Postocclusive Reactive Hyperemia Occurs in the Rat Retinal Circulation but Not in the Choroid

Guang Li,^{1,2} Jeffrey W. Kiel,³ Damon P. Cardenas,¹ Bryan H. De La Garza,¹ and Timothy Q. Duong¹⁻⁵

¹Research Imaging Institute, University of Texas Health Science Center, San Antonio, Texas

²Department of Radiology, University of Texas Health Science Center, San Antonio, Texas

³Department of Ophthalmology, University of Texas Health Science Center, San Antonio, Texas

⁴Department of Physiology, University of Texas Health Science Center, San Antonio, Texas

⁵South Texas Veterans Health Care System, San Antonio, Texas

Correspondence: Timothy Q. Duong, Department of Ophthalmology, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229; duongt@uthscsa.edu.

Jeffrey W. Kiel, Department of Ophthalmology, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229; kiel@uthscsa.edu.

Submitted: May 13, 2013

Accepted: June 21, 2013

Citation: Li G, Kiel JW, Cardenas DP, De La Garza BH, Duong TQ. Postocclusive reactive hyperemia occurs in the rat retinal circulation but not in the choroid. *Invest Ophthalmol Vis Sci*. 2013;54:5123–5131. DOI: 10.1167/iov.13-12404

PURPOSE. We tested the hypothesis that retinal blood flow has a postocclusive reactive hyperemia response modulated by occlusion duration and metabolic activity, and that choroidal blood flow does not.

METHODS. Anesthetized and paralyzed rats ($n = 34$) were studied. Retinal and choroidal blood flow was measured by laser speckle imaging and laser Doppler flowmetry, respectively. Blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) was used to measure changes in relative blood oxygenation of the retinal and choroidal circulations. Transient carotid occlusion was elicited with a hydraulic occluder on the common carotid artery. Several occlusion durations were tested during dark, constant light, and flicker light conditions to modulate metabolic demand. The hyperemia response magnitude was quantified by integrating the area above the blood flow baseline for the 3 minutes after release of the occlusion.

RESULTS. Systemic arterial pressure (108.2 ± 1.4 mm Hg) was unaffected by the carotid occlusions, and was similar among animals and conditions. Retinal blood flow had a reactive hyperemia, but choroidal blood flow did not (e.g., $14 \pm 2\% \cdot \text{sec}$ versus $0.5 \pm 4\% \cdot \text{sec}$ after 60-second occlusion). The hyperemia magnitude increased as a nonlinear function of occlusion duration and reached a plateau at occlusion durations <60 second. The hyperemia magnitude was not altered by different lighting conditions at occlusion durations of 15 and 60 seconds. BOLD fMRI results were similar to the laser-based blood flow measurements.

CONCLUSIONS. The results indicate that metabolic local control has a negligible role in choroidal blood flow regulation and only partially accounts for the blood flow behavior in the retinal circulation.

Keywords: BOLD, MRI, laser speckle imaging, laser Doppler flowmetry, retinal blood flow, choroidal blood flow, reactive hyperemia

Reactive hyperemia is the increase in blood flow above baseline that occurs after a brief arterial occlusion.¹ Reactive hyperemia occurs in denervated organs and tissues, and so it is considered an autoregulatory (i.e., intrinsic) response mediated by local control mechanisms.² Because the magnitude of reactive hyperemia varies with occlusion duration and metabolic activity, it is attributed to metabolic local control, that is, vasodilatory metabolites (e.g., adenosine) accumulate during the occlusion, thereby decreasing vascular resistance, which remains lower than baseline until the metabolites are flushed from the tissue by the hyperemia upon restoration of arterial pressure.³ Other local control mechanisms, such as the myogenic response^{4,5} or flow-mediated vasodilation^{6–8} also could cause or contribute to reactive hyperemia, but neither account readily for the modulation by occlusion duration and metabolic activity.⁹ Consequently, such modulation of reactive hyperemia may provide a means to distinguish between different mechanisms of local vascular control.

