Altered Retinal Hemodynamics and Mean Circulation Time in Spontaneously Hypertensive Rats

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PURPOSE. Although it is known that the retinal arteriolar vasculature is constricted in hypertension, the details of retinal hemodynamics and perfusion of the retinal circulation have yet to be adequately characterized.

METHODS. Male and female spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) controls were anesthetized before measurements of mean arterial blood pressure and preparation for intravital microscopy of the retinal microcirculation. Retinal vascular velocities were measured with the use of fluorescent microspheres, and diameters and mean circulation times were measured after the infusion of fluorescent dextran. Arteriolar and venular shear rates were calculated from the ratio of velocity to diameter.

RESULTS. In the retinas of SHR, velocities were elevated (compared with control WKY) in arterioles, but not in venules. Both arteriolar and venular diameters were significantly smaller in SHR versus WKY, with substantial increases in shear rates. Despite a tendency toward lower retinal blood flow rates, the mean circulation time through the SHR retina was much faster than can be explained by the measured arteriolar and venular velocities.

CONCLUSIONS. The pattern of hypertension-induced increases in blood velocity, dissipating from the arteriolar to venular side of the retinal circulation, indicates a potential transfer of the extra kinetic energy through the vasculature. The combination of elevated velocities through narrower retinal arterioles resulted in a markedly higher level of wall shear rate that may induce changes in the vessel wall. Finally, significantly more rapid transits through the hypertensive retina could be a result of altered blood flow distribution.

Keywords: hypertension, hemodynamics, shear, retinal blood flow, mean circulation time

Hypertensive retinopathy induces several vascular retinal changes, including arteriolar vasoconstriction, arteriovenous nicking (compression of a venule by an overlapping arteriole), increased vascular permeability, microhemorrhages, thickening of the basement membrane, and cotton wool spots (focal ischemia). Studying these events in an animal model enables valuable information to be gained to potentially understand and treat the pathology. One rodent model that has been used to some extent for this purpose is the spontaneously hypertensive rat (SHR), either with hyperglycemia superimposed (with streptozotocin, in a diabetes model) or in the stroke-prone spontaneously hypertensive substrain of SHR.

In the SHR strain (without streptozotocin), corrosion cast experiments from ∼20 years ago have demonstrated retinal vessel narrowing, localized constriction of veins, microaneurysms, damaged pericytes, capillary dropout, and capillary tortuosity.[1,2] McDonald et al.[3] also found microaneurysms in the retinas of SHR, as well as arteriolar smooth muscle loss. Other changes in the retinal vessels of SHR include increased arterial wall thickening,[4] a decrease in the endothelial glycocalyx,[5] an increase in vascular leukocyte adhesion,[6] and an increase in vascular permeability,[7] with the latter possibly being endothelin (ET) dependent[8] with a retinal increase in the ET-A receptor.[9] Death of

various cells occurs in the SHR retina, with an increase in terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells and acellular capillaries,[10] although one SHR study has reported the opposite finding, that is, a decrease in acellular capillaries, even with a decreased capillary density.[3]

In the SHR retina, most reports agree with significant vascular narrowing,[1,2,4,11] although one report found no change.[3] However, a limitation of several reports on changes in vascular diameters is the ex vivo nature of these measurements, with the nonperfused, nonpressurized vessel diameter in a retinal flat mount potentially not the same as the diameter in vivo. Few in vivo measurements of retinal diameters in SHR have been published, and to our knowledge, in vivo parameters of hemodynamics from the SHR retina have yet to be reported, with hemodynamics being the focus of this investigation in a comparison with normotensive Wistar-Kyoto (WKY) rats.

In addition to measuring pressurized, perfused diameters of retinal arterioles and venules, our aim in this study was to measure blood velocities in these vessels, along with mean circulation times and shear rates, with the latter two being understudied in the hypertensive retina. These measurements can serve in subsequent investigations in which interventions may be administered to return these

vascular parameters back toward normal in an effort to maintain adequate perfusion and limit changes in shear force.

