

REVIEW ARTICLE

Functional hyperemia and mechanisms of neurovascular coupling in the retinal vasculature

Eric A Newman

The retinal vasculature supplies cells of the inner and middle layers of the retina with oxygen and nutrients. Photoc stimulation dilates retinal arterioles producing blood flow increases, a response termed functional hyperemia. Despite recent advances, the neurovascular coupling mechanisms mediating the functional hyperemia response in the retina remain unclear. In this review, the retinal functional hyperemia response is described, and the cellular mechanisms that may mediate the response are assessed. These neurovascular coupling mechanisms include neuronal stimulation of glial cells, leading to the release of vasoactive arachidonic acid metabolites onto blood vessels, release of potassium from glial cells onto vessels, and production and release of nitric oxide (NO), lactate, and adenosine from neurons and glia. The modulation of neurovascular coupling by oxygen and NO are described, and changes in functional hyperemia that occur with aging and in diabetic retinopathy, glaucoma, and other pathologies, are reviewed. Finally, outstanding questions concerning retinal blood flow in health and disease are discussed.

Journal of Cerebral Blood Flow & Metabolism (2013) **33**, 1685–1695; doi:10.1038/jcbfm.2013.145; published online 21 August 2013

Keywords: arachidonic acid; diabetes; glial cells; nitric oxide; potassium channels

INTRODUCTION

Oxygen and nutrients are supplied to the retina by a dual circulatory system (Figure 1). The high metabolic needs of the retinal photoreceptors (photoreceptors have the highest metabolic rate of any cell in the body¹) are satisfied by the choroidal vasculature. The more modest metabolic demands of the neurons and glial cells of the inner portions of the retina are met by the retinal vasculature. In lower vertebrates that have thinner retinas, including amphibians, reptiles, and some mammals, the retinal vasculature is absent and the choroidal circulation supplies the entire retina.²

The choroidal circulation is supplied by the long and short ciliary arteries and the anterior ciliary arteries, which feed the large arteries in the outer portion of the choroid.³ These arteries branch into smaller vessels, which, in turn, feed the highly anastomosed choriocapillaris network lying at the inner border of the choroid, adjacent to the retinal pigment epithelium and retinal photoreceptors. The total blood flow supplying the choroid is much greater than that supplying the retina (606 vs. 25 mg/min/whole tissue⁴), to meet the high metabolic demands of the photoreceptors.⁵

The retinal vasculature is supplied by the central retinal artery, which enters the retina with the optic nerve at the optic disc. The artery branches into radial arterioles and smaller vessels on the vitreal surface of the retina.³ Pre-capillary arterioles and capillaries ramify from these surface vessels and form anastomotic networks in the ganglion cell layer, just beneath the retinal surface, and deeper in the inner nuclear layer, supplying horizontal cells, bipolar cells, amacrine cells, and Müller glial cells. Blood is returned through radial venules on the retinal surface that empty into the central retinal vein in the optic nerve.

The retinal and choroidal vasculatures differ in several respects. Retinal vessels lack autonomic innervation,^{6,7} whereas the choroidal circulation is innervated by both sympathetic and parasympathetic

nerves.^{8–10} Also, autoregulation is present in the retinal circulation^{11,12} but is less well developed in choroidal vessels.^{13,14} Responses to light also differ. As will be described below, functional hyperemia is well developed in the retinal vasculature while the choroidal blood supply does not respond well to flickering light stimuli (but see Lovasik *et al.*¹⁵). Choroidal vessels do respond to the adaptation state of the eye, however, with higher choroidal blood flow observed in the light than in the dark.^{16,17}

When the retina is stimulated by a flickering light, blood flow in the retinal vasculature increases significantly.¹⁸ This increase in blood flow, the functional hyperemia response, supplies oxygen and glucose to the active neurons in the inner and middle retinal layers. This hemodynamic response is present in the cerebral circulation as well and was described long ago in the cerebral cortex by Mosso¹⁹ and Roy and Sherrington.²⁰

It is believed that the functional hyperemia response is critical for proper retinal function.¹⁸ With increased neuronal activity in the retina, there is a need for enhanced oxygen and glucose supply and removal of metabolites. The increase in retinal blood flow serves this need. The loss of functional hyperemia that occurs in certain diseases may compromise retinal health and may contribute to the development of pathology.

This review will focus on functional hyperemia in the retinal vasculature in health and disease. Special emphasis will be placed on the signaling mechanisms responsible for generating the functional hyperemia response. The reader is referred to recent reviews^{3,18,21,22} for information about other aspects of ocular circulation.

FUNCTIONAL HYPEREMIA IN THE RETINA

Functional hyperemia in the retina is typically studied by stimulating the eye with a flickering light, which maximally activates

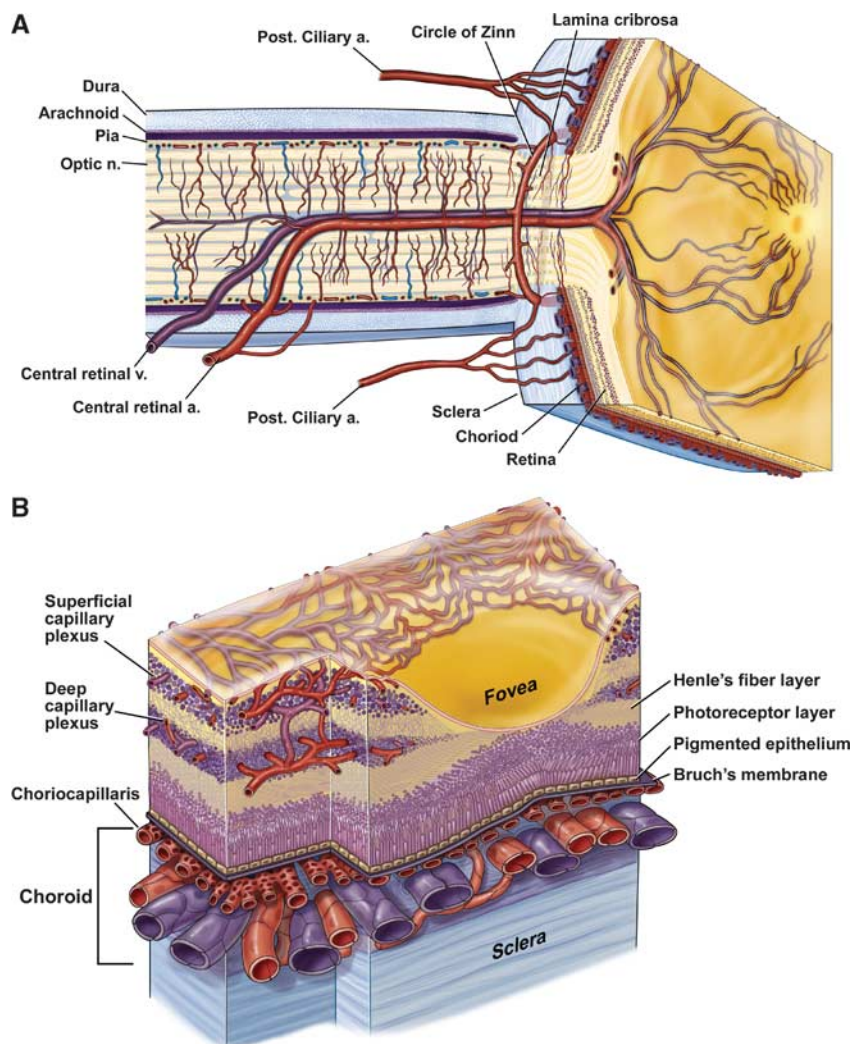


Figure 1. Anatomy of the ocular circulation. (A) Cut away drawing of the human eye along the superior–inferior axis through the optic nerve, showing the vascular supply to the retina and choroid. The retinal vessels are supplied by the central retinal artery. a, artery; v, vein; n, nerve. (B) Drawing showing the vasculature of the retina and choroid. Retinal arterioles and venules lie on the vitreal surface of the retina while capillary plexi lie in just beneath the surface and in the inner nuclear layer. Drawings by Dave Schumick from Anand-Apte and Hollyfield.¹³⁰

amacrine cells and ganglion cells in the inner retina.²³ A flickering stimulus dilates the primary arterioles on the retinal surface (Figure 2), leading to an increase in blood flow in arterioles, capillaries, and venules of the retina. Flickering light also increases blood flow in the capillaries of the optic disc (Figure 2). Increases in blood flow in retinal vessels supply active neurons in the inner and middle retinal layers with increased oxygen and glucose while blood flow increases in the capillaries of the optic disc supply the active axons of the retinal ganglion cells.

Arteriole dilation can be monitored with a modified fundus camera, such as the retinal vessel analyzer.²⁴ In humans, flickering light evokes sustained dilations ranging from 3% to 5%.^{24–26} The response has a latency of <1 s. Primary venules in humans dilate to nearly the same extent as arterioles,^{24,25} although it is unclear whether this is an active or passive response. Similar light-evoked arteriole dilations occur in cats^{27,28} and rodents.²⁹ In rats and mice, light-evoked venule dilations are much smaller than those of arterioles (TE Kornfield, AI Srienc, and EA Newman, unpublished observations).

