Increased Wall:Lumen Ratio of Retinal Arterioles in Male Patients With a History of a Cerebrovascular Event

Joanna M. Harazny, Martin Ritt, Delia Baleanu, Christian Ott, Josef Heckmann, Markus P. Schlaich, Georg Michelson, Roland E. Schmieder

Abstract—Arterial hypertension is a major risk factor for stroke, and retinal vessels can be regarded as a mirror of the cerebral vasculature. Whether vascular remodeling of retinal arterioles with ageing and hypertension plays a role in cerebrovascular risk stratification has not yet been adequately addressed. In study 1, retinal arteriolar structure was assessed in 182 normotensive volunteers and 117 patients with essential hypertension. In study 2, we compared retinal arteriolar structure among 74 normotensive volunteers, 47 patients with treated essential hypertension, and 18 subjects with a history of a cerebrovascular event. Retinal arteriolar structure was assessed using scanning laser Doppler flowmetry and automatic full-field perfusion imaging analysis. In study 1, wall:lumen ratio of retinal arterioles revealed a significant correlation with age (r = 0.198; P = 0.001). In study 2, wall:lumen ratio was highest in patients with a history of a cerebrovascular event compared with treated hypertensive and normotensive subjects (0.46 ± 0.08, 0.36 ± 0.14, and 0.35 ± 0.12; P = 0.007). When the treated group with hypertension was divided into 2 subgroups according to the quality of blood pressure control, patients with poor blood pressure control showed higher wall:lumen ratio than subjects with good blood pressure control (0.40 ± 0.13 versus 0.31 ± 0.13; P = 0.025). Thus, assessment of wall:lumen ratio of retinal arterioles emerged as an attractive tool to identify treated patients with hypertension with increased cerebrovascular risk.

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Key Words: hypertension ■ retina ■ arterioles ■ remodeling ■ cerebrovascular risk

Stroke is one of the leading causes of death and functional impairment in Western societies.1-3 Arterial hypertension is a major risk factor for both cerebral infarction and intracerebral hemorrhage.1,2 It leads to stroke by promoting atherosclerosis and lipohyalinosis in the aortic arch, viscerocerebral arteries, and arterioles and contributing to heart disease, of which stroke is a complication.3 Control of high blood pressure (BP) contributes to the prevention of stroke, as well as to the prevention or reduction of other target-organ damage. A decrease of diastolic BP of 5 to 6 mm Hg results in a 42% reduction in the incidence of stroke and a 45% reduction in the incidence of fatal stroke. In the Systolic Hypertension in the Elderly Program, reductions of 11 mm Hg in mean systolic BP in the treatment group led to a decline in the incidence of stroke of 36%.1-4

Cerebral and retinal circulation share anatomic, physiological, and embryological features.3 An autopsy study of patients with stroke showed a close correlation between cerebral and retinal arteriolar findings.6 Therefore, retinal blood vessels mirror those of the cerebral circulation. Retinal vasculature can be visualized and examined noninvasively and is, thus, an interesting tool to study cerebral circulation. In 1939, Keith et al7 demonstrated the role of retinopathy signs on risk stratification and described a widely used classification system that categorizes the signs of hypertensive retinopathy into 4 groups of increasing severity.5 In 1966, Breslin et al8 confirmed the prognostic importance of ophthalmoscopic findings in essential hypertension. However, low retinopathy grades could not be distinguished (grade 1 signs are not easily distinguished from grade 2 signs), and low grades of retinopathy are not closely related to severity of hypertension.5 Therefore, the usefulness of the classification system by Keith et al8 (and the modified version by Scheie9) and its relevance for current clinical practice has repeatedly been questioned in the last decade.5 In recent studies, a decrease in the arteriole:venule ratio and other retinal microvascular abnormalities were demonstrated to be associated with increased incidence of cardiocerebrovascular events.10,11

Vascular remodeling of small and large vessels in arterial hypertension is an early sign of atherosclerosis and lipohyalinosis. In particular, an increase in the ratio of wall thickness:lumen diameter develops and maintains hypertension by increasing vascular resistance.12 Rizzoni et al13 were able to demonstrate that an increased media:lumen ratio of subcuta-
neous small arteries was associated with an increased prevalence of cardiovascular events. In large arteries, an increased intima-media thickness of carotid arteries has repeatedly been shown to predict occurrence of both stroke and myocardial infarction.\textsuperscript{11} Ageing is associated with alterations in a number of structural and functional properties of arteries, including diameter, wall thickness, wall stiffness, and endothelial function,\textsuperscript{14} but whether ageing is associated with structural alterations of retinal arterioles is unknown. To test whether wall:lumen ratio (WLR) of retinal arterioles changes with ageing, we examined patients with hypertension and normotensive controls of various ages. In an attempt to examine whether WLR of retinal arterioles is associated with cerebrovascular disease, we investigated subjects with a history of a cerebrovascular event compared with normotensive volunteers and patients with hypertension.

