Noninvasive Evaluation of Wall Shear Stress on Retinal Microcirculation in Humans

Taiji Nagaoka and Akitosbi Yobida

PURPOSE. To evaluate wall shear stress (WSS) on retinal microcirculation noninvasively.

METHODS. Retinal vessel diameter (D) and mean centerline blood velocity ($V_{max, mean}$) were measured in the retinal arterioles and venules at first- and second-order branches in 13 subjects using laser Doppler velocimetry (LDV). Retinal blood flow (RBF) and wall shear rate (WSR) were calculated using these two parameters. Blood viscosity at the calculated shear rate was also measured using a cone-plate viscometer. WSS was calculated as the product of the WSR and the blood viscosity.

RESULTS. In the first-order branches, the averaged D, $V_{max, mean}$, RBF, and WSR mean were $108 \pm 15 \mu m$, $41 \pm 10 mm/s$, $11 \pm 4 \mu L/min$, and $1530 \pm 383 s^{-1}$ in the arterioles and $147 \pm 13 \mu m$, $23 \pm 3 mm/s$, $12 \pm 4 \mu L/min$, and $632 \pm 73 s^{-1}$ in the venules, respectively. The apparent blood viscosities at the measured shear rates were $3.5 \pm 0.3$ centipoise (cP) in the arterioles and $3.8 \pm 0.4$ cP in the venules. Therefore, the averaged WSS was $54 \pm 13$ dyne/cm$^2$ in the arterioles and $24 \pm 4$ dyne/cm$^2$ in the venules. The WSS in the second-order arterioles was significantly lower than that in the first-order branches ($P = 0.002$), but the WSS in the first-order venules was similar to that in the second-order venules.

CONCLUSIONS. The authors demonstrated that the WSS in the retinal vessels could be evaluated noninvasively in humans using LDV and cone-plate viscometry. This system may be useful for further clinical investigation of the role of shear stress in the pathogenesis of various retinal disorders. (Invest Ophthalmol Vis Sci. 2006;47:1113–1119) DOI:10.1167/iovs.05-02018

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it is important to evaluate the WSS in vivo in humans. However, there have been few clinical studies of shear stress, especially in human microcirculation, mainly because there are no adequate noninvasive, reliable methods to assess the velocity and diameter of the microvessels, especially in the arterioles, which mainly regulate total peripheral vascular resistance.

The retinal vascular system is easily accessible to noninvasive in vivo visual observation in humans. Recent population-based studies suggested that precise assessment of retinal microvascular abnormalities may provide independent information regarding cerebrovascular risk. We also recently reported that the retinal circulatory parameters measured by retinal laser Doppler velocimetry (LDV) may be associated with systemic atherosclerosis in patients with coronary artery disease; suggesting that the measurement of retinal circulation using LDV may be useful for the evaluation of systemic vascular disorders. Recently, we showed that LDV is a reliable, noninvasive, and useful tool for evaluating the retinal circulation in humans by the simultaneous measurement of vessel diameter and blood velocity, which are needed to calculate shear rate. The purpose of this study was to establish a reliable measurement system to evaluate wall shear stress in retinal vessels in humans. Because WSS is the WSR multiplied by the blood viscosity, we measured WSR and blood viscosity using retinal LDV and cone-plate viscometry to calculate the retinal WSS on retinal arterioles and venules. In addition, to examine whether WSS varies with the branching order of retinal microvessels, we examined the WSS in the first- and second-order arteriolar and venular branches in the retinal microvascular network.

METHODS

Subjects

Thirteen healthy male volunteers (age range, 19–33 years) participated in the present study. All subjects were fully informed of the procedures, risks, and benefits of the study, and written consent was obtained from all subjects before the study. The procedures used in this study were approved by the Institution Human Studies Committee and were in accordance with the tenets of the Declaration of Helsinki. Subjects had corrected visual acuity better than 20/20, clear media, and no history of ocular or systemic disease or therapy. The temperature in the examination room was maintained between 22° and 24°C. Tests were initiated at approximately 11:00 AM. Subjects were all nonsmokers and abstained from drinking coffee for at least 2 hours before the test. Each subject rested for 10 to 15 minutes in a quiet room before the test.