Arteriole dilation leads to increases in retinal blood velocity, which can be measured using techniques based on the Doppler effect. These measurement techniques include bi-directional laser

Doppler velocimetry, which yields an absolute measure of red blood cell velocity, laser Doppler flowmetry, which measures relative red blood cell velocity, blood volume, and flux in the optic nerve head and choroid, and laser speckle flowmetry, which yields two-dimensional images of relative blood velocity on the retina surface.¹⁸ Retinal blood flow can also be measured using magnetic resonance imaging, which has sufficient spatial resolution to distinguish retinal arterioles, venules, and choroidal vessels.³⁰ In humans, a flickering light results in blood flux increases of 30–38% in the optic nerve head^{31,32} and flux increases of 59% in retinal arterioles.³³ In the cat optic nerve head, flux increases of 27–59% are observed.^{27,34}

As originally noted by Roy and Sherrington,²⁰ the localized nature of blood flow control is a hallmark of the functional hyperemia response. In the cortex, activation of a small parenchymal region results in an increase in blood flow that is largely restricted to that region. The localized nature of the response demonstrates that blood flow can be controlled locally in the cerebral vasculature. The few studies that have been conducted in the retina demonstrate that the retinal vasculature is also able to control blood flow locally in response to focal photic stimulation. When one hemi-field of the cat retina is stimulated

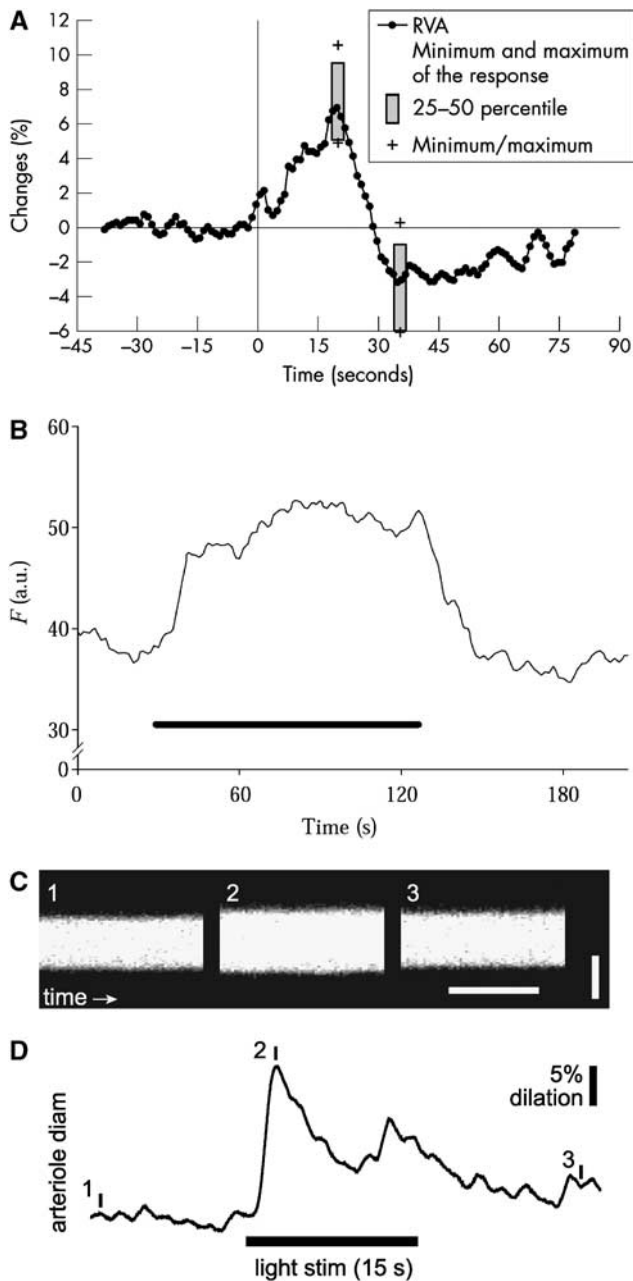


Figure 2. The functional hyperemia response in the retina. (A) Mean increase in primary arterial diameter to flicker stimulation in humans measured with the retinal vessel analyzer (RVA). Light onset is at time = 0. $^+P < 0.001$. From Nagel and Vilser.¹³¹ (B) Blood flow increase to flicker stimulation (black bar) measured at the rim of the optic disc in cats with laser Doppler flowmetry. From Riva *et al.*¹⁸ (C, D) Increase in primary arteriole diameter to flicker stimulation in a single trial in the rat measured with confocal microscopy. From Mishra and Newman.²⁹ Shown are segments of a confocal line scan image before, during, and after stimulation (C) and the diameter of the arteriole (D). Scale bars in panel C, 25 μm and 100 ms.

with a drifting grating, blood flow increases, monitored with blood oxygen level-dependent functional magnetic resonance imaging, are restricted to the stimulated half of the retina.³⁵ Similarly, when the rat retina is stimulated focally with a flickering spot, blood velocity increases, monitored with laser speckle flowmetry, are greatest near the stimulated region.³⁶

Active control of blood flow in the retinal circulation is believed to occur principally in the arterioles on the surface of the retina.

These vessels are surrounded by layers of smooth muscle cells²¹ and display large dilations ranging from 3% to 8%^{24,25,29} in response to photic stimulation. It is possible, however, that active control of blood flow also occurs in retinal capillaries, which are surrounded by contractile pericytes. Pericytes share many properties with vascular smooth muscle cells. Pericytes contain contractile proteins^{37,38} and have been shown to relax and contract in response to various signaling molecules.^{39,40} Pericytes cover a large fraction of the surface of capillaries in the retina, much larger than that in the brain,^{41,42} and active contraction of pericytes restricts blood flow through retinal capillaries after an ischemic insult.⁴³ However, it remains unclear whether pericytes actively control blood flow in the retina under normal physiologic conditions.

MECHANISMS OF NEUROVASCULAR COUPLING IN THE RETINA

Metabolic Feedback vs. Feedforward Mechanisms

Functional hyperemia is mediated, either directly or indirectly, by signaling from neurons to blood vessels. The mechanisms that mediate functional hyperemia, termed neurovascular coupling, have been of great interest for over a century. In their seminal work, Roy and Sherrington²⁰ proposed that metabolic products released from active neurons mediate functional hyperemia. According to this metabolic negative feedback mechanism,⁴⁴ neuronal activity leads to a drop in energy reserves in active neurons and to the generation of metabolic signals that dilate nearby blood vessels. The resulting increase in blood flow augments glucose and oxygen supplies and restores the energy reserves of the active neurons. Several metabolic signals could function as the neurovascular coupling signal. These include a drop in oxygen or glucose levels or increases in CO_2 (leading to acidification), adenosine, or lactate levels. All of these changes could dilate arterioles. Recent work has demonstrated, however, that some of these signals do not mediate neurovascular coupling. Specifically, functional hyperemia can occur in the absence of a drop in oxygen^{45–47} or glucose⁴⁸ levels or acidification of the parenchyma,^{49,50} demonstrating that O_2 , glucose, and CO_2 do not function as the neurovascular coupling signal. Still, neurovascular coupling could be mediated, at least in part, by adenosine or lactate metabolic signals. As discussed below, lactate may control blood flow indirectly by modulating prostaglandin E_2 (PGE_2) levels.

An alternate to the metabolic negative feedback mechanism of neurovascular coupling is a feedforward mechanism.⁴⁴ In a feedforward mechanism, active neurons release signaling molecules that directly or indirectly result in vasodilation. These signaling molecules are not directly related to cell metabolism, thus distinguishing this form of neurovascular coupling from metabolic negative feedback. Many signaling pathways may contribute to feedforward neurovascular coupling in the central nervous system. Active neurons release nitric oxide (NO) and PGE_2 , both of which relax vascular smooth muscle cells and result in vasodilation. Active neurons also release a number of transmitters that act on glial metabotropic receptors, evoking Ca^{2+} increases in glial cells. These Ca^{2+} increases lead to the release of vasodilatory agents from glial cells, including K^+ and the arachidonic acid metabolites PGE_2 and epoxyeicosatrienoic acids (EETs).

Both feedforward signals as well as metabolic negative feedback signals may contribute to functional hyperemia in the retina. Evidence supporting different neurovascular coupling signaling mechanisms is reviewed in the following sections.

Arachidonic Acid Metabolite-Mediated Neurovascular Coupling

In recent years, persuasive evidence has emerged indicating that a feedforward neurovascular coupling mechanism mediated by signaling from neurons to glial cells to blood vessels is instrumental in generating functional hyperemia in the mammalian retina.

According to this model, neurovascular coupling occurs in several steps. The release of neurotransmitters from active neurons leads to Ca^{2+} increases in glial cells, which, in turn, results in the release of vasodilatory agents from the glial cells onto blood vessels.

As Ramon y Cajal⁵¹ pointed out over a century ago, glial cells are ideally suited to regulate blood flow. Blood vessels are almost completely enveloped by the endfeet of glial cells.⁵² Thus, glial cells, more than neurons, are well situated to communicate directly with vessels. In the retina, blood vessels are surrounded by the endfeet of both astrocytes, which lie near the vitreal surface of the retina, and Müller cells, which are the principal glial cells of the retina and extend from the vitreal surface to the photoreceptors.^{21,53}

When neurons in the retina are activated by photic stimulation, intracellular Ca^{2+} increases are observed in glial cells (Figure 3).⁵⁴ The Ca^{2+} increases occur in Müller cells but not in astrocytes. This is not surprising as Müller cells directly contact neuronal synapses in the plexiform layers of the retina and thus will be stimulated by the release of transmitters from neurons.⁵⁴ Astrocytes, in contrast, are restricted to the vitreal surface of the retina and are far from neurotransmitter release sites. Light-evoked Ca^{2+} increases in

Müller cells are mediated by the release of ATP from neurons and the activation of glial purinergic receptors.⁵⁴ The Ca^{2+} increases are blocked by purinergic antagonists but not by glutamatergic, GABAergic, or cholinergic antagonists. Light-evoked glial Ca^{2+} increases are also blocked by tetrodotoxin,⁵⁴ indicating that ATP release from ganglion cells and amacrine cells, the only retinal neurons which generate tetrodotoxin-sensitive action potentials, stimulate the Müller cells. Antidromic activation of ganglion cells also evokes Ca^{2+} increases in Müller cells, supporting the role of these neurons in the generation of glial Ca^{2+} increases.⁵⁴

Calcium increases in glial cells result in the release of vasoactive agents that dilate retinal arterioles. When retinal glial cells are directly stimulated by photolysis of caged Ca^{2+} or caged inositol 1,4,5 trisphosphate, resulting in increased glial Ca^{2+} , neighboring arterioles dilate (Figure 4).⁵⁵ Signaling from glial cells to blood vessels is direct and does not involve neurons, as glial-evoked vasodilation is not reduced when transmitter release from neurons is blocked with tetanus toxin.⁵⁵ Glial-evoked vasodilation is mediated by the production of PGE_2 and EETs. When the synthetic enzymes for these two arachidonic acid metabolites are inhibited, glial-evoked vasodilation is reduced by 88%.^{55,56}

Calcium increases in glial cells can also result in the production of the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE).⁵⁵ Under physiologic conditions, production of 20-HETE is outweighed by the production of the vasodilators PGE_2 and EETs, and vasodilation results. However, as will be discussed below, under non-physiologic conditions, including hyperoxia and high NO levels, the vasodilatory mechanisms are suppressed, and 20-HETE-mediated vasoconstriction is observed.

