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Retinal Oxygen: from animals to humans

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Abstract

This article discusses retinal oxygenation and retinal metabolism by focusing on measurements made with two of the principal methods used to study O₂ in the retina: measurements of PO₂ with oxygen-sensitive microelectrodes *in vivo* in animals with a retinal circulation similar to that of humans, and oximetry, which can be used non-invasively in both animals and humans to measure O₂ concentration in retinal vessels. Microelectrodes uniquely have high spatial resolution, allowing the mapping of PO₂ in detail, and when combined with mathematical models of diffusion and consumption, they provide information about retinal metabolism. Mathematical models, grounded in experiments, can also be used to simulate situations that are not amenable to experimental study. New methods of oximetry, particularly photoacoustic ophthalmoscopy and visible light optical coherence tomography, provide depth-resolved methods that can separate signals from blood vessels and surrounding tissues, and can be combined with blood flow measures to determine metabolic rate. We discuss the effects on retinal oxygenation of illumination, hypoxia and hyperoxia, and describe retinal oxygenation in diabetes, retinal detachment, arterial occlusion, and macular degeneration. We explain how the metabolic measurements obtained from microelectrodes and imaging are different, and how they need to be brought together in the future. Finally, we argue for revisiting the clinical use of hyperoxia in ophthalmology, particularly in retinal arterial occlusions and retinal detachment, based on animal research and diffusion theory.

Keywords

oxygen; retina; retinal metabolism; animal; human; oxygen microelectrode; oximetry; hypoxia; hyperoxia; diabetes; retinal detachment; macular degeneration

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1. Importance of the oxygen supply to the retina

Like other parts of the nervous system, the retina requires a continuous supply of oxygen. If the circulation is completely cut off by a sudden increase of intraocular pressure (IOP) to levels above systemic arterial pressure, vision in humans stops in only 4 – 9 seconds (Anderson and Saltzman, 1964; Carlisle et al., 1964). The fact that this can be slightly prolonged by inspiration of pure O₂ (at 1 atmosphere absolute pressure – ATA) to 6 – 12 sec, or to 16 – 25 sec with O₂ at 2 ATA (Anderson and Saltzman, 1964; Carlisle et al., 1964), indicates that it must have been O₂ that was the limiting factor. Loss of vision during IOP elevation only reveals the limitation set by the most sensitive part of the retinal pathway, and does not indicate that all retinal function was arrested. Noell (Noell, 1951) raised IOP in his own eye, and found that he lost vision in response to a very bright flash in about 50 sec, but the b-wave of the ERG was still about half its normal amplitude at this time. He and others showed that the ERG a-wave can last for at least several minutes in primates and other animals (Fujino and Hamasaki, 1965; Noell, 1951) that are breathing air.

Complete occlusion of the entire ocular circulation is unusual clinically, but central or branch retinal artery occlusion occurs more frequently. In those conditions vision is also lost quickly, but complete recovery of retinal function, assessed by the ERG b-wave and histology, can occur after 100 min of ischemia in primates (Hayreh and Weingeist, 1980; Hayreh et al., 2004). Thus, the retina survives ischemia better than the brain. Somewhat paradoxically, it is also more susceptible to systemic hypoxia than most of the CNS, with vision being one of the first physiological functions to be impaired at high altitude or in other situations of systemic hypoxia (Hecht et al., 1946; Kobrick, 1974; McFarland and Evans, 1939; McFarland and Halperin, 1940).

In order to understand why the retina is different from the brain in ischemic and hypoxic situations, and how ischemia and hypoxia might be relevant to retinal diseases, it is important to start with normal conditions and appreciate several unique aspects of retinal physiology. These include i) the roles of the retinal and choroidal circulations in providing O₂ to the retina, ii) the roles of oxidative metabolism and anaerobic glycolysis, iii) the changes in retinal oxygenation that occur during hyperoxia, and iv) the influence of light on retinal metabolism. Research on animals and humans has explored many of these basic aspects of retinal oxygenation, and has also provided considerable knowledge about the role of O₂ in diabetes, retinal detachment, vascular occlusion, retinopathy of prematurity, and retinitis pigmentosa. However, there are still questions about all of these diseases, in terms of mechanisms, progression, and potential treatments, and our understanding of the role of O₂ in glaucoma and age-related macular degeneration is still very limited.