Mean arterial blood pressure (MABP) and heart rate were measured by electronic sphygmomanometer (EP-888i; Colin, Tokyo, Japan). Intraocular pressure (IOP) was monitored by application tonometry (Haag Streit, Bern, Switzerland). Pupil dilation was achieved using a combination of 0.5% tropicamide and 1% phenylephrine eye drops.

Measurement of Retinal Blood Flow

Retinal LDV (Canon Laser Blood Flowmeter [CLBF] model 100; Canon, Tokyo, Japan) was assessed to estimate blood flow in the major temporal retinal arterioles and adjacent venules. This retinal LDV system...
allows noninvasive measurement of the absolute values of the red blood cells (RBCs) flowing in the centerline of the vessel, based on bidirectional LDV.\textsuperscript{10} A probing red laser light (wavelength, 675 nm) is emitted from a fundus camera-like measuring head. The red Doppler-shifted light scattered from the flowing RBCs in the retinal vessels is detected simultaneously in two directions separated by a fixed angle. The signals from the two photomultiplier tube detectors undergo computer-controlled spectrum analysis, and the centerline velocity of the RBCs, which is defined as $V_{\text{max}}$, is sequentially measured automatically over 2 seconds.

This device also contains systems to measure vessel diameter and to track vessels. The green stripe (wavelength, 544 nm) is oriented perpendicularly to the axis of the vessel. During the session, a linear imaging sensor takes 15 vessel profiles of the target vessel illuminated by a green laser beam every 4 milliseconds just before and after each velocity measurement. The diameter of the retinal artery is determined automatically by computer analysis of the signal produced by the arterial image on the array sensor using the half height of the transmittance profile to define the vessel edge.\textsuperscript{11} Further detail about the LDV system is available from our previous reports.\textsuperscript{12}

**Measurement Site and Comparison of First- and Second-Order Vessels**

Laser Doppler measurements were obtained from a temporal retinal artery and vein in one eye of each subject. The arteries and veins chosen for measurement had relatively straight segments that were sufficiently distant from the adjacent vessels. We measured the retinal blood flow (RBF) at two sites in the retinal artery and vein upstream and downstream of the flow divider (Fig. 1a). The major retinal arterioles and venules that arise from the optic disc were classified as the first-order vessels, and the larger daughter arterioles and venules located 1 disc diameter away from the bifurcation point were chosen as the second-order vessels to avoid flow disturbances by the bifurcation. This measurement was repeated 5 times with an interval of 1 minute in each subject.

**Measurement of Blood Viscosity**

On the same day as the laser Doppler examination and within a few hours of blood withdrawal from the antecubital vein, blood viscosity ($\eta$) was measured in vitro at 37°C with a cone-plate viscometer (modular compact rheometer; Physica MCR-100, Paar Physica, Ostfildern, Germany). Blood was anticoagulated with EDTA (3.4 mM). After incubation of 2 mL of the blood sample for 5 minutes in a 37°C water bath, approximately 600 $\mu$L well-mixed whole blood was loaded into the viscometer. Whole blood viscosity was determined over a wide range of shear rates from 1 to 6000 s\textsuperscript{-1} by 40 discrete values (Fig. 2). Blood viscosity was expressed in centipoise.

**Calculations**

The mean centerline blood velocity ($V_{\text{max, mean}}$) was obtained by averaging $V_{\text{max}}$ during one cardiac cycle. Maximum blood velocity and minimum blood velocity, which were associated with the cardiac systolic and diastolic, were indicated as $V_{\text{max, systolic}}$ and $V_{\text{max, diastolic}}$, respectively (Fig. 1). Resistive index was defined as the following:

$$\text{Resistive index} = \frac{V_{\text{max, systolic}} - V_{\text{max, diastolic}}}{V_{\text{max, systolic}}}$$

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933440/)
Laminar flow in a tube was a prerequisite concerning Poiseuille’s law. To confirm laminar retinal blood flow, the Reynolds number ($R_e$) was calculated according to the formula

$$R_e = \frac{\rho \cdot V_{\text{mean}} \cdot (D/2)}{\eta}$$

where $\rho$ is the blood density (assumed to be 1058 kg/m$^3$), $V_{\text{mean}}$ is the mean velocity of the blood (in meters per second), $D$ is the vessel diameter (in millimeters), and $\eta$ is the blood viscosity (in centipoise). A Reynolds number value less than 1000 is considered characteristic of laminar flow.  