MATERIALS AND METHODS

Animals

SHR and WKY rats were obtained from Charles River (Wilmington, MA, USA) and housed under constant environmental conditions of 22°C with a 12-hour light–dark cycle. Experimental protocols described below were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center-Shreveport (Shreveport, LA, USA) and adhere to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgery and Preparation for Intravital Microscopy

The rats were anesthetized intraperitoneally with a ketamine/xylazine cocktail (100 mg/kg, 10 mg/kg, respectively) and kept warm on a heating pad for the remainder of the experiment. During surgery, both eyes were moistened with phosphate-buffered saline solution. An incision was made along the shaved lower abdomen, and the femoral artery and vein were cannulated with polyethylene tubing (PE50) filled with heparinized saline solution (25 U/mL). Mean arterial blood pressure was measured by cannulation of the femoral artery connected to a pressure monitor (World Precision Instruments BP-1; World Precision Instruments, Sarasota, FL, USA). Before intravital microscopy, the pupil of the left eye was dilated with a drop of Tropicamide Ophthalmic Solution USP 1% (Falcon Pharmaceuticals Ltd., Ft. Worth, TX, USA) followed with one drop of Gonak Hypromellose Ophthalmic Demulcent Solution, 2.5% (Akron Inc., Buffalo Grove, IL, USA), with the eye then covered with a 5-mm circular glass coverslip.

Measurements of Retinal Diameters and Mean Circulation Time

Rats were placed on the right side of their body with gauze sponges under the head for positioning of the left eye under the microscope. Under low magnification (×4), the retina was brought into focus through a Coolsnap ES camera (Photometrics, Tucson, AZ, USA) attached to a Nikon Eclipse E600FN microscope (Nikon Instruments, Melville, NY, USA) and a rhodamine filter. A solution of tetramethyl rhodamine isothiocyanate-labeled dextran (155 kD MW; T1287; Sigma; St. Louis, MO, USA) was infused as a bolus (35 mg/kg) while videos were recorded, to determine the vessel type (arterioles filling before venules). The fluorescent intensity curves of the fluorescent dextran in the arterioles and venules were used to calculate the mean transit times of each, as described previously,[12] with the difference in arteriolar and venular mean transit times giving the mean circulation time. In this protocol, the ends of the intensity curves are obscured by the recirculating dye returning from the heart; therefore a logarithmic fit of the declining phase of the curve is extrapolated from the time point of the recirculation, as previously described.[12] Video recording of the dye filling the retinal circulation was followed by sequential focusing at magnification × 10 of each of the arterioles and venules extending from the optic disk. Diameters were measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Measurements of Retinal Velocity, Blood Flow Rate, and Shear Rate

In a protocol similar to that described previously,[13] a solution of fluorescent microspheres (1.9 µm diameter Dragon Green; Bangs Laboratories, Inc, Fishers, IN, USA) was infused (80 µg/rat) to measure blood velocity. Videos were recorded using a 4× objective through a Nikon fluorescein filter, with a video camera exposure time of 10 ms. Images were captured and analyzed using ImageJ software. Microspheres appeared as fluorescent streaks in the vessels with the length of the streak proportional to the velocity of the microspheres. Ten consecutive microspheres streaks were averaged per vessel, with velocity calculated as the distance traveled per 10 ms frame of video and flow calculated as velocity $\times \pi \times$ diameter^2/4. Flows in individual arterioles were summed and averaged with the sum of venular flows for a calculation of total retinal blood flow rate. Shear rates of individual arterioles and venules were calculated as 8× velocity / diameter.

Statistics

Analyses of statistical data were performed with Prism software (GraphPad, La Jolla, CA, USA) using nonparametric Mann Whitney tests and linear regression. Group data are presented as means ± standard deviation, with $P < 0.05$ considered statistically significant. Both male and female SHR were included in the current study, although the study was not powered to distinguish between sexes.

Results

Data from WKY rats (N = 8; 3 male/5 female) and SHR (N = 8; 3 male/5 female) were collected at an age of approximately seven to eight months: 221 ± 12 days for WKY and 225 ± 14 days for SHR. The weight range was 375 to 410 g for males and 190 to 240 g for females, with no overall differences in body weight between WKY and SHR. Mean arterial blood pressure was significantly higher ($P < 0.001$) in SHR (133 ± 15 mm Hg) versus WKY (89 ± 18 mm Hg).