The glial cell-mediated feedforward mechanism of neurovascular coupling, summarized in Figure 5, contributes significantly to functional hyperemia in the retina. When signaling from neurons to glial cells is interrupted by the purinergic antagonist suramin, light-evoked vasodilation is nearly abolished.⁵⁵ Suramin acts by preventing glial Ca^{2+} increases rather than by interfering with neuronal activity or intrinsic vascular responsiveness. Suramin neither reduces light-evoked neuronal activity nor does it block glial-evoked vasodilation when glial cells are stimulated by photolysis of caged compounds.⁵⁵

The role of prostaglandins in mediating neurovascular coupling is supported by additional observations. PGE_2 dilates retinal arterioles in the *ex vivo* whole-mount retina,⁵⁵ and PGE_1 dilates arterioles *in vivo* when injected near retinal vessels.⁵⁷ Also, intravenous administration of the cyclooxygenase inhibitor indomethacin to block prostaglandin synthesis reduces arteriole diameter and blood flow in humans⁵⁸ and constricts arterioles in the miniature pig.⁵⁹ It should be noted, however, that ibuprofen, another cyclooxygenase inhibitor, fails to reduce retinal blood flow in the pig.⁶⁰

Potassium-Mediated Neurovascular Coupling

Neurovascular coupling in the retina may also be mediated by the release of K^+ onto blood vessels. When extracellular K^+ concentration ($[\text{K}^+]_o$) is raised from a resting level of 3–5 mmol/L up to 10–15 mmol/L, blood vessels dilate. Potassium-induced vasodilation is mediated by an increase in the conductance of inwardly rectifying K^+ channels on vascular smooth muscle cells^{61,62} and by activation of the Na^+-K^+ ATPase on the smooth muscle cells,^{63,64} both of which result in hyperpolarization and relaxation of the muscle cells. However, larger K^+ increases, above ~15 mmol/L, depolarize vascular smooth muscle cells, resulting in vasoconstriction.

Depolarizing neurons in the retina release K^+ into the extracellular space. *In vivo*, photic stimulation produces increases in $[\text{K}^+]_o$ at the surface of the optic nerve head that are well correlated with changes in blood flow. $[\text{K}^+]_o$ and flow vary similarly as the luminance and flicker frequency of the stimulus is changed.²⁷ In both mammalian⁶⁵ and amphibian^{66,67} retinas,

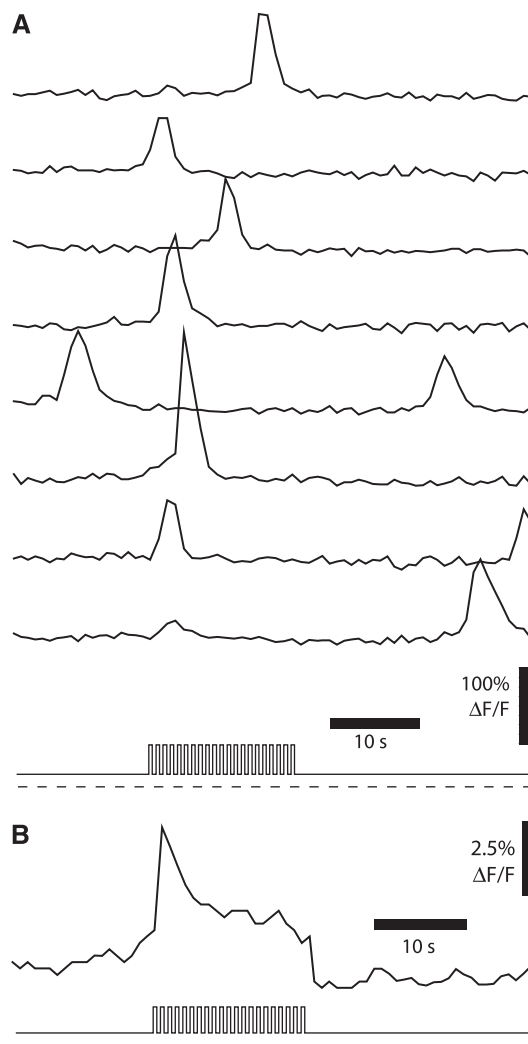


Figure 3. Cytoplasmic Ca^{2+} increases in Müller glial cells of the rat retina. **(A)** Calcium increases in eight Müller cells. The frequency of Ca^{2+} transients in individual cells increases during flicker stimulation. **(B)** Mean Müller cell Ca^{2+} increase to flicker stimulation. The time course of the flicker stimulus is shown at the bottom in **A** and **B**, with the dashed lines indicating zero intensity. From Newman.⁵⁴

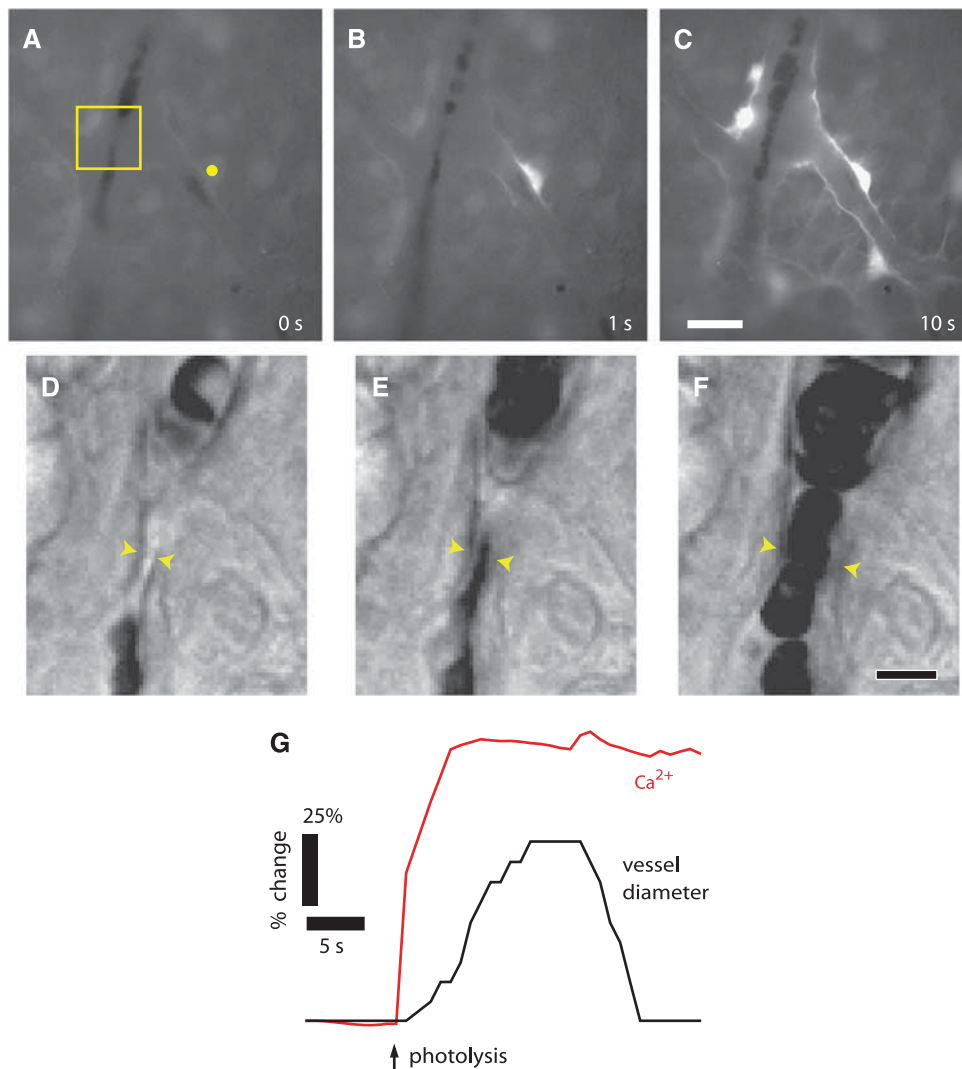


Figure 4. Calcium increases in glial cells evokes arteriole dilation in the rat retina. (A–C) Fluorescence images showing Ca^{2+} in glial cells. Photolysis of caged- Ca^{2+} within a glial cell (yellow dot in panel A) evokes an increase in glial Ca^{2+} that propagates through several glial cells adjacent to the blood vessels. (D–F) Infrared differential interference contrast images showing a higher magnification view of the small arteriole indicated by the yellow box in panel A. Each frame was acquired 0.5 s after the corresponding image above. Glial stimulation evokes arteriole dilation. Yellow arrowheads indicate luminal diameter of vessel. (G) Time course of the glial Ca^{2+} increase and vessel dilation. From Metea and Newman.⁵⁵

$[\text{K}^+]_o$ increases are the largest in the plexiform layers (increases of ≤ 1 mmol/L are observed), where K^+ is released from presynaptic and postsynaptic neuronal elements. This K^+ diffuses to blood vessels on the retinal surface where it may dilate vessels.

A number of years ago, it was proposed that neurovascular coupling is mediated by a feedforward glial cell K^+ siphoning mechanism.⁶⁸ According to this model,⁶⁹ the diffusion of K^+ from neurons to blood vessels is enhanced by a K^+ current flow through Müller cells. The $[\text{K}^+]_o$ increase due to neuronal activity generates an influx of K^+ into Müller cells and results in Müller cell depolarization. Depolarization, in turn, induces a K^+ efflux from cell endfeet, which have a very high density of K^+ channels.^{70,71} Because Müller cell endfeet terminate on blood vessels, the K^+ efflux occurs directly onto the vessels. Computer simulations of K^+ dynamics⁶⁹ demonstrate that glial K^+ siphoning results in a K^+ increase at the vessel that is larger and more rapid than would occur solely by K^+ diffusion through extracellular space. Potassium siphoned onto blood vessels could mediate neurovascular coupling.