**Reproducibility and Reliability of LDV Measurement**

To evaluate the reproducibility of the LDV system, we calculated the average coefficient of variation (CV) (100 [SD/mean]; mean $\pm$ SD). We obtained five values from the same vessel at every minute in 5 minutes, averaged them in each subject, and calculated the CV in each vessel in each subject. To demonstrate conservation of flow in the retinal microvessels using the LDV system, we examined the relation of RBF between first-order (parent) vessel and the sum of two second-order (daughter) vessels.

**Statistical Analysis**

All data are expressed as mean $\pm$ SD. Differences between the first- and second-order branches in the retinal arterioles or venules were analyzed using the Student’s paired $t$ test. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Systemic Parameters and OPP**

At the beginning of the measurements, the group-averaged values of the systolic, diastolic, and mean blood pressures were $119 \pm 13$, $63 \pm 7$, and $82 \pm 8$ mm Hg, respectively. Since the mean IOP was $15 \pm 2$ mm Hg in our subjects, the group-averaged values of the OPP were calculated to be $40 \pm 6$ mm Hg.

**Reproducibility and Reliability of LDV System**

Table 1 shows the reproducibility of the measurement of retinal circulatory parameters in the first- and second-order vessels. Variations were small for vessel diameter in arterioles and venules. CVs from $V_{\text{max}, \text{mean}}$, $V_{\text{max}, \text{systolic}}$, $V_{\text{max}, \text{diastolic}}$, and RBF were slightly higher but almost less than 15%. CVs obtained from second-order vessels were similar to those from arterioles.
first-order vessels. In addition, clear, representative velocity waveforms were obtained from first- and second-order arterioles in all subjects (Fig. 1b).

In 8 of 13 subjects, we obtained the retinal circulatory parameters in first- and second-order vessels. We excluded the data obtained from 5 subjects whose second-order vessels were smaller than 60 \( \mu \text{m} \) in diameter because of the insufficient reliability of measurement. In these 8 subjects, the average values of RBF of first- and daughter second-order vessels were 11.5 \( \pm \) 2.3, 6.9 \( \pm \) 1.8, and 4.4 \( \pm \) 1.2 \( \mu \text{L/min} \) in arterioles and 12.0 \( \pm \) 1.9, 7.1 \( \pm \) 1.1, and 4.7 \( \pm \) 2.0 \( \mu \text{L/min} \) in venules, respectively. Sums of the RBF in both second-order vessels were almost equal to those from first-order vessels. In addition, the RBF in the first-order vessels was strongly correlated with the sum of RBF in second-order vessels in arterioles \( (r = 0.94) \) and venules \( (r = 0.98) \) (Fig. 3).

### Retinal Circulatory Parameters in First and Second Branches

Vessel diameters measured in the present study ranged from 89.3 to 157.3 \( \mu \text{m} \) in first-order and from 80.1 to 132.0 \( \mu \text{m} \) in second-order arterioles and from 126.3 to 173.4 \( \mu \text{m} \) in first-order and from 109.5 to 143.1 \( \mu \text{m} \) in second-order venules. The group-averaged values of the D, \( V_{\text{max, mean}} \), RBF, and WSR were 107.9 \( \pm \) 12.6 \( \mu \text{m} \), 41.1 \( \pm \) 9.6 \( \text{mm/s} \), 11.4 \( \pm \) 4.1 \( \mu \text{L/min} \), and 1539 \( \pm \) 383 \( \text{s}^{-1} \) in the retinal arterioles and 147.3 \( \pm \) 12.6 \( \mu \text{m} \), 23.3 \( \pm \) 3.4 \( \text{mm/s} \), 12.2 \( \pm \) 3.7 \( \mu \text{L/min} \), and 652 \( \pm \) 73 \( \text{s}^{-1} \) in the venules of the first-order vessels (Table 2).