In retinal arterioles, velocities in SHR (10.6 ± 1.2 mm/s) were significantly faster ($P < 0.01$; Fig. 1A) than in WKY (8.5 ± 0.9 mm/s), and diameters in SHR (63.8 ± 5.0 µm) were significantly smaller ($P < 0.001$; Fig. 1B) than in WKY (82.3 ± 5.9 µm). With both of these changes promoting increases in shear, arteriolar shear rates were dramatically higher ($P < 0.001$; Fig. 1C) in SHR (1347 ± 126 s^{-1}) compared with WKY (848 ± 102 s^{-1}). These parameters were altered by a smaller amount in venules compared to arterioles. In retinal venules, velocity did not differ significantly (Fig. 2A; $P = 0.49$) between WKY (7.0 ± 1.3 mm/s) and SHR (7.5 ± 1.4 mm/s), but venule diameters were significantly smaller ($P < 0.01$; Fig. 2B) in SHR (82.9 ± 4.4 µm) than in WKY (98.9 ± 9.3 µm). Venular shear rates were significantly higher ($P < 0.05$; Fig. 2C) in SHR (742 ± 163 s^{-1}) than in WKY (585 ± 110 s^{-1}). As shown in Figure 3, mean circulation time (MCT) through the SHR retina (0.283 ± 0.127 s) was significantly faster ($P < 0.05$) than through the WKY retina (0.456 ± 0.101 s).

Figure 4 presents the shear rate data for the individual rats, as a function of mean arterial blood pressure (on the x-axis). As shown in Figure 4A for WKY rats, both arteriolar and venular shear rates remained essentially constant over a wide range of blood pressures in the control group,
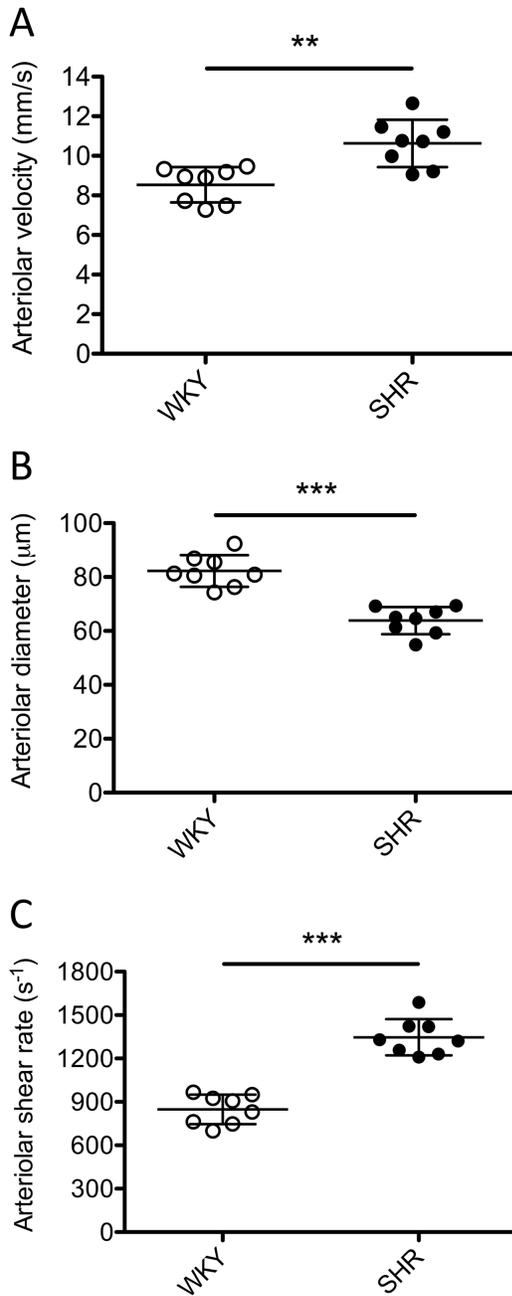
FIGURE 1. Retinal arteriolar velocity (**A**), diameter (**B**), and shear rate (**C**) in WKY and SHR. N = 8 each; means ± SD. **$P < 0.01$ and ***$P < 0.001$.

FIGURE 2. Retinal venular velocity (**A**), diameter (**B**), and shear rate (**C**) in WKY and SHR. N=8 each; means ± SD. *$P < 0.05$ and **$P < 0.01$.