Measurements in animals provide context for the newer non-invasive measurements that are now possible in humans. This review will focus on i) what we know about retinal oxygenation in normal and pathological conditions from measurements in animals and humans, ii) how we are moving toward new ways of understanding retinal oxygenation and metabolism in humans, iii) what we can predict in situations where measurements are not possible, and iv) what we still do not know and how we might learn it. It is not a review of ocular blood flow, although this will be brought in as needed, but concentrates on O₂

measurements themselves, particularly those made in mammals with a holangiotic retina, that is, one having a complete retinal circulation as in humans. Retinal O₂ is an enormous topic, and no review can completely cover it. There are several excellent sources for information on the related topic of ocular blood flow (Pemp and Schmetterer, 2008; Pournaras et al., 2008; Schmetterer and Kiel, 2012), and the reader is also referred to earlier reviews for different aspects of retinal oxygenation and metabolism (Hardarson, 2013; Linsenmeier, 2010, 2012; Wangsa-Wirawan and Linsenmeier, 2003; Yu and Cringle, 2001). Yu and Cringle (Yu and Cringle, 2001) paid particular attention to avascular retinas. There are also several reviews concerning the known and putative roles of O₂ in disease (Arjamaa and Nikinmaa, 2006; Campochiaro, 2013, 2015; Caprara and Grimm, 2012; Lange and Bainbridge, 2012; Osborne et al., 2004; Pournaras, 1995; Stefansson, 1990; Stefansson et al., 2011), some of which relate O₂ levels to molecular changes more than will be attempted here.

2. Approaches to retinal oxygen

One can take several approaches to measuring O₂ in the retina *in vivo* (Harris et al., 2003; Hogeboom van Buggenum et al., 1996; Linsenmeier, 2012). These include i) measurements of phosphorescence lifetime of an intravascular (or more recently extravascular) porphyrin-based dye whose lifetime is inversely proportional to the local PO₂ (Shahidi et al., 2006; Shahidi et al., 2010), ii) MRI measurements of relative PO₂ (Berkowitz, 1996; Berkowitz et al., 2001; Cheng et al., 2006), iii) optical probes using dyes embedded on the surface of a fiberoptic probe (Holekamp et al., 2005; Stefansson et al., 1989), and iv) histological measurements with a chemical probe, pimonidazole, that labels hypoxic regions (de Gooyer et al., 2006; Wright et al., 2010). There have also been many studies designed to measure O₂ utilization *in vitro* (e.g. (Ames et al., 1992; Winkler, 1995)), but these generally have not had any spatial resolution, and could only characterize the metabolism of the entire retina. This review will incorporate results from many of these methods, but it will focus on the use of O₂-sensitive microelectrodes, which uniquely have high spatial resolution, and on oximetry to measure hemoglobin saturation, which uniquely has the ability to measure O₂ levels in the retinal vasculature of humans.

2.1. Oxygen microelectrodes

Oxygen microelectrodes work by reducing O₂ at the surface of a gold or platinum electrode (cathode) when a negative voltage is applied. This generates a current that is proportional to the flux of O₂ from the medium to the electrode surface, because the electrode is operated at a voltage at which all the O₂ is reduced and the PO₂ at the cathode is zero. This also makes the electrode current proportional to the PO₂ in the medium. The principle is the same as that used in all polarographic O₂ measurements, for instance those in blood gas analyzers (Buerk, 2004). The electrode current is also proportional to the surface area of the cathode, so there is an advantage in keeping the electrode small to limit its disturbance of the PO₂ in the local environment. The microelectrodes used by our group and others (e.g. (Buerk et al., 1993; Pournaras et al., 1989; Riva et al., 1986; Tsacopoulos and Lehmenkuhler, 1977; Yu and Cringle, 2001)) have a cathode slightly recessed into the glass micropipette and are called Whalen electrodes (Whalen et al., 1967). They have high spatial resolution