Methods

Study Population and Study Design

These observational studies were based on collaboration between the University of Erlangen-Nürnberg Departments of Neurology, Ophthalmology, and Nephrology and Hypertension. The study participants were patients referred to one of these collaborating departments or recruited via advertisements in local newspapers.

Study 1

This study population was composed of patients with essential hypertension (defined as systolic BP $\geq 140$ mm Hg or diastolic BP $\geq 90$ mm Hg or on antihypertensive treatment) and normotensive control subjects (systolic BP $< 140$ mm Hg and diastolic BP $< 90$ mm Hg). BP was measured in a sitting position according to World Health Organization criteria 3 times, and the mean was calculated. Inclusion criteria were male gender and age of $\geq 18$ years. Exclusion criteria were renal impairment (defined by serum creatinine $> 1.2$ mg/dL), hepatic disease (determined by any of the following: GOT or GPT values exceeding $> 150\%$ of the upper limit of the reference range or GGT, AP, or bilirubin levels $> 200\%$ of the upper limit of the reference range), diabetes mellitus (defined by a fasting glucose $\geq 126$ mg/dL or on antidiabetic treatment), any form of secondary arterial hypertension, any significant eye disease (in particular, hypertensive retinopathy grades III and IV), atrial fibrillation, atrioventricular block grade II or higher, dilatative cardiomyopathy, a history of vasculitis, seizure disorder, and regular smoking.

Study 2

This study population consisted of 3 groups. First, patients with a history of a cerebrovascular event (determined as transient ischemic attack, prolonged reversible ischemic neurologic deficit, or stroke diagnosed by a neurologist within 1 to 7 days before examination of retinal vasculature) represented the cerebrovascular event group. Second, patients with essential hypertension (as defined in study 1) receiving antihypertensive treatment with $\geq 1$ antihypertensive drug according to the European Guidelines of Hypertension and the discretion of the individual primary care physician. No patient in this group had a history or clinical signs of a cerebrovascular event. Subjects in this group were further subdivided according to the quality of BP control into those with good BP control (systolic BP $< 140$ mm Hg and diastolic BP $< 90$ mm Hg) and those with poor BP control (systolic BP $\geq 140$ mm Hg and/or diastolic BP $\geq 90$ mm Hg).

Third, normotensive subjects (as determined in study 1) served as a control group, and none of the participants had a history or clinical signs of a cerebrovascular event. Inclusion criteria were male gender and age between 40 and 80 years. Exclusion criteria did not differ from study 1.

Before enrollment in the study, informed written consent was obtained from each participant. The study protocol was approved by the clinical investigations ethics committee of the University of Erlangen-Nürnberg. The study was conducted in accordance with Good Clinical Practice guidelines and in compliance with the Declaration of Helsinki and Title 45 of the US Code of Federal Regulations, Part 46.

Measurement of WLR of Retinal Arterioles

Retinal WLR was assessed using scanning laser Doppler flowmetry (SLDF) at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering). Briefly, an arteriole with a size between 80 and 140 $\mu$m of the superficial retinal layer in a retinal sample of 2.56x0.64x0.30 mm was scanned within 2 seconds at a resolution of 256 points x 64 lines x 128 lines. Measurements were performed in the juxtapapillary area of the right eye, 2 to 3 mm temporal superior to the optic nerve; the mean from 3 measurements was taken. Analyses of diameters were performed offline with automatic full-field perfusion imaging analysis (SLDF version 3.7 by Welzenbach).\textsuperscript{15–17} Outer arteriole diameter (AD) was measured in reflection images, and lumen diameter (LD) was measured in perfusion images. WLR was calculated using the formula (AD – LD)/LD (Figure 1).

![Figure 1. Assessment of WLR of retinal arterioles.](http://hyper.ahajournals.org/Downloaded from/March 31, 2017)
The examination was performed in sitting position after 20 minutes of rest, at room temperature and daylight conditions between 8 AM and 2 PM but before lunch. The readers (J.M.H., Christiane Koehler, Susanne Avendano, and Ullrike Heinritz) of retinal vasculature were blinded to the BP and metabolic and cerebral disease status of the patients.