FIGURE 3. Mean circulation time through the retinas of WKY and SHR. N = 6 each; means ± SD. *$P < 0.05$.

with no change in shear with pressure. However, in arterioles of the SHR group (Fig. 4B), not only was the average shear rate higher than in the WKY group, but also, shear continued to escalate with higher blood pressures ($P < 0.05$). The same escalation also might be present in SHR venules (Fig. 4B), although the regression slope was not quite statistically significant ($P = 0.10$).

Total retinal blood flow rate was calculated from velocities and diameters and are presented in Figure 5. As shown in the figure, retinal blood flow did not change substantially between SHR vs WKY, with a nonstatistically significant tendency ($P = 0.13$) toward lower rates of flow in SHR.
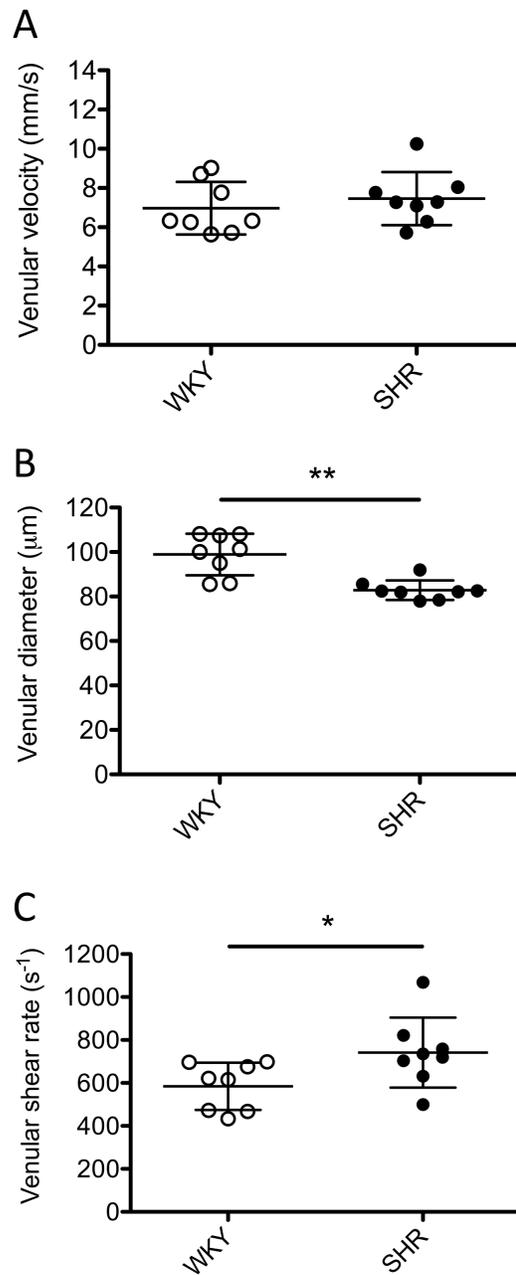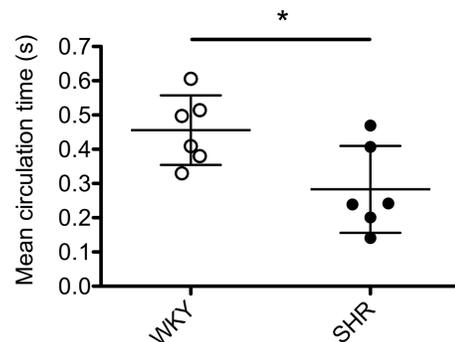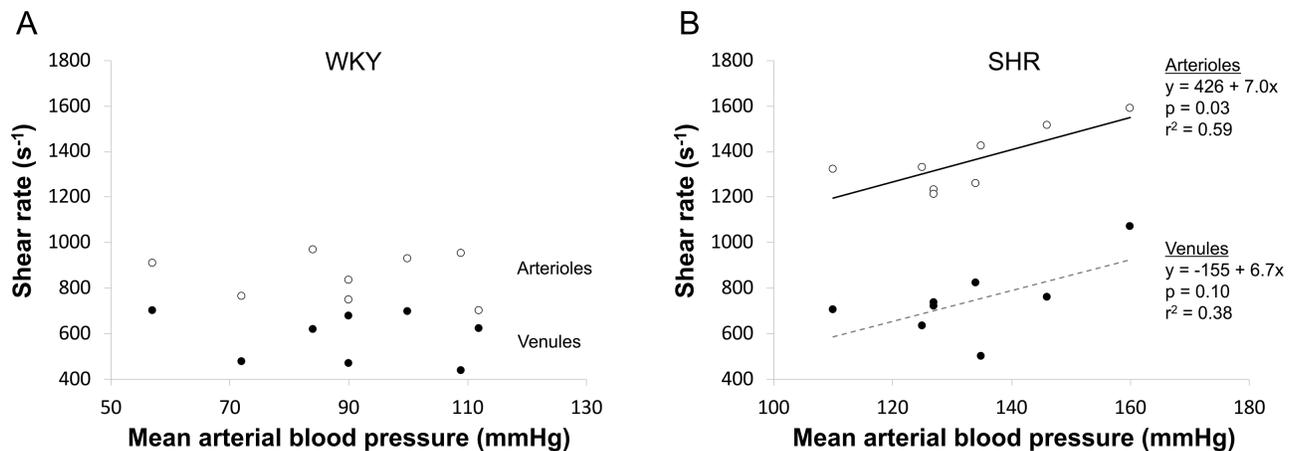
FIGURE 4. Retinal arteriolar and venular shear rates as a function of mean arterial blood pressure in WKY (**A**) and SHR (**B**). The slope of the linear regression for arterioles in the SHR group is significantly different from 0 ($P < 0.05$).
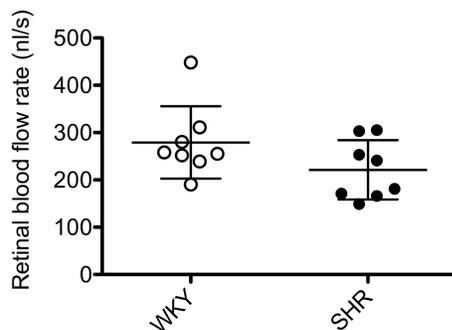