(approximately 1 μm) (Schneiderman and Goldstick, 1978), which is important because retinal PO_2 varies on that spatial scale. The only other method with potentially high spatial resolution is the use of pimonidazole, but this allows only a single histological measurement from an animal, so it is not suitable for understanding O_2 dynamics. In addition, its PO_2 resolution is considered to be binary (i.e., absence of pimonidazole adducts above approximately 10 mm Hg and presence of the adducts below 10 mmHg) (Arteel et al., 1995; Raleigh et al., 1996). The temporal response of oxygen microelectrodes is also very fast, a few msec (Schneiderman and Goldstick, 1978), which is much faster than any PO_2 changes due to metabolism. Oxygen electrodes do consume O_2 , but at a current of 6 pA, which is typical, one can calculate that this is only about the amount that one retinal neuron uses (Linsenmeier, 2012), so they are not thought to markedly disturb tissue PO_2 . Because O_2 is normally the only substance that undergoes net reduction in tissue, the current is purely due to O_2 , unlike electrodes that operate in the oxidizing direction, where one has to separate signals from several compounds that are oxidized at the electrode (e.g. NO, ascorbic acid, and catecholamines). The behavior of O_2 electrodes does not depend on light, and they can be used in the dark-adapted retina, unlike methods such as oximetry that require illumination. The microelectrodes we use are like ion-selective electrodes in having a second barrel that is used to record local voltages (Linsenmeier and Yancey, 1987). This allows recording of the local electroretinogram (ERG) in response to flashes, which provides a good indication of retinal depth (Brown, 1968; Lau and Linsenmeier, 2012; Steinberg et al., 1980) and whether the retina is healthy. The disadvantages of microelectrodes are that they require an invasive approach, require calibration outside the eye, and can only sample from a small number of locations in one animal. In our application, the electrode is inserted through a needle in the wall of the eye, and is driven through the vitreous, striking the retina at an angle of about 45° to the surface. We generally penetrate the retina in steps of 3 μm , recording the ERG b- and c-waves at 30 μm intervals until the electrode crosses the retinal pigment epithelium (RPE) and the local ERG is dramatically reduced. The short duration flashes used to evoke ERGs are below rod saturation and allow the retina to remain dark-adapted. The microelectrode is then withdrawn steadily at 2 $\mu\text{m}/\text{sec}$ to the vitreous, generating a graph of PO_2 vs. depth, called a profile. Our convention is to put the choroid at the left and the vitreous at the right of these graphs. Figure 1 shows examples of oxygen profiles from different animals, which will be described below.

2.2. Diffusion modeling

By fitting mathematical models of retinal O_2 diffusion and consumption to the PO_2 profiles it is possible to derive values for O_2 utilization in vivo. The models generally apply only to the outer, avascular retina, because the mathematics are more tractable, although full retinal models are considered below. The avascular retina can reasonably be assumed to be a slab of tissue that consumes O_2 , with the supply of O_2 coming from the boundaries, one at the choroid and the other at the deep capillary layer of the retinal circulation. Under these conditions it is possible to use the simplest one-dimensional steady state form of the diffusion equation, with O_2 diffusing along the photoreceptors, and no substantial diffusion component perpendicular to them:

$$QO_2 = Dk \left(d^2 PO_2 / dx^2 \right) \quad (1)$$

Where x is the dimension representing retinal depth, D is the O_2 diffusion coefficient ($1.97 \times 10^{-5} \text{ cm}^2/\text{sec}$ in the retina (Roh et al., 1990)), k is O_2 solubility ($2.4 \times 10^{-5} \text{ ml } O_2\text{-ml tissue}^{-1} \text{-mmHg}^{-1}$ (Linsenmeier and Braun, 1992)), and QO_2 is oxygen utilization per unit weight of tissue ($\text{ml } O_2\text{-}100\text{g}^{-1}\text{-min}^{-1}$). The first modeling of the outer retina was done by Dollery et al (Dollery et al., 1969), but it treated the outer retina as a single layer with uniform QO_2 , which does not fit the observed oxygen profiles. Instead, it is better to divide the outer retina into three layers, because only part of it consumes O_2 . The structure of the three layer model is shown in Figure 2. This model was developed without assuming that three layers would be ideal (Haugh et al., 1990), but two layers was not enough, and four did not offer any improvement in fits. A three layer model for the outer retina does make sense. There are no mitochondria in layer 1, which we interpret as outer segments, or layer 3, which corresponds to the outer nuclear layer, so these layers do not consume O_2 . PO_2 decreases through these layers linearly as can be appreciated in Figure 1, toward layer 2, which does have O_2 consumption, called Q_2 . This corresponds to photoreceptor inner segments, where all the mitochondria (except those in the synapse) are located. The details of this model have been presented previously (e.g. (Haugh et al., 1990; Lau and Linsenmeier, 2012; Linsenmeier and Braun, 1992)), so the solution of equation 1, i.e. PO_2 vs. distance, will not be repeated here. It has been used to fit measured PO_2 profiles in rat (Lau and Linsenmeier, 2012), cat (Braun and Linsenmeier, 1995; Linsenmeier and Braun, 1992; Padnick-Silver et al., 2006a; Yancey and Linsenmeier, 1989), and macaque (Birol et al., 2007).