Interobserver and Intraobserver Error for the Assessment of WLR
To assess intraobserver variation, 1 examiner measured WLR of retinal arterioles of 10 different subjects with 4 measurements per subject. To assess interobserver variation, WLR of retinal arterioles of 10 different subjects were evaluated by 4 different examiners with 1 measurement per subject. The coefficients of variation expressed in percentages were calculated according to the formula (SD/ mean)×100. The intraobserver coefficient of variation was 3.5%, and the interobserver coefficient of variation was 8.9%.

Statistics
All of the statistical analyses were performed using SPSS 14.0 (SPSS Inc.). Results are given as mean±SD. For comparison between groups Mann–Whitney U test, Kruskal–Wallis test, or 1-way ANOVA (with Bonferroni correction where appropriate) were used. χ² test was used for comparison with respect to frequency of medical drug classes used in the good and poor BP control group. Correlation analyses were performed by Pearson’s correlation coefficient. A 2-tailed P<0.05 was considered statistically significant.

Results
Study 1
Clinical characteristics of subjects of study 1 are shown in Table 1. Among the 117 subjects in the hypertensive group in study 1, 11 subjects (9.4%) were newly diagnosed and untreated. Within the remaining group of 106 patients with hypertension who were on treatment, 52 (49%) did not reach target BP levels, which were defined as a systolic BP <140 mm Hg and diastolic BP <90 mm Hg. The subjects with hypertension were older (P=0.003) and had higher systolic, diastolic, and mean BP levels (all P<0.0001) than normotensive control subjects. Body mass index (BMI) was also higher in hypertensive than normotensive subjects (P<0.0001). WLR of retinal arterioles did not differ between the study groups (P=0.735). In normotensive subjects, WLR correlated with age (r=0.227; P=0.002; Figure 2). Dividing the normotensive group in 3 age groups, WLR was higher in subjects 60 to 79 years of age compared with subjects 40 to 59 or 20 to 39 years of age (0.37±0.13 versus 0.33±0.12 versus 0.26±0.11; P=0.004). In the hypertensive group, no such clear relation between age and WLR was observed, irrespective whether correlation analyses (r=0.150; P=0.102; Figure 3) or the categorization into 3 age groups (0.38±0.17 versus 0.34±0.14 versus 0.29±0.11; P=0.327) was performed (Figure 4). Thus, WLR clearly increased with ageing in the control group, but no such relation was evident in hypertensive subjects. However, no differences in WLR of retinal arterioles were observed between the hypertensive group and normotensive subjects at each age category (all P>0.2). Correlation analyses revealed a significant correlation between WLR of retinal arterioles and age (r=0.198;
agents in various combinations (β-blocker and angiotensin converting enzyme [ACE] inhibitor), 5 patients used 2 antihypertensive agents in various combinations (β-blockers, ACE inhibitors, calcium antagonists, and diuretics were used in 100%, 80%, 20%, and 20% of these subjects, respectively; Table 3). The treated essential hypertensive group consisted of 47 subjects. Twenty of these patients were on monotherapy (β-blockers, ACE-inhibitors, angiotensin receptor blockers [ARBs], and calcium antagonists were used in 35%, 30%, 10%, and 25%, respectively), whereas 27 patients used ≥2 agents (β-blockers, ACE inhibitors, ARBs, calcium antagonists, and diuretics were used in 44%, 56%, 11%, 41%, and 81% of these subjects, respectively). The normotensive group consisted of 74 volunteers. Mean duration of hypertension was 5.36 years in the hypertensive group and 9.67 years in the normotensive group. Systolic, diastolic, and mean arterial BP levels in the hypertensive group (despite treatment) were significantly higher than in the normotensive group (139±14 versus 124±9 mm Hg, 84±13 versus 73±10 mm Hg, and 103±12 versus 90±9 mm Hg; all P<0.0001) but did not differ significantly, although they were numerically higher, from patients in the cerebrovascular event group (130±16, 80±12, and 97±13 mm Hg).