The retinal and choroidal circulations clearly differ in their neural regulation—the choroidal circulation is under autonomic neural control and the retinal circulation is not.¹⁰ Less clear are the local mechanisms regulating retinal and choroidal blood flow. The increase in retinal blood flow in response to flickering light stimulation^{11–13} is similar to the functional hyperemia that occurs in other tissues during increased metabolic activity,³ which suggests metabolic local control is present in the retinal circulation. By contrast, the negligible choroidal blood flow response to flickering light suggests little metabolic local control¹⁴; instead, the choroidal blood flow responses to perfusion pressure change at different intraocular pressures suggest myogenic local control is present in the choroidal circulation.^{15,16} While this evidence is suggestive of different local control mechanisms in the retinal and choroidal circulations, it is not definitive.

The reactive hyperemia response has not been studied previously in the retinal and choroidal circulations to our knowledge. In our study, we tested the hypothesis that retinal

FIGURE 1. Typical experimental recordings from the blood flow (BF, *left*) and BOLD MRI (BOLD, *right*) protocols under light adapted conditions using the 60-second carotid occlusion.

blood flow has a reactive hyperemia response modulated by occlusion duration and metabolic activity, and that choroidal blood flow does not.

METHODS

Animal Preparation

The animal experiments were performed with Institutional Animal Care and Use Committee (IACUC) approval, and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A total of 34 Long-Evans rats (300–340 g; Charles River Laboratories International, Inc., Wilmington, MA) were used: 13 rats were studied using laser speckle imaging (LSI)¹⁷ to measure retinal blood flow (RBF) responses, 7 rats were studied using LSI to measure RBF responses and laser Doppler flowmetry (LDF)¹⁸ to measure choroidal blood flow (ChBF) responses simultaneously in the same animals, and 14 rats were used in the blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI)¹⁹ study to measure retinal and choroidal responses.

The rats were anesthetized by an initial dose of 5% isoflurane and a maintenance dose of approximately 1.7 to 2.3% isoflurane during surgery. The right femoral artery was cannulated with a polyethylene tube (PE-50) to monitor systemic arterial blood pressure (BP) continuously. Then, a custom-made occluder was wrapped around the common carotid artery such that it could be occluded reversibly from outside of the MRI scanner or on the bench top. The left eye was dilated with topical 1% atropine. The rats then were switched to urethane (0.8 g/kg, intraperitoneally [IP]) anesthesia and placed on mechanical ventilation. Pancuronium bromide (3 mg/kg first dose, then 1.5 mg/kg/h, IP) was used to paralyze the animals and prevent eye movement.^{20,21} End-tidal CO₂ (Surgivet capnometer; Surgivet, Dublin, OH), arterial oxygen saturation (MouseOx oximeter; Starr Life Sciences Corp., Oakmont, PA), heart rate (MouseOx oximeter; Starr Life Sciences Corp.), and rectal temperature were maintained within normal physiologic ranges. Small supplement doses of urethane were given as needed up to a maximum cumulative dose of 1.5 g/kg for the entire experiment, which typically lasted 4 to 6 hours. For the LSI and LDF experiments, the

animals were mounted in a stereotaxic instrument. For the MRI experiments, the animals were mounted in a custom-made head-holder.

LSI and LDF Methods

An LSI system similar to that described by Cheng et al.^{22,23} was used to measure RBF in rats with the optical probe positioned on the optical axis ≈ 1 cm from the cornea. Each image was the average of 30 frame captures. Each trial consisted of a total of 200 images with 44 images acquired during preocclusion baseline when using the exposure time of 84 ms per frame, or a total of 400 images with 88 preocclusion baseline images when using the exposure time of 42 ms per frame.

A commercial LDF system (PF 4001; Perimed, Stockholm, Sweden) employing an infrared laser diode (780 nm, 1 mW) was used to measure anterior ChBF.²⁴ With the upper eyelid retracted slightly, the tip of the LDF probe (Probe 415, 0.25 mm fiber separation; Perimed) was placed with a micromanipulator to touch the sclera lightly ≈ 4 mm posterior to the limbus from the dorsal side. The light sources of the LSI and LDF were turned on and off alternatively, while the other was measuring blood flow continuously, to confirm that the light source of one instrument did not affect the measurement of the other.