In the second-order branches, the D, \( V_{\text{max, mean}} \), RBF, and WSR were 100.6 \( \pm \) 13.8 \( \mu \text{m} \), 27.7 \( \pm \) 5.4 \( \text{mm/s} \), 6.7 \( \pm \) 2.2 \( \mu \text{L/min} \), and 1119 \( \pm \) 277 \( \text{s}^{-1} \), respectively, in the retinal arterioles and 127.5 \( \pm \) 9.9 \( \mu \text{m} \), 19.4 \( \pm \) 4.3 \( \text{mm/s} \), 7.6 \( \pm \) 2.4 \( \mu \text{L/min} \), and 606 \( \pm \) 115 \( \text{s}^{-1} \), respectively, in the venules.

In the retinal arterioles, the vessel diameter in the first-order branches tended to be higher than that in the second-order branches, but the difference did not reach significance \( (P = 0.07) \). There were significant differences in \( V_{\text{max, mean}} \), \( V_{\text{max, systolic}} \), \( V_{\text{max, diastolic}} \), pulse amplitude, RBF, and Reynold’s number between the first- and second-order branches in retinal arterioles (Table 2). In the retinal venules, the values of the D, \( V_{\text{max, mean}} \), and RBF were significantly higher in the first-order branches than in the second-order branches. The WSR in

#### Table 2. Values of Retinal Circulatory Parameters of Arterioles and Venules in First- and Second-Order Branches

<table>
<thead>
<tr>
<th></th>
<th>Arterioles</th>
<th></th>
<th>Venules</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (( \mu \text{m} ))</td>
<td>107.9 ( \pm ) 12.6</td>
<td>100.6 ( \pm ) 13.3</td>
<td>0.07</td>
<td>147.3 ( \pm ) 12.6</td>
</tr>
<tr>
<td>( V_{\text{max, mean}} ) (mm/s)</td>
<td>41.1 ( \pm ) 9.6</td>
<td>27.7 ( \pm ) 5.4</td>
<td>0.0001</td>
<td>23.3 ( \pm ) 3.4</td>
</tr>
<tr>
<td>( V_{\text{max, systolic}} ) (mm/s)</td>
<td>66.0 ( \pm ) 14.9</td>
<td>45.7 ( \pm ) 9.6</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>( V_{\text{max, diastolic}} ) (mm/s)</td>
<td>24.8 ( \pm ) 7.3</td>
<td>16.7 ( \pm ) 4.0</td>
<td>0.0002</td>
<td>—</td>
</tr>
<tr>
<td>Pulse amplitude (mm/s)</td>
<td>42.2 ( \pm ) 12.6</td>
<td>29.1 ( \pm ) 8.2</td>
<td>0.0002</td>
<td>632 ( \pm ) 73</td>
</tr>
<tr>
<td>Resistive index</td>
<td>0.62 ( \pm ) 0.09</td>
<td>0.63 ( \pm ) 0.08</td>
<td>0.45</td>
<td>—</td>
</tr>
<tr>
<td>RBF (( \mu \text{L/min} ))</td>
<td>11.4 ( \pm ) 4.1</td>
<td>6.6 ( \pm ) 2.1</td>
<td>0.0005</td>
<td>12.2 ( \pm ) 3.7</td>
</tr>
<tr>
<td>RVR (mmHg \cdot \text{min/( \mu \text{L} ))}</td>
<td>5.3 ( \pm ) 1.7</td>
<td>—</td>
<td>—</td>
<td>0.93 ( \pm ) 0.25</td>
</tr>
<tr>
<td>Reynold’s number</td>
<td>1.29 ( \pm ) 0.43</td>
<td>0.79 ( \pm ) 0.22</td>
<td>0.001</td>
<td>632 ( \pm ) 73</td>
</tr>
<tr>
<td>WSR (s)</td>
<td>1539 ( \pm ) 383</td>
<td>1119 ( \pm ) 277</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>WSR (s)</td>
<td>2498 ( \pm ) 529</td>
<td>1838 ( \pm ) 423</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>WSS (dyne/cm(^2))</td>
<td>939 ( \pm ) 306</td>
<td>680 ( \pm ) 214</td>
<td>0.002</td>
<td>—</td>
</tr>
<tr>
<td>Apparent viscosity at WSR (cP)</td>
<td>3.52 ( \pm ) 0.30</td>
<td>3.60 ( \pm ) 0.32</td>
<td>0.001</td>
<td>3.82 ( \pm ) 0.38</td>
</tr>
<tr>
<td>Apparent viscosity at WSR (cP)</td>
<td>939 ( \pm ) 306</td>
<td>680 ( \pm ) 214</td>
<td>0.002</td>
<td>—</td>
</tr>
<tr>
<td>Apparent viscosity at WSR (cP)</td>
<td>3.68 ( \pm ) 0.35</td>
<td>3.76 ( \pm ) 0.34</td>
<td>0.01</td>
<td>24.1 ( \pm ) 3.5</td>
</tr>
<tr>
<td>WSS (dyne/cm(^2))</td>
<td>54.0 ( \pm ) 13.2</td>
<td>40.2 ( \pm ) 10.6</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>WSS (dyne/cm(^2))</td>
<td>85.8 ( \pm ) 16.8</td>
<td>63.8 ( \pm ) 15.3</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>WSS (dyne/cm(^2))</td>
<td>34.3 ( \pm ) 11.3</td>
<td>25.5 ( \pm ) 8.3</td>
<td>0.002</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as mean \( \pm \) SD. RBF, retinal blood flow; WSR, wall shear rate; WSS, wall shear stress; RVR, retinal vascular resistance.
the first-order branch arterioles was significantly higher than that in the second-order branch arterioles ($P=0.002$). On the other hand, there was no significant difference in the WSR of the retinal venules between the first- and second-order branches ($P=0.44$). At the level of retinal WSR, after taking into account the Fahraeus-Lindqvist effect (equation 5), the apparent blood viscosity was $3.52 \pm 0.30$ cP in the first-order arterioles and $3.82 \pm 0.38$ cP in the first-order venules, respectively. Therefore, the retinal WSS\textsubscript{mean} was $54.0 \pm 13.2$ dyne/cm\textsuperscript{2} in the first-order arterioles and $24.1 \pm 3.5$ dyne/cm\textsuperscript{2} in the first-order venules. The WSS\textsubscript{mean} in the second-order arterioles was significantly lower than that in the first-order arterioles ($P=0.002$), but the WSS\textsubscript{mean} in the second-order venules was similar to that in the first-order venules.