More recently, the glial K^+ siphoning hypothesis of neurovascular coupling was tested in the retina.⁷² The results do not

support the K^+ siphoning hypothesis. Glial cells were depolarized by current injection through patch pipettes. This depolarization should evoke K^+ efflux from the cell endfeet and result in vasodilation. Although vessels dilated in response to bath-applied increases in $[\text{K}^+]_o$, current injection did not induce vasodilation. In a second test of the K^+ siphoning hypothesis, light-evoked vasodilation was assessed in mice where Kir4.1 K^+ channels were genetically knocked out. Kir4.1 is the principal K^+ channel in retinal glial cells,⁷³ and K^+ siphoning fluxes should be nearly abolished in Kir4.1 knockout animals. However, light-evoked vasodilation was as large in the knockout animals as in wild-type controls. The experiments demonstrate that glial cell K^+ siphoning does not contribute significantly to neurovascular coupling in the retina.

Although neurovascular coupling in the retina is not mediated by K^+ siphoning, it could be mediated by a different K^+ mechanism. Retinal Müller cells express BK Ca^{2+} -activated K^+ channels as well as Kir channels.⁷⁴ When neuronal activity evokes Ca^{2+} increases in Müller cells, BK K^+ channels will open.⁶¹ BK channels are also modulated by arachidonic acid metabolites, and Ca^{2+} -dependent increases in EETs will open the channels.^{75,76} BK channel opening

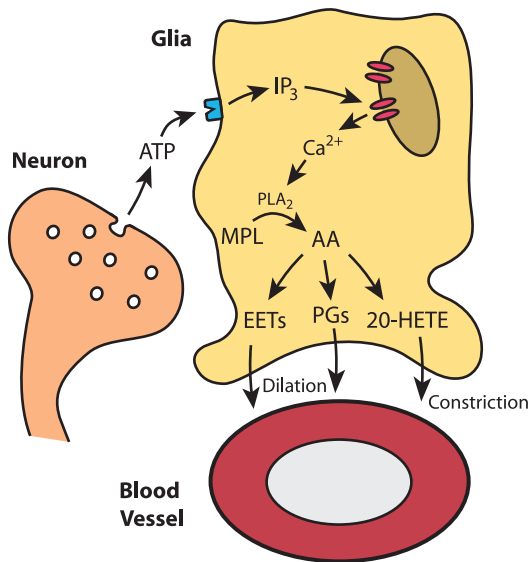


Figure 5. Summary of the arachidonic acid metabolite-mediated neurovascular coupling mechanism. ATP released from active neurons stimulates P2Y receptors on glial cells, resulting in the production of inositol 1,4,5 trisphosphate (IP₃) and the release of Ca²⁺ from internal stores. Glial Ca²⁺ activates phospholipase A₂ (PLA₂) resulting in the production of arachidonic acid (AA) from membrane phospholipids (MPL). Increased AA levels lead to the production of its metabolites, including the vasodilators epoxyeicosatrienoic acids (EETs) and prostaglandin E₂ (PGE₂) and the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE). Under physiologic conditions, the vasodilators have a stronger influence on vessels than the vasoconstrictor, leading to vessel dilation and increased blood flow. Drawing by Anusha Mishra, unpublished.

will result in an efflux of K⁺ from glial cell endfeet onto blood vessels, which could lead to vessel dilation. This K⁺-glial BK channel hypothesis of neurovascular coupling has received support in experiments in the cortex,⁶¹ but the hypothesis has not been tested in the retina.

Nitric Oxide-Mediated Neurovascular Coupling

Nitric oxide is a potent vasodilator and is believed to have an important role in mediating neurovascular coupling in the cerebellum.⁷⁷ Activity-dependent Ca²⁺ increases within neurons activate neuronal nitric oxide synthase (NOS), leading to the production of NO. The membrane-permeant NO can then diffuse to blood vessels, where it opens K⁺ channels and relaxes vascular smooth muscle cells,⁷⁸ leading to vasodilation and increased blood flow.

In the retina, NO levels have been measured *in vivo* with NO-sensitive microelectrodes positioned in the vitreous humor. A flickering light produces an increase in NO at the retinal surface⁷⁹ and at the optic nerve head,³⁴ suggesting that it could mediate neurovascular coupling. In both humans²⁵ and cats,²⁸ systemic administration of non-selective NOS inhibitors reduces flicker-evoked increases in blood flow, consistent with a neurovascular coupling mechanism mediated by NO.

These results would appear to support NO as an important mediator of neurovascular coupling in the retina. However, additional studies indicate that the effects of NO are more complicated. In the *ex vivo* rat retina, non-selective inhibition of NOS results in an increase, not a decrease, in flicker-evoked vasodilation.⁵⁵ As will be discussed below, the principal effect of NO in the retina may be as a modulator of neurovascular coupling, not as a mediator. A similar conclusion has been reached in the brain. Reduction of NOS activity in the cerebral cortex by topical administration of a neuronal NOS inhibitor results in a reduction in

the functional hyperemia response.⁸⁰ However, the response is restored when NO levels are raised by addition of an NO donor. Also, functional hyperemia in the cortex is not diminished when neuronal NOS activity is reduced by genetic manipulation.⁸¹ These results demonstrate that in the cerebral cortex NO modulates neurovascular coupling and must be present for functional hyperemia to occur. However, NO does not mediate the response. NO may have a similar modulatory role in the retina.

Lactate-Mediated Neurovascular Coupling

Lactate is a vasodilator that is a product of anaerobic glycolysis and is released by both glial cells and neurons. Lactate production increases substantially in the rabbit retina in response to flickering light.^{82,83} In the miniature pig, intravitreal injection of lactate near the retinal surface evokes arteriole dilation.⁵⁹ This effect is not due to acidification, as lactate injection at a neutral pH produces the same effect. A similar response is seen in isolated, pressurized retinal arterioles of the pig, which dilate in response to lactate.⁸⁴ In humans, increased serum levels of lactate, produced either by exercise or by intravenous injection, reduces flicker-evoked arteriole dilation.²⁶ This suggests that lactate contributes to neurovascular coupling, although the interpretation is complicated by the fact that lactate could have systemic effects and was administered on the luminal rather than on the abluminal side of blood vessels. Together these experiments demonstrate that lactate could contribute to neurovascular coupling as a metabolic negative-feedback mediator. However, definitive evidence for its role as a mediator of neurovascular coupling is lacking.

Adenosine-Mediated Neurovascular Coupling

Adenosine is another vasodilatory metabolic byproduct released by neurons and glial cells that could mediate functional hyperemia in the retina. Intravitreal but not intravenous administration of adenosine in the rabbit produces vasodilation and increased basal blood flow.⁸⁵ Adenosine also relaxes cultured pericytes isolated from the bovine retina.⁸⁶ In the cat, intravenous administration of adenosine increases baseline blood flow and enhances flicker-induced increases in blood flow.³⁴ This would argue against adenosine being a mediator of neurovascular coupling, as raising baseline levels of adenosine would be expected to decrease an adenosine-mediated vasodilation during flicker stimulation.

Summary of Neurovascular Coupling Mechanisms

As reviewed above, many vasoactive agents that are produced during photic stimulation dilate retinal arterioles and could mediate neurovascular coupling. It is likely that several of these agents act in concert to produce functional hyperemia in the retina. A similar phenomenon is seen in the brain, where a number of neurovascular coupling mechanisms are believed to operate concurrently to produce functional hyperemia.⁴⁵ However, recent studies suggest that one particular neurovascular coupling mechanism, the feedforward mechanism where glial cells release vasodilatory PGE₂ and EETs, is a principal and perhaps dominant mechanism mediating functional hyperemia in the retina. Determining the relative importance of this and other neurovascular coupling mechanisms awaits further experimentation.

MODULATION OF FUNCTIONAL HYPEREMIA IN THE RETINA

Oxygen Modulation of Neurovascular Coupling

Oxygen as well as CO₂ is a well-known modulator of blood flow. In humans,^{87,88} monkeys,⁸⁹ and miniature pigs,²² hyperoxia constricts retinal arterioles and venules and reduces retinal blood flow. Similarly, hypoxia dilates arterioles and venules and increases blood flow in the retina.

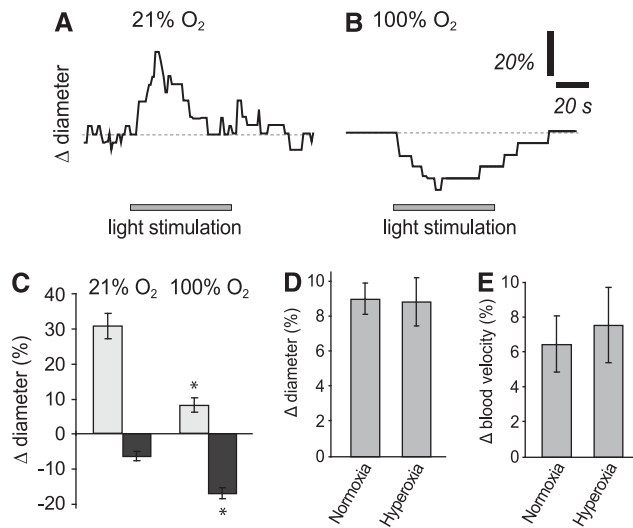


Figure 6. Oxygen depresses flicker-induced vasodilation in the *ex vivo* whole-mount retina but not *in vivo*. (A) Under normoxic conditions, when the *ex vivo* rat retina is superfused with saline equilibrated with air, flicker stimulation dilates arterioles. (B) When exposed to 100% O₂, vasodilations are depressed, unmasking a flicker-induced vasoconstriction. (C) Summary of results in the *ex vivo* preparation. In 21% O₂, flicker-induced vasodilations (gray bars) and much larger than vasoconstrictions (black bars). In 100% O₂, vasodilations are depressed and vasoconstrictions enhanced. * $P < 0.005$. (D, E) In the *in vivo* preparation, hyperoxia, induced by breathing 100% O₂, does not depress flicker-induced arteriole dilation (D) nor increase blood velocity (E). From Mishra *et al.*⁵⁶

In addition to modifying basal blood flow, oxygen modulates neurovascular coupling in the retina. In the *ex vivo* whole-mount preparation of the rat retina, raising O₂ reduces flicker-induced vasodilation.⁵⁶ Under normoxic conditions (superfusate bubbled with air), a flickering stimulus evokes a large (30%) vasodilation, which is sometimes followed by a small (7%) vasoconstriction (Figures 6A and 6C). When O₂ is raised to 100%, vasodilations are reduced to 8% whereas vasoconstrictions increase to 17% (Figures 6B and 6C). Pharmacological inhibition of the synthetic enzymes of arachidonic acid metabolites demonstrates that O₂ acts by blocking PGE₂-mediated but not EETs-mediated dilation of blood vessels.