MRI Methods

The MRI studies were performed on an 11.7 T Bruker Biospec (Bruker Corporation, Billerica, MA). A surface coil (ID = 1 cm) was used for retinal imaging of the left eye. BOLD fMRI^{19,25–27} of a single axial slice bisecting the optic nerve head was acquired using 4-segment gradient-echo EPI with FOV = 7.7×7.7 mm, TR/TE = 1500/12 ms, resolution = $51 \times 51 \times 800$ μ m. For each trial, a total of 100 images (10 minutes) was acquired, with 20 images acquired during preocclusion baseline.

Protocols

Four sets of data were obtained. The initial set was obtained under light adapted conditions using 60-second carotid occlusions. Simultaneous measurements of RBF and ChBF were made in one subgroup of animals, and BOLD measurements were made in another subgroup of animals. The second

FIGURE 2. Responses (normalized to baseline) during and after 60-second carotid occlusion under light adapted conditions. (A) Mean retinal and choroidal BF and (B) retinal and choroidal BOLD during the occlusion period. (C) Magnitude of the BF reactive hyperemia and (D) BOLD reactive hyperemia after the occlusion.

data set was obtained in separate subgroups for RBF and ChBF, and for retinal BOLD (RBOLD) under light adapted conditions using carotid occlusion durations of 15, 30, 60, or 90 seconds. RBF also was tested with a 6-second occlusion; however, this was deemed too short a duration for the RBOLD temporal resolution, so it was not attempted. Because choroidal reactive hyperemia was negligible in the first two sets, the remaining sets focused on retinal responses. The third data set was obtained in separate subgroups for RBF and RBOLD with the animals dark adapted, light adapted, and during flickering light stimulation using a carotid occlusion duration of 60 seconds. To determine if a shorter duration carotid occlusion (i.e., with a less than maximal reactive hyperemia) would reveal an effect of flicker stimulation, the fourth and final data set was obtained under light adapted conditions and during flickering light stimulation using an occlusion duration of 15 seconds. The animal numbers for each test are indicated in the Figures.

For each animal in the optical imaging and MRI studies, approximately 2 to 3 trials were collected under each light condition for each occlusion duration. An approximately 5- to 20-minute break was given between trials, depending on the length of occlusion durations and animal stability. Before each trial under the dark condition, the animals were dark adapted for 40 minutes. The three light conditions and the lengths of occlusion duration were randomized. For the RBF measurements, the light source for the flicker condition was a white LED light with an intensity of 1.35×10^8 cd/m², pointed at the eye from a distance of approximately 25 cm with an angle of approximately 45° to the center axis of the rat body. For the RBOLD measurements, the light source for the flicker and constant light conditions was a white LED light guided through a 2-mm fiberoptic cable bundle,^{19,28} where the maximum luminance at the end of the fiberoptic cable bundle was 3.54×10^4 cd/m². The end of the cable bundle was mounted on the coil and pointed to the equator of eye. During the constant light condition, the LED light was set at half of the maximum

level. During the flicker condition, the LED light was set at maximum level. The flicker frequency was 10 Hz.

Data Analysis

Regions-of-interest (ROIs) were drawn manually on LSI images to avoid large retinal vessels.¹⁷ The mean value of the pixels in the ROIs from the sequentially acquired images formed a time course of mean relative retinal blood flow measurements. The LDF technique measured the perfusion from a tissue sampling volume of approximately 1 mm³, and provided a continuous recording of relative ChBF. The LSI and LDF measurements were normalized to baseline.

Time-series MRI data were coregistered using Statistical Parametric Mapping 5 (SPM5, available in the public domain at <http://www.fil.ion.ucl.ac.uk/spm/>) software, and the RBOLD and choroidal BOLD (ChBOLD) signals were extracted as described elsewhere.²⁹ The extracted BOLD time courses were detrended³⁰ and normalized to their baseline periods.