FIGURE 5. Total retinal blood flow rate in WKY and SHR. N = 8 each; means ± SD. No statistically significant difference between groups was found ($P = 0.13$).

DISCUSSION

To our knowledge, this is the first study of in vivo retinal hemodynamics in SHR. In addition to smaller retinal arterioles, our findings in SHR included smaller retinal venules, faster arteriolar velocities, and markedly elevated vascular shear rates and more rapid mean circulation times despite a tendency toward a reduction in total retinal blood flow.

The microvascular response to an acute increase in velocity is an increase in diameter to limit shear forces on the vessel wall[14,15] in flow-mediated dilation. Normal levels of shear can be beneficial, mediating the release of the vasodilator nitric oxide and limiting leukocyte and platelet adhesion. However, elevated shear can induce increases in permeability (seen in the hypertensive retinal circulation) and in cell signaling, with an increase in endothelial intracellular calcium.[16] With the sustained hypertension in SHR (in contrast to the acute flow-mediated dilation scenario), elevations in arteriolar blood velocity are associated with smaller rather than larger diameters because of arteriolar wall remodeling, causing a substantial elevation in shear force on the vessel wall.

The elevated shear rates shown in Figures 1 and 2 are likely to be underestimates of the increases in shear stress, as shear stress is the product of shear rate and viscosity. Blood viscosity has been found to be significantly increased

in SHR[17,18] and in hypertensive individuals,[19] because of increases in both plasma viscosity and hematocrit. It is possible that the transmission of this force to the vasculature is the mechanism by which the elevated kinetic energy is dissipated going from the arterioles to venules in the SHR retina, with elevations in velocity seen primarily on the arteriolar side. In the WKY retina, shear rates are well controlled over a wide range of mean arterial blood pressures, but they are found to be higher (possibly in a linear relationship) with increasing blood pressures in SHR.