A similar O₂ inhibition of PGE₂ signaling is seen in hippocampal slices. Under normoxic conditions, vasodilation evoked by neuronal activity is mediated by PGE₂ release from astrocytes.⁹⁰ Under hyperoxic conditions, O₂ inhibits PGE₂ signaling by increasing the uptake of PGE₂ into cells via the prostaglandin transporter, which exchanges PGE₂ for lactate. When pO₂ is raised, glycolytic production of lactate is reduced, lowering extracellular lactate levels. This enhances PGE₂ transport into cells, lowering extracellular PGE₂ levels and thus reducing PGE₂-induced vasodilation.

Although O₂ modulates neurovascular coupling in *ex vivo* preparations of the retina as well as the brain, it does not modulate functional hyperemia in the retina *in vivo*. Flicker-evoked dilation of rat retinal arterioles and increases in blood velocity are not reduced when the O₂ level of inspired air is increased to 100%⁵⁶ (Figures 6D and 6E). A similar insensitivity to hyperoxia is seen in the cerebral cortex *in vivo*, where hyperbaric hyperoxygenation has no effect on functional hyperemia.⁴⁶ These seemingly conflicting results arise due to the different pO₂ levels reached in hyperoxic *ex vivo* and *in vivo* preparations. When the *ex vivo* whole-mount retina is exposed to 100% O₂, pO₂ within the retina reaches 548 mm Hg.⁵⁶ In contrast, when an animal breathes 100% O₂, pO₂ within the retina only increases to 53 mm Hg.⁹¹ This

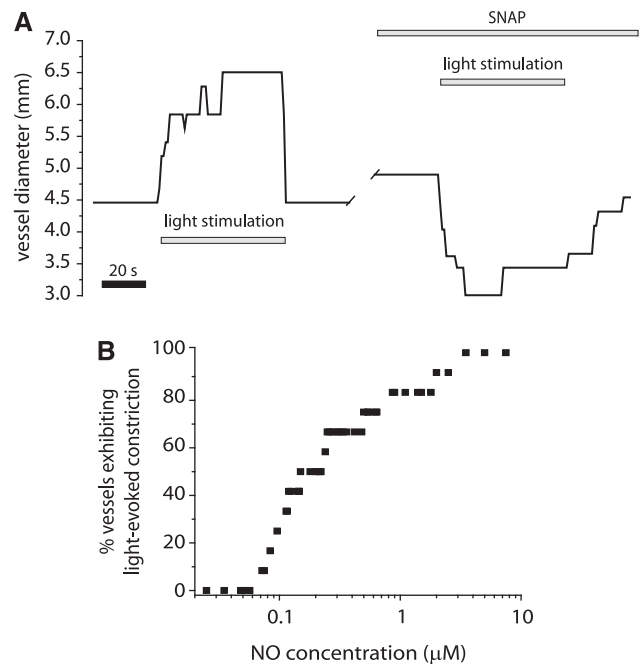


Figure 7. Nitric oxide (NO) depresses light-induced vasodilation and unmasks vasoconstriction in the retina. (A) Flicker stimulation evokes arteriole dilation in the *ex vivo* whole-mount rat retina. When NO levels are raised by the addition of an NO donor (S-nitroso-N-acetylpenicillamine (SNAP)), the vasodilation is depressed, revealing a flicker-induced vasoconstriction. (B) When NO concentration is low, flicker evokes vasodilations but not vasoconstrictions. As NO levels are raised by an NO donor, the fraction of vessels displaying vasoconstrictions increases. From Metea and Newman.⁵⁵

level of pO₂ is not sufficient to block flicker-evoked vasodilation in the *ex vivo* retina.⁵⁶ Thus, although high O₂ does modulate neurovascular coupling and block glial-evoked vasodilation, pO₂ levels in the hyperoxic retina *in vivo* do not rise high enough for this effect to occur.

Nitric Oxide Modulation of Neurovascular Coupling

NO, in addition to being a potent vasodilator, also exerts a strong effect on blood flow by modulating neurovascular coupling in the retina. The modulatory effects of NO have been characterized in the whole-mount rat retina.⁵⁵ When NO levels are <100 nmol/L, flicker-evoked vasodilations, but not vasoconstrictions, are observed. As NO levels are raised, however, vasodilations become smaller and vasoconstrictions more common (Figure 7). At 10 μmol/L NO, large flicker-evoked vasoconstrictions, mediated by 20-HETE, occur. The mechanism by which NO suppresses flicker-evoked vasodilation is not known. The modulatory effect of NO could be due, however, to its inhibition of P450 epoxygenase, which synthesizes the vasodilator EETs. With less EETs being made, vasodilations will be smaller and vasoconstrictions will be unmasked.

As discussed in the following section, NO modulation of neurovascular coupling also occurs *in vivo*. In diabetic retinopathy, increased levels of NO are responsible for suppressing flicker-evoked vasodilation.⁹²

FUNCTIONAL HYPEREMIA IN THE PATHOLOGIC RETINA

Aging

Systematic changes in blood flow are observed as the retina ages. In humans, basal blood volume, velocity, and blood flow all

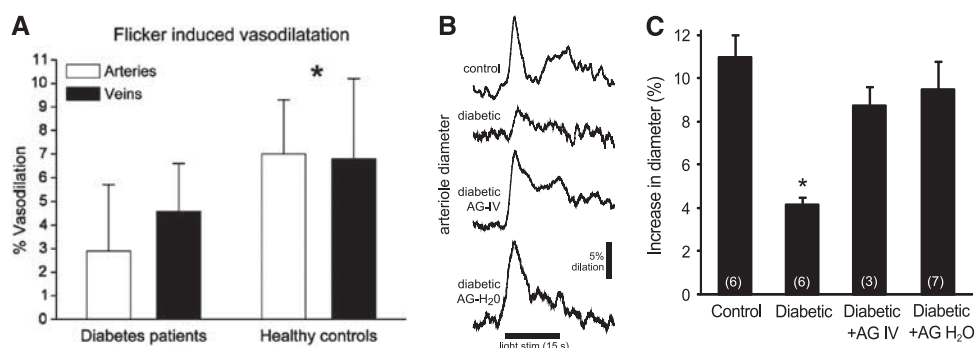


Figure 8. Flicker-induced vasodilation is depressed in diabetic retinopathy. **(A)** Flicker-induced vasodilation of primary arterioles and venules is depressed in patients with type 1 diabetes. $*P < 0.02$. From Pemp *et al.*¹⁰⁷ **(B, C)** The depression of flicker-induced vasodilation in diabetic rats is reversed by the inducible nitric oxide synthase inhibitor aminoguanidine (AG). **(B)** Both acute AG administration (AG-IV) and chronic administration in drinking water (AG-H₂O) reverses the loss of flicker-induced vasodilation in diabetic animals *in vivo*. **(C)** Summary of results. $*P < 0.001$. From Mishra and Newman.²⁹

decrease as subjects age.^{93,94} A reduction in flicker-evoked arteriole dilation is also observed in older subjects, although the effect was not significant.⁹⁵ Multiple factors could account for reductions in basal blood flow and light-evoked increases in blood flow in the aging retina. Age-dependent changes in the responsiveness of vascular smooth muscle cells could have a principal role in the reduction of basal and flicker-evoked blood flow.

Another possible mechanism may involve changes in Ca^{2+} signaling in retinal glial cells, which, as discussed above, mediate the release of vasodilators and vasoconstrictors onto blood vessels. Glial cells in the retina^{96,97} as well as the brain⁹⁸ generate spontaneous intercellular Ca^{2+} waves that propagate through networks of glial cells. These glial Ca^{2+} waves can dilate or constrict retinal arterioles as they propagate past vessels.^{55,96} In the rat, the frequency at which these glial Ca^{2+} waves are generated increases substantially with age.⁹⁶ This increase in glial Ca^{2+} signaling may influence retinal blood flow and contribute to the changes in basal and light-evoked blood flow that are observed as the retina ages.

Diabetic Retinopathy

Changes in both basal blood flow and flicker-evoked increases in blood flow occur during the course of diabetic retinopathy. Both increases and decreases in basal blood flow have been reported (reviewed in Pemp and Schmetterer⁹⁹). An increase in arteriole cross-sectional area and a decrease in blood velocity, leading to a net reduction in blood flow, are seen in patients in early stages of diabetic retinopathy.¹⁰⁰ However, another study showed increased blood flow in the earliest stages of the disease.¹⁰¹ A trend towards increased blood flow is observed with longer duration of diabetes.¹⁰² A decrease in basal blood flow is observed in the rat streptozotocin model of diabetic retinopathy just 2 weeks after induction of diabetes¹⁰³ while a decrease in both retinal and choroidal blood flow is seen in the *Ins2^{Akita}* mouse model of diabetes at 7 months.¹⁰⁴ These inconsistencies in retinal blood flow changes could be due to several factors, including the stage of diabetic retinopathy at which the observations are made, glycemic control, the techniques used to monitor blood flow, and demographic parameters.

Changes in the functional hyperemia response in diabetic patients are less ambiguous. There is a pronounced decrease in flicker-evoked vasodilation in patients in the early stages of diabetic retinopathy^{105–107} (Figure 8A). The loss of vasodilation occurs before overt signs of clinical retinopathy are observed. A similar reduction in flicker-evoked vasodilation is seen in a rat model of type 1 diabetes,⁹² where light-evoked vasodilations are reduced from 11% to 4%²⁹ (Figures 8B and 8C). As in patients, this decrease in functional hyperemia in the rat occurs in the early

stages of retinopathy, before morphologic changes in the vasculature are seen. The loss of the functional hyperemia response observed in both diabetic patients and in an animal model of diabetes could result in retinal hypoxia and may contribute to the development of retinopathy.²⁹

Another change that occurs in the early stages of diabetic retinopathy in animal models of diabetes is an upregulation of inducible NOS (iNOS). iNOS increases are seen in both glial cells and neurons in the retina^{92,108} and result in increased NO levels.^{108,109} As described above, NO, in addition to acting on vessels to induce vasodilation, is a modulator of neurovascular coupling that suppresses flicker-evoked vasodilation.⁵⁵ The upregulation of iNOS and the resulting increase in NO could be responsible for the decrease in flicker-evoked vasodilation observed in diabetic retinopathy. If this is the case, then inhibition of iNOS should result in the restoration of the functional hyperemia response in the diabetic retina.