The magnitude of the reactive hyperemia was calculated as the area under the curve during the reactive hyperemia period relative to baseline, where the hyperemia period was defined as a 3-minute period starting immediately after the occlusion was released. The mean BOLD and BF changes during the entire occlusion also were determined.

Statistical analyses were performed in STATA 11 (StataCorp LP, College Station, TX) and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). The data were analyzed by unpaired and paired *t*-tests, ANOVA, and nonlinear regression. *P* values less than 0.05 were taken to be statistically significant. Values reported are mean $\pm$ SE.

RESULTS

Figure 1 (left) shows the BP, RBF, and ChBF recordings from a typical trial. BP was stable during the entire trial. At the start of

FIGURE 3. (A) Group-averaged normalized RBF and (B) RBOLD time courses under light adapted conditions with various occlusion durations. (C) The relationship between occlusion duration and the magnitude of the reactive hyperemia for RBF and (D) BOLD. The data were fit to an arbitrary nonlinear function ($Y = K1 \cdot [X^{K2} / (K3 + X^{K2})]$).

the occlusion, RBF and ChBF decreased sharply as expected. RBF gradually returned toward baseline, but ChBF did so to a lesser extent. Immediately after release of the occlusion, RBF overshoot its baseline (i.e., the reactive hyperemic response), but ChBF simply returned to baseline. The BOLD measurements (Fig. 1, right) showed similar trends as the BF measurements albeit at a lower SNR.

During the 60-second occlusion, the average RBF over the entire occlusion duration decreased less than the average ChBF ($-33.3\% \pm 14.4\%$ vs. $-49.8\% \pm 19.2\%$, $P = 0.014$; Fig. 2A). However, the average RBOLD signals during the occlusion decreased significantly more than the average ChBOLD signals ($-12.4\% \pm 4.8\%$ vs. $-4.4\% \pm 2.7\%$, $P = 0.0001$; Fig. 2B). The magnitudes of the RBF and RBOLD reactive hyperemias were significantly larger than those of ChBF and ChBOLD ($P = 0.0007$ and 0.0135 , respectively; Figs. 2C, 2D). The ChBF and

ChBOLD reactive hyperemias were quite small and not statistically different from zero.

Figure 3 shows the effects of varying the occlusion duration on RBF and RBOLD reactive hyperemia under light adapted conditions. The averaged time course traces for RBF (Fig. 3A) and RBOLD (Fig. 3B) showed smaller reactive hyperemias after the shorter occlusion durations. When quantified, the magnitude of the RBF reactive hyperemia increased as a nonlinear function of occlusion duration at durations less than ≈ 60 seconds, but did not increase further with longer duration occlusions (Fig. 3C). The RBOLD reactive hyperemia magnitudes had a similar relationship with occlusion duration (Fig. 3D). In contrast to the retina, there was no indication of a choroidal reactive hyperemic response at different occlusion durations (Fig. 4).

Before testing the effects of flickering light stimulation on reactive hyperemia, we confirmed that flicker stimulation elicited increases in RBF and RBOLD (Fig. 5) consistent with previous retinal studies and the functional hyperemia seen in other tissues during increased metabolic activity. Repeated flicker stimulation induced no obvious change in arterial blood pressure, but did elicit detectable increases in retinal perfusion by LSI and BOLD.

Figure 6 shows the effects of a 60-second occlusion performed under dark and light adapted conditions, and during flickering light stimulation. The averaged time course traces for RBF and RBOLD (Figs. 6A, 6B) suggested subtle effects during the occlusion and the reactive hyperemia. However, during the occlusion, there were no significant differences in the RBF decreases among the three light conditions (Fig. 6C, $P > 0.05$). Similarly, although there was a trend for RBOLD to be lower during flicker than in the light- and dark-adapted conditions, the RBOLD occlusion responses also were not significantly different among the three light conditions (Fig. 6D, $P > 0.05$). After the occlusion, there also

FIGURE 4. Group-averaged normalized ChBF time courses under light adapted conditions and different occlusion durations ($n = 7$).