**DISCUSSION**

The results of the present study demonstrate that in vivo measurements of the WSS in the retinal arterioles and venules of healthy men are reliable and reproducible using a system that combines a retinal LDV measurement and the cone-plate viscometry. Our results also showed that the WSS in the retinal arteriole was about twice as high as that in the venules and that there was a difference in the WSS between the first- and second-order branches in the retinal arterioles but not in the venules. These results suggest that the distribution of WSS is not constant in the vascular tree, at least not in the retinal microcirculation.

In this study, we presented a new approach to the noninvasive assessment of WSS in human retinal microcirculation. The implications of Poiseuille’s law are that flow is inversely proportional to the viscosity of the fluid. For a Newtonian fluid such as water, viscosity has been defined as the ratio of shear stress to the shear rate of the fluid. On the other hand, for a non-Newtonian fluid such as blood, the ratio of shear stress to shear rate is not constant. Therefore, it is important to measure or estimate the apparent viscosity of the blood in the retinal microcirculation for precise evaluation of WSS in the retinal microvessels. However, few studies have been conducted of shear rate measurement in human vascular disorders because, until now, there have been no adequate, noninvasive methods to assess WSR and WSS.

The first step in estimating WSS requires velocity measurements. A parabolic velocity profile was a critical assumption for the calculation of blood velocity and WSR in retinal microcirculation in the present study. Recently, Logean et al.\textsuperscript{17} showed an excellent velocity profile that was very close to parabolic in retinal arterioles and venules, probably first-order vessels. In addition, Eiju et al.\textsuperscript{30} observed parabolic flow in the rat mesentery artery (diameter, 40 $\mu$m) using high-resolution laser Doppler velocimetry. This study seems to support our assumption that velocity profiles are close to parabolic flow in second-order vessels (diameters greater than 80 $\mu$m). Because a Reynolds number less than 1000 is considered characteristic of laminar flow,\textsuperscript{25} the present result—that the mean value of the Reynolds number in the retinal microcirculation was approximately 1.0—seems to support the concept that the retinal circulation is a parabolic flow. Logean et al.\textsuperscript{17} also reported that the velocity profile was still slightly blunted at five vessel diameters after bifurcation, suggesting that a sufficient distance is required to reestablish steady flow after a junction. Although we could not confirm the velocity profile was parabolic in first- and second-order vessels in the present study, we measured the vessels that had relatively straight segments and were located 1 disc diameter upstream and downstream from the bifurcation to minimize the influence of branching on the velocity profile. Taken together, it is reasonable to consider that we can analyze the present data under the assumption that the velocity profile might be parabolic in the retinal microcirculation.