The effect of inhibiting iNOS has been tested in an animal model of type 1 diabetes. In the rat *ex vivo* whole-mount preparation, administration of aminoguanidine or 1400 W, which are both blockers of iNOS, results in the recovery of flicker-evoked vasodilation in diabetic retinas.⁹² Aminoguanidine, administered acutely by intravenous injection or chronically in the drinking water, restores the functional hyperemia response *in vivo* as well (Figures 8B and 8C).²⁹

Aminoguanidine, by reversing the loss of functional hyperemia, could be a useful therapeutic tool for treating diabetic retinopathy. Indeed, aminoguanidine has been shown to dramatically slow the progression of diabetic retinopathy in animal models of diabetes.^{108–112} A human trial of aminoguanidine also demonstrates a slowing of diabetic retinopathy progression, although the trial was terminated early due to side effects of aminoguanidine when administered at high doses.¹¹³ In addition to inhibiting iNOS, aminoguanidine also blocks formation of advanced glycation endproducts and its beneficial effect on diabetic retinopathy could be due to this effect on advanced glycation endproducts. However, aminoguanidine slows the progression of diabetic retinopathy without affecting advanced glycation endproduct levels,^{111,112} suggesting that it acts by inhibiting iNOS. It remains to be determined whether the beneficial effect of iNOS inhibitors in slowing the progression of diabetic retinopathy is due to the restoration of the functional hyperemia response.

Other Pathologies

Changes in retinal blood flow have been observed in a number of other pathologies, although relatively little research has been done in this area. In patients with open-angle glaucoma, a reduction in basal blood flow in the optic nerve head and retina is

observed.^{114–116} Similarly, both retinal and choroidal blood flow is reduced in the DBA/2 J mouse model of glaucoma.¹¹⁷ Also, in the early stages of the disease, a reduction in flicker-evoked dilation of retinal venules but not arterioles is observed in patients.¹¹⁸ These observations support the hypothesis that the pathologic changes associated with glaucoma may be due, at least in some instances, to retinal ischemia caused by the increase in intraocular pressure and the resulting decrease in perfusion pressure, leading to a decrease in retinal blood flow.¹¹⁹ It should be noted, however, that glaucoma can develop in patients with normal intraocular pressure.^{119,120}

In patients with age-related macular degeneration, reductions in choroidal blood flow, but not retinal flow, have been observed.^{121–124} These reports support the hypothesis that a primary cause of age-related macular degeneration may be a diminished supply of oxygen and nutrients and a decrease in the clearance of waste products from the pigment epithelium and the outer retina caused by reduced choroidal blood flow, possibly arising from stiffening of the sclera and Bruch's membrane due to lipid deposition.¹²⁵

Changes in functional hyperemia occur in hypertensive patients,¹²⁶ where a reduction in flicker-evoked arteriole dilation is seen. In Alzheimer's patients, a narrowing of retinal venules and a reduction in blood flow occurs.¹²⁷ A more detailed characterization of blood flow changes in retinal diseases awaits additional research.

FUTURE DIRECTIONS

Although much is known about blood flow and functional hyperemia in the retinal vasculature, many questions remain to be addressed.

Research during the past decades has implicated many different mechanisms that may mediate neurovascular coupling in the retina. These mechanisms include production of arachidonic acid metabolites by glial cells, release of K⁺ from glial cells and neurons, release of NO, and production of neuronal and glial metabolites, including lactate and adenosine. The relative importance of each of these signaling mechanisms in generating the functional hyperemia response remains to be determined.

Photic stimulation of the retina results in large, rapid dilations of retinal arterioles. These dilations are presumably responsible for generating the increases in blood flow that occur in response to flickering light. However, pericytes, which are intrinsically contractile and surround capillaries, could contribute to generating the functional hyperemia response in the microvasculature. Future research will determine whether capillaries and their associated pericytes actively contribute to generating functional hyperemia.

Reductions in basal blood flow and in flicker-evoked increases in blood flow are observed in aging and in many retinal pathologies. These reductions could result in retinal hypoxia and possibly contribute to the development of retinal pathology. The retina is particularly susceptible to hypoxia caused by compromised blood flow, as it has an extremely high metabolism and O₂ consumption. Arden^{128,129} has proposed that compromised retinal blood flow coupled with the increase in O₂ consumption of photoreceptors in the dark may be a primary cause of diabetic retinopathy. Similarly, the decrease in basal blood flow observed in glaucoma and the resulting hypoxia has been suggested to be a primary cause of retinal damage seen in the disease.¹¹⁵ It remains to be determined whether these changes in retinal blood flow are responsible for the development of diabetes, glaucoma, and other retinal pathologies. An additional challenge will be to determine whether therapies based on reversing reductions in basal blood flow and functional hyperemia are successful in treating these retinal diseases.

DISCLOSURE/CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

The author thanks Gerhard Garhöfer for his helpful comments on the manuscript.

REFERENCES

- 1 Braun RD, Linsenmeier RA, Goldstick TK. Oxygen consumption in the inner and outer retina of the cat. *Invest Ophthalmol Vis Sci* 1995; **36**: 542–554.
- 2 Battered RG, Hinrichsen CF, Weller WL, Haight JR. How thick should a retina be? A comparative study of mammalian species with and without intraretinal vasculature. *Vision Res* 1991; **31**: 169–187.
- 3 Riva CE, Alm A, Pourmaras CJ. Ocular circulation. In: Kaufman PL, Alm A, Levin LA, Nilsson SFE, ver Hoeve J, Wu SM (eds). *Adler's Physiology of the Eye*. Elsevier: Edinburgh, 2011, pp 243–273.
- 4 Alm A, Bill A. Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys (*Macaca irus*): a study with radioactively labelled microspheres including flow determinations in brain and some other tissues. *Exp Eye Res* 1973; **15**: 15–29.
- 5 Linsenmeier RA, Padnick-Silver L. Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. *Invest Ophthalmol Vis Sci* 2000; **41**: 3117–3123.
- 6 Laties AM. Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. *Arch Ophthalmol* 1967; **77**: 405–409.
- 7 Ye XD, Laties AM, Stone RA. Peptidergic innervation of the retinal vasculature and optic nerve head. *Invest Ophthalmol Vis Sci* 1990; **31**: 1731–1737.
- 8 Alm A. The effect of sympathetic stimulation on blood flow through the uvea, retina and optic nerve in monkeys (*Macaca irus*). *Exp Eye Res* 1977; **25**: 19–24.
- 9 Kawarai M, Koss MC. Sympathetic vasoconstriction in the rat anterior choroid is mediated by α_1 -adrenoceptors. *Eur J Pharmacol* 1998; **363**: 35–40.
- 10 Nilsson SF. Nitric oxide as a mediator of parasympathetic vasodilation in ocular and extraocular tissues in the rabbit. *Invest Ophthalmol Vis Sci* 1996; **37**: 2110–2119.
- 11 Grunwald JE, Riva CE, Kozart DM. Retinal circulation during a spontaneous rise of intraocular pressure. *Br J Ophthalmol* 1988; **72**: 754–758.
- 12 Riva CE, Sinclair SH, Grunwald JE. Autoregulation of retinal circulation in response to decrease of perfusion pressure. *Invest Ophthalmol Vis Sci* 1981; **21**: 34–38.
- 13 Bill A. Intraocular pressure and blood flow through the uvea. *Arch Ophthalmol* 1962; **67**: 336–348.
- 14 Riva CE, Titze P, Hero M, Petrig BL. Effect of acute decreases of perfusion pressure on choroidal blood flow in humans. *Invest Ophthalmol Vis Sci* 1997; **38**: 1752–1760.
- 15 Lovasik JV, Kergoat H, Wajszilber MA. Blue flicker modifies the subfoveal choroidal blood flow in the human eye. *Am J Physiol Heart Circ Physiol* 2005; **289**: H683–H691.
- 16 Fuchsjäger-Mayrl G, Polska E, Malec M, Schmetterer L. Unilateral light-dark transitions affect choroidal blood flow in both eyes. *Vision Res* 2001; **41**: 2919–2924.
- 17 Longo A, Geiser M, Riva CE. Subfoveal choroidal blood flow in response to light-dark exposure. *Invest Ophthalmol Vis Sci* 2000; **41**: 2678–2683.
- 18 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.
- 19 Mosso A. Sulla circolazione del sangue nel cervello dell'uomo. *R Accad Lincei* 1880; **5**: 237–358.
- 20 Roy CS, Sherrington CS. On the regulation of the blood-supply of the brain. *J Physiol* 1890; **11**: 85–108.
- 21 Kur J, Newman EA, Chan-Ling T. Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. *Prog Retin Eye Res* 2012; **31**: 377–406.
- 22 Pourmaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res* 2008; **27**: 284–330.
- 23 Werblin FS, Dowling JE. Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J Neurophysiol* 1969; **32**: 339–355.
- 24 Polak K, Schmetterer L, Riva CE. Influence of flicker frequency on flicker-induced changes of retinal vessel diameter. *Invest Ophthalmol Vis Sci* 2002; **43**: 2721–2726.