FIGURE 5. (A) Femoral arterial BP and RBF traces in a trial of flicker visual stimuli with the LSI technique. (B) A representative BP trace along with the mean RBOLD traces from five repeated trials of flicker stimuli. The flicker visual stimulation (10 Hz) was on for 30 seconds (gray shaded areas) with intervening 90-second resting periods.

were no significant differences among the three light conditions for the magnitude of the reactive hyperemia in RBF and RBOLD (Figs. 6E, 6F; $P > 0.05$). To corroborate the findings further from the 60-second occlusion, a similar study was performed using a shorter (15 seconds) occlusion duration under light adapted and flickering light conditions (Fig. 7). The results from the 15-second occlusion also indicated a trend towards a greater decrease in RBOLD during the occlusion with flicker stimulation, but no difference in RBF or RBOLD reactive hyperemia magnitude between the light adapted and flicker conditions.

DISCUSSION

Our study used the reactive hyperemia response and its modulation by occlusion duration and metabolic activity as an indicator of metabolic local vascular control. The study hypothesis was that metabolic local control occurs in the retinal circulation, but not in the choroid. The results with LSI, LDF, and BOLD MRI partly support this hypothesis, that is, reactive hyperemia occurs in the retinal circulation, but not in the choroidal circulation, and the magnitude of the retinal reactive hyperemia was modulated by occlusion duration. However, the retinal reactive hyperemia was not modulated by lighting conditions that should have altered retinal metabolic activity, which is inconsistent with the metabolic local control hypothesis.

The study has some weaknesses that need mentioning before discussing the results. First, for anatomic reasons, BP was measured at the femoral artery, and so the arterial pressures at the entrance to the retinal and choroidal circulations during and after the occlusions are unknown. IOP also could not be measured invasively or noninvasively without disturbing the BF measurements, and so the perfusion pressure also is unknown. Mitigating this concern somewhat, the IOP decrease during the occlusions likely was small and consistent, and the femoral BP was stable before, during, and

after the occlusions, and was similar between animals, but the actual pressure challenge to the ocular circulations is ambiguous. This made it difficult to interpret the return toward baseline during the occlusions evident in several of the Figures (Figs. 1, 3, 4, 6, 7), and so only the average BFs during the occlusions were quantified. Second, also for anatomic reasons, ocular BP was decreased by occluding the common carotid artery. Consequently, brain perfusion also was affected, and a vascular steal effect from the cerebral circulation may have influenced the ocular BF responses during and after the occlusions (e.g., a concomitant cerebral reactive hyperemia may have blunted the reactive hyperemia in the retinal and choroidal circulations). Third, the measurement depths of the LSI and LDF techniques potentially are deep enough that choroidal perfusion may have influenced the LSI RBF measurement, and retinal perfusion may have influenced the LDF ChBF measurement. The light from the LSI and LDF instruments did not affect each other's flow signals and so it seems the retinal pigment epithelium constrained their measurement volumes, such that LSI measured only RBF and LDF measured only ChBF, but this is difficult to verify, although the confirmatory BOLD results suggested this was the case. If the choroid contributed to the LSI measurement of RBF, the lack of reactive hyperemia in the choroid also potentially would blunt the RBF reactive hyperemia. Similarly, a partial volume effect may have blunted the RBOLD reactive hyperemia if the choroid contributed to the RBOLD signal.

The overarching concern from these three weaknesses is that the potential maximum magnitude of the retinal reactive hyperemic response may be larger than the results indicate. Nonetheless, the results indicate clearly that reactive hyperemia occurs in the retinal circulation and that it is modulated by the duration of the occlusion. This behavior is consistent with the metabolic theory of local vascular control,³ which holds that tissues regulate their perfusion in accordance with their metabolic needs via a metabolism-linked vasodilatory feedback signal (e.g., adenosine) from the parenchymal cells to

FIGURE 6. (A) Group-averaged normalized RBF and (B) RBOLD time courses under dark, constant light, and flicker conditions with 60-second occlusions. (C) Mean RBF and (D) RBOLD changes during the occlusion period. (E) Magnitude of the reactive hyperemia in RBF and (F) RBOLD under the three light conditions.

