal circulation to be studied. Recent developments in imaging techniques have improved spatial and temporal resolution of fluorescein angiograms to the extent that it allows quantification of retinal capillary perfusion. Findings in patients with diabetic retinopathy have shown the value of these measurements for microvascular research.

Retinal vascular alterations in hypertension provide a clue to the status of various organs, especially the central nervous system, the cardiovascular system, and the kidneys. The similarity in structure and function between retinal vessels and cerebral vessels is striking. Both tissues exhibit blood-tissue barriers. The tissue reactions of the retina to vascular changes are very similar to those of the central nervous system. The retinal capillary network is a complex network that is arranged in distinct layers. Three-dimensional vascular casts show well-defined layered patterns, with an outer capillary layer, an inner layer, and a radial peripapillary capillary layer around the optic disc. In the macular area only a capillary monolayer is present. Different vascular disorders seem to affect preferentially either the deeper or superficial capillary layers. Retinal hypertensive alterations mainly arise from the superficial capillaries. In vivo imaging of the retina projects the three-dimensional vascular system on a two-dimensional image. Therefore, only the perifoveal monolayer of the capillary network allows for detailed analysis of the capillary network from fluorescein angiograms. Capillary density in the perifoveal monolayer can be assessed by measurement of perifoveal intercapillary areas. The size of intercapillary areas is inversely correlated to capillary density.

The results of the present study demonstrate retinal microcirculatory changes in the perifoveal capillary network of hypertensive patients. Both the capillary bed and capillary hemodynamics show significant alterations. It seems unlikely that all of the differences in microvessel density and flow velocity were due to the medication patients had received. Only two patients were receiving angiotensin-converting enzyme inhibitors, which have been shown to reduce microvessel density in rats. Most patients in the present study were receiving β-adrenergic or calcium channel blockers, which are unlikely to produce capillary rarefaction.

The significant increase of perifoveal intercapillary areas indicates decreased capillary density. This finding is similar to previous observations of capillary rarefaction in human hypertension and in several rat models of hypertension. The coefficient of variation of the perifoveal intercapillary areas shows no significant difference between hypertensive patients and the control group. The unchanged homogeneity in capillary network in combination with decreased capillary density indicate global capillary loss in the perifoveal capillary network.

Different features may lead to capillary rarefaction in hypertension. Functional rarefaction of the capillary bed may result from a primary increase in sympathetic activity or local autoregulatory mechanisms. Studies in hypertensive rats have shown an increase of vasoconstriction and closure of smaller arterioles after a rise in blood pressure. However, application of sodium nitroprusside has been shown to open the functionally closed arterioles. The state of functional closure of microvessels progresses to one of anatomic rarefaction in chronic hypertension. At this stage, structural changes in the vessel walls have been demonstrated by light and electron microscopy. Results of mathematical analysis have suggested that rarefaction in the microvascular bed may contribute to the increase in total peripheral resistance observed in hypertension.

The mean capillary flow velocity measured among the hypertensive patients was reduced by approximately 22% compared with the results in the control group. The coefficients of variation were comparable in both groups, indicating a global reduction of flow velocity in the perifoveal capillary network among hypertensive patients. The present findings are in good agreement with results from flow velocity measurements in nailfold capillaries. The reduction of flow velocity may be due to decreased capillary perfusion caused by increased resistance in the precapillary arteries as well as to an increase of plasma viscosity. The combination of decreased capillary flow velocity and capillary density in the perifoveal network indicates reduced blood flow in the macular area. Previous studies measuring retinal blood flow velocities and arteriovenous passage times indicated no significant changes of retinal hemodynamics in hypertensive patients with minor retinal vascular alterations. This difference in the findings may be explained by the formation of preferential channels in the multilayer capillary network of the peripheral retina. Shunt flow through preferential channels would lead to constant or increased total retinal blood flow in combination with reduced local flow.

The present study in patients with arterial hypertension elucidates the alteration of capillary blood flow in a vascular network that is very similar to that of the brain. Further studies with this technique may help to identify patients at high risk for cerebrovascular diseases.
Acknowledgment
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References
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