- 25 Dorner GT, Garhofer G, Kiss B, Polska E, Polak K, Riva CE *et al.* Nitric oxide regulates retinal vascular tone in humans. *Am J Physiol Heart Circ Physiol* 2003; **285**: H631–H636.
- 26 Garhofer G, Zawinka C, Huemer KH, Schmetterer L, Dorner GT. Flicker light-induced vasodilatation in the human retina: effect of lactate and changes in mean arterial pressure. *Invest Ophthalmol Vis Sci* 2003; **44**: 5309–5314.
- 27 Buerk DG, Riva CE, Cranston SD. Frequency and luminance-dependent blood-flow and K^+ ion changes during flicker stimuli in cat optic nerve head. *Invest Ophthalmol Vis Sci* 1995; **36**: 2216–2227.
- 28 Kondo M, Wang L, Bill A. The role of nitric oxide in hyperaemic response to flicker in the retina and optic nerve in cats. *Acta Ophthalmol Scand* 1997; **75**: 232–235.
- 29 Mishra A, Newman EA. Aminoguanidine reverses the loss of functional hyperemia in a rat model of diabetic retinopathy. *Front Neuroener* 2012; **3**: 10.
- 30 Shih YY, Muir ER, Li G, De la Garza BH, Duong TQ. High-resolution 3D MR microangiography of the rat ocular circulation. *Radiology* 2012; **264**: 234–241.
- 31 Garhofer G, Huemer KH, Zawinka C, Schmetterer L, Dorner GT. Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow. *Curr Eye Res* 2002; **24**: 109–113.
- 32 Riva CE, Logean E, Falsini B. Temporal dynamics and magnitude of the blood flow response at the optic disk in normal subjects during functional retinal flicker-stimulation. *Neurosci Lett* 2004; **356**: 75–78.
- 33 Garhofer G, Zawinka C, Resch H, Huemer KH, Dorner GT, Schmetterer L. Diffuse luminance flicker increases blood flow in major retinal arteries and veins. *Vision Res* 2004; **44**: 833–838.
- 34 Buerk DG, Riva CE. Adenosine enhances functional activation of blood flow in cat optic nerve head during photic stimulation independently from nitric oxide. *Microvasc Res* 2002; **64**: 254–264.
- 35 Duong TQ, Ngan SC, Ugurbil K, Kim SG. Functional magnetic resonance imaging of the retina. *Invest Ophthalmol Vis Sci* 2002; **43**: 1176–1181.
- 36 Srienic AI, Kurth-Nelson ZL, Newman EA. Imaging retinal blood flow with laser speckle flowmetry. *Front Neuroener* 2010; **2**: 128.
- 37 Herman IM, D'Amore PA. Microvascular pericytes contain muscle and nonmuscle actins. *J Cell Biol* 1985; **101**: 43–52.
- 38 Hughes S, Chan-Ling T. Characterization of smooth muscle cell and pericyte differentiation in the rat retina *in vivo*. *Invest Ophthalmol Vis Sci* 2004; **45**: 2795–2806.
- 39 Peppiatt CM, Howarth C, Mobbs P, Attwell D. Bidirectional control of CNS capillary diameter by pericytes. *Nature* 2006; **443**: 700–704.
- 40 Puro DG. Physiology and pathobiology of the pericyte-containing retinal microvasculature: new developments. *Microcirculation* 2007; **14**: 1–10.
- 41 Frank RN, Dutta S, Mancini MA. Pericyte coverage is greater in the retinal than in the cerebral capillaries of the rat. *Invest Ophthalmol Vis Sci* 1987; **28**: 1086–1091.
- 42 Frank RN, Turczyn TJ, Das A. Pericyte coverage of retinal and cerebral capillaries. *Invest Ophthalmol Vis Sci* 1990; **31**: 999–1007.
- 43 Yemisci M, Gursay-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med* 2009; **15**: 1031–1037.
- 44 Attwell D, Buchan AM, Chrapak S, Lauritzen M, MacVicar BA, Newman EA. Glial and neuronal control of brain blood flow. *Nature* 2010; **468**: 232–243.
- 45 Leithner C, Roysl G, Offenhauser N, Fuchtemeier M, Kohl-Bareis M, Villringer A *et al.* Pharmacological uncoupling of activation induced increases in CBF and CMRO₂. *J Cereb Blood Flow Metab* 2010; **30**: 311–322.
- 46 Lindauer U, Leithner C, Kaasch H, Rohrer B, Foddis M, Fuchtemeier M *et al.* Neurovascular coupling in rat brain operates independent of hemoglobin deoxygenation. *J Cereb Blood Flow Metab* 2010; **30**: 757–768.
- 47 Mintun MA, Lundstrom BN, Snyder AZ, Vlassenko AG, Shulman GL, Raichle ME. Blood flow and oxygen delivery to human brain during functional activity: theoretical modeling and experimental data. *Proc Natl Acad Sci USA* 2001; **98**: 6859–6864.
- 48 Powers WJ, Hirsch IB, Cryer PE. Effect of stepped hypoglycemia on regional cerebral blood flow response to physiological brain activation. *Am J Physiol* 1996; **270**: H554–H559.
- 49 Astrup J, Heuser D, Lassen NA, Nilsson B, Norberg K, Siesjo BK. Evidence against H^+ and K^+ as main factors for the control of cerebral blood flow: a microelectrode study. In: Ciba Foundation Symposium (ed). *Cerebral Vascular Smooth Muscle and its Control*. Elsevier Scientific: Amsterdam, The Netherlands, 1978, pp 313–333.
- 50 Makani S, Chesler M. Rapid rise of extracellular pH evoked by neural activity is generated by the plasma membrane calcium ATPase. *J Neurophysiol* 2010; **103**: 667–676.
- 51 Cajal SRy. *Histology of the Nervous system*. Oxford: New York, NY, USA, 1995, pp 1–805.
- 52 Kacem K, Lacombe P, Seylaz J, Bonvento G. Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 1998; **23**: 1–10.
- 53 Dreher Z, Wegner M, Stone J. Muller cell endfeet at the inner surface of the retina: light microscopy. *Vis Neurosci* 1988; **1**: 169–180.
- 54 Newman EA. Calcium increases in retinal glial cells evoked by light-induced neuronal activity. *J Neurosci* 2005; **25**: 5502–5510.
- 55 Metea MR, Newman EA. Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J Neurosci* 2006; **26**: 2862–2870.
- 56 Mishra A, Hamid A, Newman EA. Oxygen modulation of neurovascular coupling in the retina. *Proc Natl Acad Sci USA* 2011; **108**: 17827–17831.
- 57 Pournaras C, Tsacopoulos M, Chapuis P. Studies on the role of prostaglandins in the regulation of retinal blood flow. *Exp Eye Res* 1978; **26**: 687–697.
- 58 Weigert G, Berisha F, Resch H, Karl K, Schmetterer L, Garhofer G. Effect of unspecific inhibition of cyclooxygenase by indomethacin on retinal and choroidal blood flow. *Invest Ophthalmol Vis Sci* 2008; **49**: 1065–1070.
- 59 Brazitikos PD, Pournaras CJ, Munoz JL, Tsacopoulos M. Microinjection of L-lactate in the preretinal vitreous induces segmental vasodilation in the inner retina of miniature pigs. *Invest Ophthalmol Vis Sci* 1993; **34**: 1744–1752.
- 60 Parys-Van Ginderdeuren R, Malcolm D, Varma DR, Aranda JV, Chemtob S. Dissociation between prostaglandin levels and blood flow to the retina and choroid in the newborn pig after nonsteroidal antiinflammatory drugs. *Invest Ophthalmol Vis Sci* 1992; **33**: 3378–3384.
- 61 Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW *et al.* Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat Neurosci* 2006; **9**: 1397–1403.
- 62 Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R546–R552.
- 63 Bunger R, Haddy FJ, Querengasser A, Gerlach E. Studies on potassium induced coronary dilation in the isolated guinea pig heart. *Pflugers Arch* 1976; **363**: 27–31.
- 64 Haddy FJ. Potassium effects on contraction in arterial smooth muscle mediated by Na^+ , K^+ -ATPase. *FASEB J* 1983; **42**: 239–243.
- 65 Dick E, Miller RF, Bloomfield S. Extracellular K^+ activity changes related to electroretinogram components. II. Rabbit (E-type) retinas. *J Gen Physiol* 1985; **85**: 911–931.
- 66 Dick E, Miller RF. Extracellular K^+ activity changes related to electroretinogram components. I. Amphibian (I-type) retinas. *J Gen Physiol* 1985; **85**: 885–909.
- 67 Karwoski CJ, Newman EA, Shimazaki H, Proenza LM. Light-evoked increases in extracellular K^+ in the plexiform layers of amphibian retinas. *J Gen Physiol* 1985; **86**: 189–213.
- 68 Newman EA, Frambach DA, Odette LL. Control of extracellular potassium levels by retinal glial cell K^+ siphoning. *Science* 1984; **225**: 1174–1175.
- 69 Paulson OB, Newman EA. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science* 1987; **237**: 896–898.
- 70 Newman EA. Regional specialization of retinal glial cell membrane. *Nature* 1984; **309**: 155–157.
- 71 Newman EA. Distribution of potassium conductance in mammalian Müller (glial) cells: a comparative study. *J Neurosci* 1987; **7**: 2423–2432.
- 72 Metea MR, Kofuji P, Newman EA. Neurovascular coupling is not mediated by potassium siphoning from glial cells. *J Neurosci* 2007; **27**: 2468–2471.
- 73 Kofuji P, Ceelen PW, Zahs KR, Surbeck LW, Lester HA, Newman EA. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. *J Neurosci* 2000; **20**: 5733–5740.
- 74 Newman EA. Voltage-dependent calcium and potassium channels in retinal glial cells. *Nature* 1985; **317**: 809–811.
- 75 Dunn KM, Nelson MT. Potassium channels and neurovascular coupling. *Circ J* 2010; **74**: 608–616.
- 76 Gebremedhin D, Yamaura K, Zhang C, Bylund J, Koehler RC, Harder DR. Metabotropic glutamate receptor activation enhances the activities of two types of Ca^{2+} -activated K^+ channels in rat hippocampal astrocytes. *J Neurosci* 2003; **23**: 1678–1687.
- 77 Yang G, Zhang Y, Ross ME, Iadecola C. Attenuation of activity-induced increases in cerebellar blood flow in mice lacking neuronal nitric oxide synthase. *Am J Physiol Heart Circ Physiol* 2003; **285**: H298–H304.
- 78 Feletou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* 2006; **26**: 1215–1225.
- 79 Donati G, Pournaras CJ, Munoz JL, Poitry S, Poitry-Yamate CL, Tsacopoulos M. Nitric oxide controls arteriolar tone in the retina of the miniature pig. *Invest Ophthalmol Vis Sci* 1995; **36**: 2228–2237.
- 80 Lindauer U, Megow D, Matsuda H, Dirnagl U. Nitric oxide: a modulator, but not a mediator, of neurovascular coupling in rat somatosensory cortex. *Am J Physiol Heart Circ Physiol* 1999; **277**: H799–H811.
- 81 Ma J, Ayata C, Huang PL, Fishman MC, Moskowitz MA. Regional cerebral blood flow response to vibrissal stimulation in mice lacking type I NOS gene expression. *Am J Physiol Heart Circ Physiol* 1996; **270**: H1085–H1090.
- 82 Ames III A, Li YY, Heher EC, Kimble CR. Energy metabolism of rabbit retina as related to function: high cost of Na^+ transport. *J Neurosci* 1992; **12**: 840–853.