the nearby vascular smooth muscle cells controlling arteriolar resistance and capillary flow distribution. The metabolic theory provides a mechanistic explanation for blood flow behaviors, such as pressure-flow autoregulation, functional hyperemia, and reactive hyperemia. In the specific case of reactive hyperemia, the mismatch of perfusion and metabolism during transient occlusion leads to accumulation of the feedback signal, which dilates the vessels so that upon release of the occlusion, blood flow overshoots baseline and remains elevated until the excess feedback signal is flushed from the tissue. Given such a mechanism, it follows that the amount of feedback signal accumulated depends on the duration of the ischemic period, so that the magnitude of the reactive hyperemia is modulated by the occlusion duration (limited by the maximum vasodilation achievable by the supply vessels). This explanation has a venerable history going back at least as far as Roy and Brown in 1880.¹ The present results are consistent with their results in frogs, and the reactive hyperemia responses observed in numerous other species and tissues by other investigators.³¹⁻³⁷

The metabolic theory of local control also predicts the functional hyperemia response to increased metabolic activity that occurs in many tissues, that is, if metabolism increases and blood flow is no longer sufficient, the production of the vasodilatory feedback signal increases to match once again perfusion to metabolism.³ In the eye, this also is called neurovascular coupling, and the hyperemic response to flickering light stimulation is a commonly cited example, since flicker stimulation seems to increase retinal metabolism as indicated by increased retinal glucose use and oxygen extraction.^{12,13,38,39} If the increased retinal perfusion during flicker stimulation is a functional hyperemia, then it follows that the magnitude of the reactive hyperemia during flicker stimulation should be greater than when the retina is at rest, that is, the faster rate of production of the vasodilatory feedback signal during a functional hyperemia should cause a greater accumulation of the feedback signal during an occlusion and a larger hyperemia upon release of the occlusion. Such behavior occurs in the gut and other tissues.^{40,41}

FIGURE 7. (A) Group-averaged normalized RBF and RBOLD time courses under the constant light and flicker conditions with 15-second occlusions. (C) Mean RBF and (D) RBOLD changes during the occlusion period. (E) Magnitude of the reactive hyperemia in RBF and (F) RBOLD under the two light conditions.

Although no direct index of retinal metabolism was measured in our study, preliminary experiments confirmed that RBF and RBOLD increased in response to flicker stimulation in the preparation used in our study (Fig. 5). Consequently, it was surprising that the lighting conditions had no significant effect on the magnitude of the reactive hyperemia after the 60-second occlusion (Fig. 6). A shorter duration 15-second occlusion also was tried to see if an effect would be evident in a submaximal reactive hyperemia; there was none (Fig. 7).

One possible explanation for this finding is that the increase in retinal perfusion during flicker stimulation may not be a functional hyperemia as explained by the metabolic theory of local control,¹² but rather a direct neurogenic vasodilatory response, perhaps mediated by astrocytes or glial cells with direct projections to the resistance vessels and pericytes.⁴² This mechanism is hard to reconcile with the modulation of reactive hyperemia by occlusion duration, and so perhaps a local neurogenic and a metabolic mechanism work in parallel. This is speculative and requires further study. However, the results do indicate that the metabolic theory alone cannot

account for the retinal reactive hyperemia response during flicker stimulation.

The lack of choroidal reactive hyperemia is consistent with the study hypothesis and other evidence indicating a lack of choroidal metabolic local control, such as the negligible choroidal blood flow responses to flicker stimulation, hypoxia, and hyperoxia.² The distance from the retina to the choroidal resistance vessels also argues against the effectiveness and timeliness of a mechanism based on diffusion of a feedback signal. Instead, neural and other forms of local control likely dominate choroidal blood flow regulation.

CONCLUSIONS

In summary, the results showed that reactive hyperemia occurs in the retinal circulation, but not in the choroid, and that retinal reactive hyperemia is modulated by the duration of the preceding occlusion, but not the lighting conditions. We concluded that metabolic local control has a negligible role in

choroidal blood flow regulation and only partially accounts for the blood flow behavior in the retinal circulation.