The model supplements the microelectrode measurements in several important ways. Because it is grounded in measurements, it is an accurate description of O_2 diffusion and consumption, and allows us to obtain parameters and draw conclusions that we would not otherwise be able to make. First, it is the only way to obtain a value for the O_2 consumption of the inner segments *in vivo*. Second, it allows us to obtain a value for the O_2 consumption of the outer retina as a whole, which can be compared to measurements made by various methods *in vitro* or with the Fick Principle (section 2.3) *in vivo*. We have called this overall outer retinal O_2 consumption Q_{av} or Q_{OR} previously, but here we call it $OR-QO_2$ for reasons that will be apparent shortly. $OR-QO_2$ is a weighted average of QO_2 of the different layers, which can be shown to be

$$OR - QO_2 = Q_2 \cdot (L_2 - L_1) / L \quad (2)$$

where L_1 and L_2 are the boundaries of layer 2 and L is the thickness of the avascular outer retina. We have also shown that $OR-QO_2$ is less sensitive than Q_2 to the quality of the PO_2 profiles and to fitting, and has a smaller variance than Q_2 (Haugh et al., 1990; Linsenmeier and Pournaras, 2008). The third reason that the modeling is useful is that allows us to determine the fractions of O_2 supplied to the photoreceptors by the retinal circulation and

the choroidal circulation. This amounts to computing the flux of O_2 through layer 3 compared to the flux through layer 1. The fraction of O_2 used in layer 2 that comes from the retinal circulation is

$$Fr = 100 \cdot [(L_1 + L_2) / 2L + (P_L - P_C) \cdot Dk / (Q_2 O_2 \cdot L^2)] \quad (3)$$

where P_L and P_C are the PO_2 s at the inner boundary of the avascular layer and at the choriocapillaris, respectively. Fourth, the model gives us a compact way to compare parameters across different conditions, for example light vs. dark, or normoxia vs. hypoxia, as well as across different animals. It is important to realize that this model applies only to the outer retina, and that the QO_2 of the photoreceptor synapses, which are at the border of the inner and outer retina, is not included in this model.

In the inner retina the gradients of O_2 are three dimensional, due to the presence of capillaries, and there is not a separation of supply and demand into a simple one-dimensional geometry with supply at the boundaries and consumption in the tissue between the boundaries, as there is in the outer retina. The inner retinal O_2 supply is three-dimensional, and an appropriate model would have to be three dimensional as well. The one-dimensional PO_2 gradients obtained with a microelectrode are therefore normally insufficient to derive values of QO_2 in the inner retina. An attempt has been made to extend the three layer model in rat retina by adding 5 additional layers (Cringle and Yu, 2002), assuming that the vasculature is well behaved and can be identified with two discrete layers, but because the model and measurements are still one-dimensional, it is difficult to place much confidence in this model.

Another way to extend the model into the inner retina is to occlude the retinal circulation and supply all the O_2 needed from the choroid, eliminating the complex geometry of inner retinal O_2 supply. In that case only one additional layer proved to be necessary, and it was possible to derive a value for inner retinal QO_2 , called Q_{IR} (Braun et al., 1995), or IR- QO_2 in our present terminology. This is not a perfect solution, however, because, while we and others (see Section 7) have been able to supply O_2 to the entire inner retina during vascular occlusion, the ERG was only half of normal during occlusion and 100% O_2 inspiration (Braun and Linsenmeier, 1995). In addition, the inner retina was unusually acidic under these conditions (Birol et al., 2005), probably because there was no clearance of acid by the retinal circulation, so the values of IR- QO_2 derived during occlusion may not be entirely normal. To address the limitations of these approaches, we believe that it may be possible to obtain a three-dimensional (3-D) O_2 map of the inner retina under normal conditions, and to fit a 3-D model to the data, but this remains to be shown.

The models just described are the basis for other O_2 models used to derive physiological parameters (Cringle et al., 1996; Cringle and Yu, 2002, 2004; Cringle et al., 2002; Haugh-Scheidt et al., 1995b). They can also be used for predictive simulations, as described in later sections.