WLR was greatest in patients with a history of a cerebrovascular event compared with hypertensive and normotensive control subjects (0.46±0.08 versus 0.36±0.14 versus 0.35±0.12; P=0.007; Figure 6). One-way ANOVA with Bonferroni correction showed that WLR was significantly higher in the cerebrovascular event group when compared with the hypertensive group (P=0.014) or with normotensive subjects (P=0.006) and that WLR was not different between hypertensive and normotensive subjects. We further divided the subjects in the hypertensive group into subjects with good BP control (n=20) and subjects with poor BP control (n=27). Accordingly, BP levels (systolic BP 135±6 versus 153±10 mm Hg; diastolic BP 86±5 versus 100±8 mm Hg; and mean arterial BP 102±5 versus 118±8 mm Hg) differed between this 2 groups (all P<0.0001), whereas heart rate (76±10 versus 69±3 bpm), age (54±6 versus 54±6 years), and BMI (27±2 versus 28±3 kg/m²) were similar (P=0.279, P=0.96, and P=0.12, respectively). Patients with poor BP control showed significantly higher WLR than subjects with good BP control (P=0.025; Figure 7). Seventy-five percent of patients with good BP control and 74% of patients with poor BP control were treated with ≥1 antihypertensive agent with vasoprotective properties (ACE inhibitor, ARB, or a calcium antagonist).

**Study 2**

Clinical characteristics of participants are shown in Table 2. In the cerebrovascular event group, 9 patients had a history of transient ischemic attack, and 9 patients had a history of stroke. All of the patients in this group had essential hypertension, and 8 patients in this group were treated with antihypertensive medication (3 subjects were on monotherapy [2× β-blockers and 1× angiotensin converting enzyme [ACE] inhibitor], 5 patients used ≥2 antihypertensive agents in various combinations (β-blockers, ACE inhibitors, calcium antagonists, and diuretics were used in 100%, 80%, 20%, and 20% of these subjects, respectively; Table 3). The treated essential hypertensive group consisted of 47 subjects. Twenty of these patients were on monotherapy (β-blockers, ACE-inhibitors, angiotensin receptor blockers [ARBs], and calcium antagonists were used in 35%, 30%, 10%, and 25%, respectively), whereas 27 patients used ≥2 agents (β-blockers, ACE inhibitors, ARBs, calcium antagonists, and diuretics were used in 44%, 56%, 11%, 41%, and 81% of these subjects, respectively). The normotensive group consisted of 74 volunteers. Mean duration of hypertension was 7.51±3.63 years in the hypertensive group and 9.67±4.63 years in the cerebrovascular event group (P=0.117). Mean duration of hypertension did not differ between the good BP control group and the poor BP control group (7.65±5.56 and 7.41±5.32 years; P=0.881). Mean duration of treatment period was 6.13±4.5 years in the 8 treated subjects with hypertension of the cerebrovascular event group. Mean duration of treatment period did not differ between the good and poor BP control groups (5.30±3.8 and 5.63±4.5 years; P=0.787).

Age and BMI did not differ among the group with a history of a cerebrovascular event, the hypertensive group, and normotensive group. Systolic, diastolic, and mean arterial BP
Discussion

To our knowledge, this is the first study to evaluate WLR of retinal arterioles in normotensive and treated subjects with hypertension, as well as in patients with a history of a cerebrovascular event. We found that WLR increased with ageing in normotensive subjects. WLR did not differ at any age group between normotensive and hypertensive subjects. Of note, no significant correlation between age and vascular structure was found in subjects with hypertension. However, when the normotensive and hypertensive subjects were analyzed, combined WLR of retinal arterioles showed a significant correlation with age. The main result of our study is that increased WLR is associated with cerebrovascular disease: WLR was highest in the cerebrovascular event group, whereas in patients with treated essential hypertension and in the normotensive control group, WLR was significantly lower. Interestingly, retinal vascular structure was not different between treated hypertensive and normotensive subjects. This is in line with recent reports suggesting that antihypertensive agents can correct vascular remodeling of subcutaneous and omental small arteries and arterioles.18 Our data suggest that these agents probably show similar effects on vascular structure in retinal vessels as those observed in the subcutaneous and omental vascular bed. Because we divided our treated hypertensive group into 2 subgroups according to the quality of BP control, we could demonstrate that good BP control was associated with a lower WLR as opposed to poor BP control. Each antihypertensive drug class was used to a similar frequency in the good and poor BP control groups (Table 3). Thus, it seems as if the reduction of BP achieved is the major factor to influence vascular structure in retinal arterioles of treated patients with hypertension. However, no overall correlation was found between BP and WLR of retinal arterioles when both study cohorts were analyzed combined. Systolic \( r = -0.19; \) diastolic \( r = 0.37; \) and mean arterial BP \( r = 0.16; \) were not correlated with WLR of retinal arterioles. The lack of a relation between WLR of retinal arterioles and BP may well be the result of the demonstrated effect of some antihypertensive agents to beneficially influence vascular structure. In this context, the large number of subjects on antihypertensive treatment also needs to be taken into account. To clarify this issue, a cohort of previously untreated subjects would need to be studied. In our study cohort, however, the number of untreated hypertensive subjects is too small to adequately address this issue.