We examined the relation of the WSS between first- and second-order branches in the present study. Because the reliability of the measurement of second-order vessels using an LDV system had not been demonstrated, we had to examine whether our system was capable of performing reliable measurements of retinal blood flow in first- and second-order vessels. The present results showed that CVs from retinal circulatory parameter in second-order vessels were similar to those in first-order vessels (Table 1), that a similar and representative blood velocity waveform was obtained from first- and second-order arterioles, and that the RBF in first-order vessels was almost equal to the sum of the RBF in both second-order daughter arterioles. Collectively, these findings suggest that WSS in the retinal microcirculation can be reliably estimated, at least in first- and second-order retinal vessels.

We used cone-plate viscometry to measure whole blood viscosity. Previous studies using cone-plate viscometry reported that whole blood viscosity was approximately 4.5 cP at a WSR of 225 s\textsuperscript{-1}\textsuperscript{27} in healthy human volunteers. In our study, the estimated whole blood viscosity was $4.6 \pm 0.6$ cP at 225 s\textsuperscript{-1}, suggesting that our data are similar to those reported previously. Although it was reported that the blood viscosity almost reached a plateau at a shear rate of 90 s\textsuperscript{-1},\textsuperscript{25} we demonstrated that the blood viscosity gradually decreased by approximately 30% from 100 s\textsuperscript{-1} (5.16 $\pm$ 0.93 cP) to 3000 s\textsuperscript{-1} (3.64 $\pm$ 0.30 cP) (Fig. 2). Because WSS is a function of WSR and viscosity, the measurement of WSR and viscosity in the retinal microvessel was needed for the precise determination of WSS in the retinal microcirculation. Fahraeus and Lindqvist\textsuperscript{22} found that the apparent viscosity at constant flow rate was reduced in tubes of radii smaller than 0.2 mm. Therefore, we calculated the apparent blood viscosity according to equation 5, which has been previously defined. The calculated apparent viscosity, $\eta_{app}$, was lower than $\eta_{vis}$ by approximately 5% and was obtained in vitro by cone-plate viscometry (data not shown). Although such a small decrease in apparent blood viscosity with the decreased vessel radius might have had little effect on the present results, it was needed to calculate the apparent blood viscosity for the reliable evaluation of retinal WSS.

Murray’s law\textsuperscript{26} posits that shear stress on the walls of vessels is constant and equal throughout the circulatory system.\textsuperscript{26} Kimaya et al.\textsuperscript{9} roughly estimate the WSS in various vascular beds based on their and other studies. They found that WSS values ranged from 10 to 20 dyne/cm\textsuperscript{2} from large artery to capillary, suggesting that WSS in the entire arterial tree and its capillary bed is controlled at an approximately constant level, probably by the same autoregulatory mechanism. However, the present results, which showed a difference in WSR and WSS between the first- and second-order arterioles in the retinal microcirculation (Table 2), also suggest that shear stress does not seem to be constant throughout the vascular system in the human retinal microcirculation. Because WSS decreases with decreasing intravascular pressure in the microcirculation\textsuperscript{50} and at least 50% of the pressure gradient arises from the arterioles,\textsuperscript{31} it is reasonable to think that intravascular pressure might be higher in first-order arterioles than in second-order arterioles in the retinal microcirculation. Therefore, the differences in WSS between first- and second-order arterioles may be the result of differences in the intravascular pressure in retinal microcirculation.