Acknowledgments

The authors thank Joseph M. Harrison for assistance with the light source intensity measurements.

Supported in part by the NIH/NEI (R01 EY014211, EY018855, and EY009702), MERIT Award from the Department of Veterans Affairs, and San Antonio Life Science Institute (TQD), and CTSA Pilot Project & Translational Technology (CTSA-8UL1TR000149) and AHA SWA 2012 Predoctoral Fellowship (AHA-13PRE14680087; GL).

Disclosure: **G. Li**, None; **J.W. Kiel**, None; **D.P. Cardenas**, None; **B.H. De La Garza**, None; **T.Q. Duong**, None

References

- Roy CS, Brown JG. the blood-pressure and its variations in the arterioles, capillaries and smaller veins. *J Physiol*. 1880;2:323-446.
- Kiel JW. Local determinants of ocular blood flow. In: Schmetterer L, Kiel J, eds. *Ocular Blood Flow*. Berlin, Germany: Springer; 2012.
- Granger HJ, Shepherd AP. Intrinsic microvascular control of tissue oxygen delivery. *Microvasc Res*. 1973;5:49-72.
- Bayliss WM. On the local reactions of the arterial wall to changes of internal pressure. *J Physiol*. 1902;28:220-231.
- Johnson PC. The myogenic response. In: Bohr D, Somlyo A, Sparks H, Geiger S, eds. *Handbook of Physiology: The Cardiovascular System*. Bethesda, MD: American Physiological Society; 1980:409-442.
- Hilton SM. A peripheral arterial conducting mechanism underlying dilatation of the femoral artery and concerned in functional vasodilatation in skeletal muscle. *J Physiol*. 1959; 149:93-111.
- Kuo L, Davis MJ, Chilian WM. Endothelium-dependent, flow-induced dilation of isolated coronary arterioles. *Am J Physiol*. 1990;259:H1063-H1070.
- Koller A, Sun D, Kaley G. Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles in vitro. *Circ Res*. 1993;72:1276-1284.
- Shepherd AP, Granger DN. Metabolic regulation of the intestinal circulation. In: Shepherd AP, Granger DN, eds. *Physiology of the Intestinal Circulation*. New York: Raven Press, 1984.
- Lates AM. Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. *Arch Ophthalmol*. 1967;77:405-409.
- Riva CE, Harino S, Shonat RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett*. 1991;128:291-296.
- Riva CE, Logean E, Falsini B. Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. *Prog Retin Eye Res*. 2005;24:183-215.
- Riva CE, Buerk DG. Dynamic coupling of blood flow to function and metabolism in the optic nerve head. *Neuro-Ophthalmol*. 1998;20:45-54.
- Garhofer G, Huemer KH, Zawinka C, Schmetterer L, Dörner GT. Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow. *Curr Eye Res*. 2002;24:109-113.
- Kiel JW, Shepherd AP. Autoregulation of choroidal blood flow in the rabbit. *Invest Ophthalmol Vis Sci*. 1992;33:2399-2410.
- Schmidl D, Garhofer G, Schmetterer L. The complex interaction between ocular perfusion pressure and ocular blood flow - relevance for glaucoma. *Exp Eye Res*. 2011;93: 141-155.
- Sugiyama T, Araie M, Riva CE, Schmetterer L, Orgul S. Use of laser speckle flowgraphy in ocular blood flow research. *Acta Ophthalmol*. 2010;88:723-729.
- Kiel JW, Reitsamer HA. Laser Doppler flowmetry. In: Schmetterer L, Kiel J, eds. *Ocular Blood Flow*. Berlin, Germany: Springer; 2012.
- De La Garza BH, Muir ER, Li G, Shih YY, Duong TQ. Blood oxygenation level-dependent (BOLD) functional MRI of visual stimulation in the rat retina at 11.7 T. *NMR Biomed*. 2011;24: 188-193.