- 83 Wang L, Bill A. Effects of constant and flickering light on retinal metabolism in rabbits. *Acta Ophthalmol Scand* 1997; **75**: 227–231.
- 84 Hein TW, Xu W, Kuo L. Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. *Invest Ophthalmol Vis Sci* 2006; **47**: 693–699.
- 85 Hiraio M, Oku H, Goto W, Sugiyama T, Kobayashi T, Ikeda T. Effects of adenosine on optic nerve head circulation in rabbits. *Exp Eye Res* 2004; **79**: 729–735.
- 86 Matsugi T, Chen Q, Anderson DR. Adenosine-induced relaxation of cultured bovine retinal pericytes. *Invest Ophthalmol Vis Sci* 1997; **38**: 2695–2701.
- 87 Hickam JB, Frayser R. Studies of the retinal circulation in man: observations on vessel diameter, arteriovenous oxygen difference, and mean circulation time. *Circulation* 1966; **33**: 302–316.
- 88 Luksch A, Garhofer G, Imhof A, Polak K, Polska E, Dorner GT *et al*. Effect of inhalation of different mixtures of O₂ and CO₂ on retinal blood flow. *Br J Ophthalmol* 2002; **86**: 1143–1147.
- 89 Eperon G, Johnson M, David NJ. The effect of arterial P_{O2} on relative retinal blood flow in monkeys. *Invest Ophthalmol* 1975; **14**: 342–352.
- 90 Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, MacVicar BA. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* 2008; **456**: 745–749.
- 91 Yu DY, Cringle SJ, Alder VA, Su EN. Intraretinal oxygen distribution in rats as a function of systemic blood pressure. *Am J Physiol* 1994; **267**: H2498–H2507.
- 92 Mishra A, Newman EA. Inhibition of inducible nitric oxide synthase reverses the loss of functional hyperemia in diabetic retinopathy. *Glia* 2010; **58**: 1996–2004.
- 93 Embleton SJ, Hosking SL, Roff Hilton EJ, Cunliffe IA. Effect of senescence on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry. *Eye* 2002; **16**: 156–162.
- 94 Grunwald JE, Piltz J, Patel N, Bose S, Riva CE. Effect of aging on retinal macular microcirculation: a blue field simulation study. *Invest Ophthalmol Vis Sci* 1993; **34**: 3609–3613.
- 95 Nagel E, Vilser W, Lanzl I. Age, blood pressure, and vessel diameter as factors influencing the arterial retinal flicker response. *Invest Ophthalmol Vis Sci* 2004; **45**: 1486–1492.
- 96 Kurth-Nelson ZL, Mishra A, Newman EA. Spontaneous glial calcium waves in the retina develop over early adulthood. *J Neurosci* 2009; **29**: 11339–11346.
- 97 Newman EA, Zahs KR. Calcium waves in retinal glial cells. *Science* 1997; **275**: 844–847.
- 98 Mathiesen C, Brazhe A, Thomsen K, Lauritzen M. Spontaneous calcium waves in Bergman glia increase with age and hypoxia and may reduce tissue oxygen. *J Cereb Blood Flow Metab* 2013; **33**: 161–169.
- 99 Pemp B, Schmetterer L. Ocular blood flow in diabetes and age-related macular degeneration. *Can J Ophthalmol* 2008; **43**: 295–301.
- 100 Feke GT, Buzney SM, Ogasawara H, Fujio N, Goger DG, Spack NP *et al*. Retinal circulatory abnormalities in type 1 diabetes. *Invest Ophthalmol Vis Sci* 1994; **35**: 2968–2975.
- 101 Grunwald JE, DuPont J, Riva CE. Retinal haemodynamics in patients with early diabetes mellitus. *Br J Ophthalmol* 1996; **80**: 327–331.
- 102 Konno S, Feke GT, Yoshida A, Fujio N, Goger DG, Buzney SM. Retinal blood flow changes in type I diabetes. A long-term follow-up study. *Invest Ophthalmol Vis Sci* 1996; **37**: 1140–1148.
- 103 Higashi S, Clermont AC, Dhir V, Bursell SE. Reversibility of retinal flow abnormalities is disease-duration dependent in diabetic rats. *Diabetes* 1998; **47**: 653–659.
- 104 Muir ER, Renteria RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. *Invest Ophthalmol Vis Sci* 2012; **53**: 6488–6494.
- 105 Garhofer G, Zawinka C, Resch H, Kothly P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. *Br J Ophthalmol* 2004; **88**: 887–891.
- 106 Mandecka A, Dawczynski J, Blum M, Muller N, Kloos C, Wolf G *et al*. Influence of flickering light on the retinal vessels in diabetic patients. *Diabetes Care* 2007; **30**: 3048–3052.
- 107 Pemp B, Garhofer G, Weigert G, Karl K, Resch H, Wolzt M *et al*. Reduced retinal vessel response to flicker stimulation but not to exogenous nitric oxide in type 1 diabetes. *Invest Ophthalmol Vis Sci* 2009; **50**: 4029–4032.
- 108 Du Y, Smith MA, Miller CM, Kern TS. Diabetes-induced nitrate stress in the retina, and correction by aminoguanidine. *J Neurochem* 2002; **80**: 771–779.
- 109 Kowluru RA, Engerman RL, Kern TS. Abnormalities of retinal metabolism in diabetes or experimental galactosemia VIII. Prevention by aminoguanidine. *Curr Eye Res* 2000; **21**: 814–819.
- 110 Hammes HP, Martin S, Federlin K, Geisen K, Brownlee M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci USA* 1991; **88**: 11555–11558.
- 111 Kern TS, Engerman RL. Pharmacological inhibition of diabetic retinopathy: aminoguanidine and aspirin. *Diabetes* 2001; **50**: 1636–1642.
- 112 Kern TS, Tang J, Mizutani M, Kowluru RA, Nagaraj RH, Romeo G *et al*. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. *Invest Ophthalmol Vis Sci* 2000; **41**: 3972–3978.
- 113 Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K *et al*. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004; **24**: 32–40.
- 114 Findl O, Rainer G, Dallinger S, Dorner G, Polak K, Kiss B *et al*. Assessment of optic disk blood flow in patients with open-angle glaucoma. *Am J Ophthalmol* 2000; **130**: 589–596.
- 115 Michelson G, Langhans MJ, Groh MJ. Perfusion of the juxtapapillary retina and the neuroretinal rim area in primary open angle glaucoma. *J Glaucoma* 1996; **5**: 91–98.
- 116 Wang Y, Fawzi AA, Varma R, Sadun AA, Zhang X, Tan O *et al*. Pilot study of optical coherence tomography measurement of retinal blood flow in retinal and optic nerve diseases. *Invest Ophthalmol Vis Sci* 2011; **52**: 840–845.
- 117 Lavery WJ, Muir ER, Kiel JW, Duong TQ. Magnetic resonance imaging indicates decreased choroidal and retinal blood flow in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 2012; **53**: 560–564.
- 118 Garhofer G, Zawinka C, Resch H, Huemer KH, Schmetterer L, Dorner GT. Response of retinal vessel diameters to flicker stimulation in patients with early open angle glaucoma. *J Glaucoma* 2004; **13**: 340–344.
- 119 Flammer J, Orgul S, Costa VP, Orzalesi N, Kriegelstein GK, Serra LM *et al*. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res* 2002; **21**: 359–393.
- 120 Caprioli J, Coleman AL. Blood pressure, perfusion pressure, and glaucoma. *Am J Ophthalmol* 2010; **149**: 704–712.
- 121 Ciulla TA, Harris A, Chung HS, Danis RP, Kagemann L, McNulty L *et al*. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. *Am J Ophthalmol* 1999; **128**: 75–80.
- 122 Grunwald JE, Metelitsina TI, DuPont JC, Ying GS, Maguire MG. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. *Invest Ophthalmol Vis Sci* 2005; **46**: 1033–1038.
- 123 Schmetterer L, Kruger A, Findl O, Breiteneder H, Eichler HG, Wolzt M. Topical fundus pulsation measurements in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 1998; **236**: 160–163.
- 124 Uretmen O, Akkin C, Erakgun T, Killi R. Color Doppler imaging of choroidal circulation in patients with asymmetric age-related macular degeneration. *Ophthalmologica* 2003; **217**: 137–142.
- 125 Friedman E, Krupsky S, Lane AM, Oak SS, Friedman ES, Egan K *et al*. Ocular blood flow velocity in age-related macular degeneration. *Ophthalmology* 1995; **102**: 640–646.
- 126 Riva CE, Salgarello T, Logean E, Colotto A, Galan EM, Falsini B. Flicker-evoked response measured at the optic disc rim is reduced in ocular hypertension and early glaucoma. *Invest Ophthalmol Vis Sci* 2004; **45**: 3662–3668.
- 127 Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. *Invest Ophthalmol Vis Sci* 2007; **48**: 2285–2289.
- 128 Arden GB. The absence of diabetic retinopathy in patients with retinitis pigmentosa: implications for pathophysiology and possible treatment. *Br J Ophthalmol* 2001; **85**: 366–370.
- 129 Arden GB, Sivaprasad S. The pathogenesis of early retinal changes of diabetic retinopathy. *Doc Ophthalmol* 2012; **124**: 15–26.
- 130 Anand-Apte B, Hollyfield JG. Developmental anatomy of the retinal and choroidal vasculature. In: Besharse J, Bok D (eds). *Encyclopedia of the Eye*. Academic Press, Elsevier Books: London, UK, 2010, pp 9–15.
- 131 Nagel E, Vilser W. Flicker observation light induces diameter response in retinal arterioles: a clinical methodological study. *Br J Ophthalmol* 2004; **88**: 54–56.