The results of the present studies also showed that half the WSS in the arterioles is exerted on the retinal venules. It is reported that shear stress ranges from 1 to 6 dyne/cm\textsuperscript{2} in the venous system.\textsuperscript{32} The present data—the group-averaged values
in the first and second venules of 25.1 and 24.3 dyne/cm², respectively, suggest that high WSS is exerted on the retinal venules compared with that in the veins in other vascular beds. Because recent in vitro studies reported that approximately 20 dyne/cm² of shear stress stimulates the retinal endothelial cells to release nitric oxide (NO), the NO released from the endothelium in the arterioles and the venules may play an important role in vasoregulation, especially in the retinal microcirculation, where the retinal arterioles are adjacent to the paired venules. Because venules continuously produce NO under resting conditions and during hyperemia, NO can diffuse to and dilate nearby paired arterioles. Donati et al. reported that experimental branch vein occlusion induces impaired release of constitutive NO and arteriolar constriction in the affected retina. In addition, the characteristic changes—venous bedding, looping, and duplication—in patients with diabetic retinopathy were mainly observed in retinal venules. Given that the retinal circulation is impaired in patients with diabetes, it is important to evaluate WSS because it affects not only arterioles but also venules in the retinal circulation.

In vitro and in vivo experiments have shown that vessels tend to maintain constant shear stress in response to flow changes, called flow-mediated regulation. Flow-mediated brachial arterial reactivity, in which endothelium-derived NO plays an important role, is impaired in patients with overt atherosclerosis and in asymptomatic persons with risk factors for coronary disease. We recently showed that the WSR increases during hypoxia using this method in anesthetized cats, suggesting that flow-induced vasodilation may be involved in the increased RBF in response to systemic hypoxia. Given that endothelium-derived NO is considered necessary to maintain adequate vascular shear stress, our system for measuring the retinal WSS may be useful for further investigation of endothelial function in the human microcirculation.

It is now possible to estimate WSS in humans with noninvasive imaging techniques such as magnetic resonance imaging and Doppler sonography. Many studies of shear stress in humans have focused on large arteries, such as the aorta, carotid artery, and brachial artery, whereas data regarding shear stress in the microcirculation seem to be rare. Previous studies showed that the values of the peak (systolic) and mean WSS were reported to be 2.7 to 5.4 dyne/cm² and 2.8 to 10.4 dyne/cm² in human abdominal aorta, 14.7. 18.7 to 29.5 dyne/cm² and 7.0 to 15.3 dyne/cm² in the common carotid artery,5.24.46.47 and 17.0 dyne/cm² and 4.65 dyne/cm² in the brachial artery,48 respectively. The present results—that is, the estimated peak and mean WSS of 90.8 and 57.1 dyne/cm², respectively, in the first-order retinal arteriole also revealed that the WSS in the retinal arterioles (resistance artery) is higher than in other tissue vascular beds, such as the aorta, carotid (elastic artery), and brachial (muscular artery) artery. The differences in WSS between retinal and other vascular beds may be dependent on the inherent characteristics of some types of arteries. Further study is needed to investigate the clinical meaning of high shear stress in retinal vessels.

The present study had some limitations. First, we measured only first- and second-order retinal vessels. To examine how WSS distributes throughout the retinal microvascular network, we had to measure the smaller vessels further downstream. However, the instrument was limited in that measurements on vessels smaller than 60 μm in diameter were difficult to achieve. Second, in the LDV system, the measurement of vessel diameter is performed by taking 15 vessel images every 4 milliseconds just before and after each velocity measurement. The vessel diameter measurement might have been affected by the pulsation. Although we cannot completely exclude this possibility, the CVS from 30 vessel images obtained before and after each velocity measurement, which is calculated automatically in the LDV system, were less than 3% (data not shown), suggesting that there might have been little influence of pulsation on the shear stress calculation. Third, correction factors 1.649 and 2.013 for the estimation of V_mean have both been used for the calculation of retinal blood flow in previous papers by different laboratories using LDV. In the present study, we used a correction factor of 2.0 (V_mean = V_mean / 2.0), in accordance with a previous study in which this issue has been discussed in detail. We could not determine the true factor to correct the centerline blood velocity obtained through LDV because we did not evaluate the blood velocity profile in this study. Further study is needed to elucidate which correction factor for V_mean should be used for the calculation of retinal blood flow.

In conclusion, we demonstrated that WSS in retinal vessels could be measured in humans using a system that combines retinal LDV and cone-plate viscometry. Because decreases in the WSS in the common carotid artery and the brachial artery were previously reported in patients with diabetes and hypertension, it is possible that abnormalities of the WSS may be associated with retinal vascular disorders, such as diabetic and hypertensive retinopathy. Therefore, this system may be useful for further clinical investigation of the role of shear stress in the pathogenesis of various retinal disorders.

References