- Duong TQ, Pardue MT, Thule PM, et al. Layer-specific anatomical, physiological and functional MRI of the retina. *NMR Biomed*. 2008;21:978-996.
- Duong TQ, Muir ER. Magnetic resonance imaging of the retina. *Jpn J Ophthalmol*. 2009;53:352-367.
- Cheng HY, Duong TQ. Simplified laser-speckle-imaging analysis method and its application to retinal blood flow imaging. *Opt Lett*. 2007;32:2188-2190.
- Cheng H, Yan Y, Duong TQ. Temporal statistical analysis of laser speckle images and its application to retinal blood-flow imaging. *Opt Express*. 2008;16:10214-10219.
- Kiel JW, Reitsamer HA, Walker JS, Kiel FW. Effects of nitric oxide synthase inhibition on ciliary blood flow, aqueous production and intraocular pressure. *Exp Eye Res*. 2001;73: 355-364.
- Duong TQ, Ngan SC, Ugurbil K, Kim SG. Functional magnetic resonance imaging of the retina. *Invest Ophthalmol Vis Sci*. 2002;43:1176-1181.
- Cheng H, Nair G, Walker TA, et al. Structural and functional MRI reveals multiple retinal layers. *Proc Natl Acad Sci U S A*. 2006;103:17525-17530.
- Shih YY, Li G, Muir ER, et al. Pharmacological MRI of the choroid and retina: blood flow and BOLD responses during nitroprusside infusion. *Magn Reson Med*. 2012;68:1273-1278.
- Shih YY, De la Garza BH, Muir ER, et al. Lamina-specific functional MRI of retinal and choroidal responses to visual stimuli. *Invest Ophthalmol Vis Sci*. 2011;52:5303-5310.
- Li G, De La Garza B, Shih YY, Muir ER, Duong TQ. Layer-specific blood-flow MRI of retinitis pigmentosa in RCS rats. *Exp Eye Res*. 2012;101:90-96.
- Cook ER, Peters K. Calculating unbiased tree-ring indices for the study of climatic and environmental change. *The Holocene*. 1997;7:361-370.
- Lewis T, Grant R. Observations upon reactive hyperaemia in man. *Heart*. 1925;12:73-120.
- Randall JE, Horvath SM. Relationship between duration of ischemia and reactive hyperemia in a single vessel. *Am J Physiol*. 1953;172:391-398.
- Zimmer R, Lang R, Oberdorster G. Post-ischemic reactive hyperemia of the isolated perfused brain of the dog. *Pflugers Arch*. 1971;328:332-343.
- Shepherd AP, Riedel GL. Differences in reactive hyperemia between the intestinal mucosa and muscularis. *Am J Physiol*. 1984;247:G617-G621.
- Fairchild HM, Ross J, Guyton AC. Failure of recovery from reactive hyperemia in the absence of oxygen. *Am J Physiol*. 1966;210:490-492.
- Barcroft H. The mechanism of vasodilatation in the limbs during and after arrest of the circulation. *Angiology*. 1972;23: 595-599.
- Gidday JM, Esther JW, Ely SW, Rubio R, Berne RM. Time-dependent effects of theophylline on myocardial reactive

- hyperaemias in the anaesthetized dog. *Br J Pharmacol*. 1990;100:95-101.
38. Bill A, Sperber GO. Control of retinal and choroidal blood flow. *Eye*. 1990;4:319-325.
39. Shakoor A, Blair NP, Mori M, Shahidi M. Chorioretinal vascular oxygen tension changes in response to light flicker. *Invest Ophthalmol Vis Sci*. 2006;47:4962-4965.
40. Kiel JW, Riedel GL, Shepherd AP. Local control of canine gastric mucosal blood flow. *Gastroenterology*. 1987;93:1041-1053.
41. Valteau JD, Granger DN, Taylor AE. Effect of solute-coupled volume absorption on oxygen consumption in cat ileum. *Am J Physiol*. 1979;236:E198-E203.
42. Attwell D, Buchan AM, Charpak S, et al. Glial and neuronal control of brain blood flow. *Nature*. 2010;468:232-243.