To our knowledge, the shear rate measurements made in this study are the first to be published for the in vivo SHR retina. However, acute responses to increased pressure in the retinal circulation have been made in other models, and are consistent with an autoregulatory limitation of blood flow over a range of pressures. For example, in rats in which arterial pressure was acutely elevated to 153 mm Hg (from 114 mm Hg baseline) by a balloon catheter partially occluding the descending aorta, retinal blood flow increased marginally before trending back toward basal levels over a period of a few minutes.[20] Acute increases in blood pressure in miniature pigs, using infusion of tyramine, increased retinal blood flow by only 8% with an increase in mean arterial blood pressure of 22%, and no measurable increase in blood flow was found with an increase in blood pressure of 31%.[21] In the same study, the range for autoregulation was exceeded when an acute 50% increase in blood pressure increased retinal blood flow by 57%. In cats, when ocular perfusion pressure increases acutely by more than 40 mm Hg (with inflation of a balloon in the descending aorta), substantial increases in both arteriolar shear rates and blood flow rates ensue.[22] In humans injected with tyramine, the range of autoregulation was found to be exceeded between a 30% and 40% increase in blood pressure, causing retinal blood flow increases of 11% and 32%, respectively.[23] A similar result in humans was found in which increases in blood pressure (induced by acute exercise) resulted in an increase in retinal blood flow only when the increase in blood pressure exceeded 41%.[24] The autoregulatory control is believed to be important for the retinal circulation, because of the lack of autonomic innervation. In contrast, sustained hypertension is typically associated with decreases in retinal blood flow. For example, in one

study, capillary flow was found to decrease by ~10% in long duration hypertensive patients.[25] In our study of the SHR retina, flow also tended to be lower.

The significantly more rapid mean circulation time through the SHR retina compared to WKY (Fig. 3) would not be expected solely from the measured values of entering and exiting velocities in the arterioles and venules (Figs. 1, 2). MCT can be defined as a length (L) divided by velocity (V), with L being a flow-weighted measure of the multitude of different pathways that can be taken between the arteriolar and venular sites. If the value of L does not change between SHR and WKY, we can say that $(MCT \times V)_{WKY} = (MCT \times V)_{SHR}$. Inserting our measured values of MCT into this equation (0.283 s for SHR; 0.456 s for WKY) and solving for SHR velocity, we have $V_{SHR} = (0.456/0.283) \times V_{WKY} = 1.61 \times V_{WKY}$. As shown in Figures 1 and 2, the arteriolar and venular velocities in SHR compared to WKY were not higher by a factor of 1.61 (1.24 for arterioles; 1.07 for venules). Although we did not measure the extent to which retinal capillary velocities may have changed in SHR, it should be noted that in human hypertension, 10% to 22% decreases (rather than increases) in retinal capillary velocity have been reported.[26] If capillary velocity is not higher in SHR (than in WKY) by at least a factor of 1.61, then it must be considered that the microvascular pathways (L) may change.

One such change in the hypertensive retina that could alter the arterio-venular transit is vascular constriction in the arteriolar delivery from the superficial retina to the intermediate and deep capillary layers of the retina. As we have found previously for the diabetic rat retina,[12] this "shunting" of flow away from the deeper capillaries would result in considerably faster mean circulation times, at the expense of decreased red blood cell distribution to the deeper capillaries. In a potentially connected mechanism, it is possible that some capillaries will stop flowing, as reported in human hypertensive retinopathy,[25,27] with optical coherence tomographic angiography indicating a reduced capillary density in the deep retinal plexus with poorly controlled blood pressure.[28] Moreover, the decreases in capillary density can occur even in patients in whom blood pressure has been reasonably well controlled via medication.[29] In these scenarios explaining the significantly faster mean circulation times in SHR compared to WKY (exceptionally high capillary velocity; shunting of flow; decreases in capillary density), red blood cell distribution or adequate oxygenation of the retina are vulnerable.

One limitation of the study was that we did not include measures of intraocular pressure, which influences retinal blood flow and hemodynamics, and therefore could explain some of the differences between SHR and WKY rats. Although statistical power with N = 8 per group was sufficient for most comparisons in the study, the limited sample size was likely a contributing factor in our inability to state with certainty whether retinal blood flow was indeed lower in SHR compared to WKY rats (Fig. 5). Another limitation of our study was the use of ketamine/xylazine anesthesia, which can affect retinal blood flow.[30] Although we have no reason to believe that this effect of anesthesia differs between WKY rats and SHR, this is a consideration that can be examined with other choices of anesthesia in future studies.

The hemodynamic changes seen in the SHR retina can be further investigated to examine the effects of elevated shear on permeability, endothelial signaling, and the development of a thickened basement membrane. Furthermore,

the potential consequences of more rapid circulation times, and either highly elevated capillary velocities and/or shunting of flow, can be investigated in future studies with regard to oxygenation and function of the retina.

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