Because of the importance of target organ damage in determining the overall cardiovascular and cerebrovascular risk in hypertensive patients, evidence for organ involvement is crucial. Recently published guidelines recommend identifying abnormal structure and function of blood vessels as one tool to identify individuals with hypertension with advanced risk. In a recent study evaluating subjects with mild hypertension, media:lumen ratio of small subcutaneous arteries and arterioles was increased in all of the participants, whereas \( \approx 60\% \) had impaired endothelial function (tested with acetylcholine-induced relaxation) and 45% had left ventricular hypertrophy.19 Therefore, altered small arterial structure may represent the most prevalent and perhaps the earliest form of target organ damage in essential hypertension, occurring before proteinuria or cardiac hypertrophy is evident. In addition, structural alterations in small arteries and arterioles may be closely related to target organ damage, especially at the cardiac level, because a linear relation between the media:lumen ratio of subcutaneous small arteries and arterioles and left ventricular mass index or relative wall thickness has been detected in patients with hypertension.20 However, whether these findings of subcutaneous and omental resistance arteries also hold true in the retinal blood vessels is unknown.

Table 3. Frequencies of Antihypertensive Drug Classes Used in Good and Poor Blood Pressure Control Group (Study 2)

<table>
<thead>
<tr>
<th>Antihypertensive Drug Class</th>
<th>Good BP Control Group, Yes/No</th>
<th>Poor BP Control Group, Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td>10/10</td>
<td>12/15</td>
</tr>
<tr>
<td>ARBs</td>
<td>1/9</td>
<td>3/24</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>7/13</td>
<td>9/18</td>
</tr>
<tr>
<td>( \beta )-Blockers</td>
<td>7/13</td>
<td>12/15</td>
</tr>
<tr>
<td>Diuretics</td>
<td>10/10</td>
<td>12/15</td>
</tr>
<tr>
<td>ACE inhibitors + ARBs</td>
<td>11/9</td>
<td>15/12</td>
</tr>
<tr>
<td>ACE inhibitors + ARBs + calcium antagonists</td>
<td>15/5</td>
<td>20/7</td>
</tr>
</tbody>
</table>

No significant differences in the frequency of the use of antihypertensive drug classes were evident (all \( P > 0.450 \)).
A major limitation of our study is that we cannot provide detailed data concerning medical treatment and duration of hypertension in study 1. Study 1 was the initial study, its primary goal was to evaluate the new SLDF-technique, and it was not designed to draw any conclusion regarding the association of any drug with vascular structure. The effect of some antihypertensive agents on vascular structure might have influenced WLR in treated patients with hypertension. In study 1, WLR showed a wide range of dispersion in patients with hypertension that might be because of the high dispersion of BP levels on one hand and the effect of different antihypertensive agents on vascular structure on the other hand. Drugs that interfere with the renin-angiotensin system\(^2\)\(^{21–23}\) and calcium antagonists\(^2\)\(^{24}\) have been shown to correct or at least improve vascular remodeling in subcutaneous small arteries and arterioles in subjects with hypertension, whereas such an effect was not found in patients treated with \(\beta\)-blockers or diuretics. The wide range of dispersion in WLR might also be responsible for why our data did not show an expected correlation of age with WLR in patients with hypertension. Thus, future studies are needed that focus on the impact of different antihypertensive agents on retinal arteriolar structure and that evaluate the association of age with WLR of retinal arterioles in treated and untreated patients with hypertension.

**Perspectives**

We found that WLR of retinal arterioles increases with age in normotensive subjects, whereas this relation did not reach the level of statistical significance in treated subjects with hypertension. WLR was significantly increased in patients with overt cerebrovascular disease. In addition, WLR of retinal arterioles was higher in treated subjects with hypertension with poor BP control than in patients with good BP control and is, thus, in accordance with the increased risk for stroke in poorly controlled subjects with hypertension. Our data suggest that the noninvasive measurement of the WLR of retinal arterioles with SLDF represents a novel interesting research and potentially clinical tool for cerebrovascular risk stratification.

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**Disclosures**

None.

**References**


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