Before leaving diffusion models, however, two other points should be discussed. First, in our models, QO_2 is treated as zero order, that is, it is independent of PO_2 . In other work, QO_2 is allowed to vary with PO_2 in a first order approximation or stepwise, with a small half maximal PO_2 of 1 or 2 mmHg (Roberts et al., 2015; Roos, 2004, 2006). While undoubtedly more accurate, the models fit the data well without this, and it makes no real difference to the parameters derived from modeling. Second, the retina contains the putative O_2 carrier neuroglobin, as do other neural tissues (Schmidt et al., 2003), and it was hypothesized that this would enhance retinal O_2 transport. Subsequent calculations suggested that neuroglobin would be unlikely to serve as a significant reservoir of O_2 , and it may have a function related to apoptosis instead (Fago et al., 2004; Fago et al., 2008). Roberts et al. (Roberts et al., 2015), however, have done modeling to show some enhancement of O_2 transport in regions of the retina with very low PO_2 . One can see some theoretical effects, but the differences with and without neuroglobin are small, and fitting models to data is not likely to resolve the role of neuroglobin in retinal oxygen transport. In the steady state models, the three parameters QO_2 , D , and k always occurs as QO_2/Dk , and, to derive QO_2 itself, we multiply QO_2/Dk by a measured value of D , and an assumed solubility, k . If the actual effective value of k is higher, due to binding of O_2 to neuroglobin, then the values of QO_2 , that we have reported would be a few percent too low.

2.3. Fick Principle

Fitting diffusion models to PO_2 data can provide values for QO_2 in animals, but it cannot be done in humans, and imaging, the other principal measure discussed here, relies on a different model. As for any tissue, this model is the Fick Principle, which is a mass balance on O_2 . If one knows the total amount of O_2 entering the retina per unit time in the arteries and the total amount leaving in the veins, the difference must be what stayed in the tissue. Since the retina does not store O_2 , apart from the small amount that is dissolved (or bound to neuroglobin), this difference must also be the amount of O_2 utilized by the tissue. In practice the total O_2 extraction can be obtained by multiplying the quantity of blood flowing per minute by the difference in O_2 concentration (often called saturation) between arteries and veins. If the arteries and veins are those of the retinal circulation, we arrive at the inner retinal metabolic rate of O_2 (IR-MRO₂):

$$IR-MRO_2 = RBF \times (s_{ra}O_2 - s_{rv}O_2) \quad (4)$$

where RBF is the total retinal blood flow rate (volume of blood per unit time) and sO_2 is the oxygen concentration (volume of O_2 /volume of blood). The subscripts *ra* and *rv* represent retinal arteries and veins respectively. Correspondingly, a value for the outer retina, OR-MRO₂, can be obtained from choroidal blood flow rate (ChBF) times the arteriovenous difference in the choroidal circulation.

$$OR-MRO_2 = ChBF \times (s_{ca}O_2 - s_{cv}O_2) \quad (5)$$

where ChBF is choroidal blood flow. The exact form of these equations will depend on how the parameters are measured, but in all cases Eq. 4 and 5 represent the amount of O₂ taken out of the retinal or choroidal circulation. If the weight of tissue supplied by each circulation is known, then IR-MRO₂ and OR-MRO₂ can be expressed in terms of the volume of O₂ per unit weight of tissue per unit time. The most straightforward assumption is that the relevant weights are the weights of the inner and outer retina, each very close to half the total retinal weight (Werkmeister et al., 2015). It might seem then that the inner and outer retinal O₂ utilization values would match the corresponding inner and outer retinal metabolic rate of O₂, that is:

$$IR-MRO_2 = IR - QO_2 \quad (6)$$

and

$$OR-MRO_2 = OR - QO_2 \quad (7)$$

If these were true, it would simplify comparing microelectrode measurements and imaging measurements, but as described in Section 5, we know that the situation is more complicated, and these equations are generally not true. The main issue is that O₂ taken out of the retinal circulation (IR-MRO₂) is not all used for metabolism in the inner retina (IR-QO₂). Some O₂ derived from the retinal circulation supplies the photoreceptors instead.

2.4. Oxygen measurements with imaging

In contrast to other techniques, imaging methods give values for the concentration of oxygen, rather than PO₂. Because they rely on absorption of light by hemoglobin, these techniques always involve measurements in blood vessels, in contrast to the microelectrode measurements, which are generally extravascular. Saturation is related to PO₂ in a non-linear way that depends on the shape of the hemoglobin saturation curve, but, except in hyperoxia where arterial PO₂ increases without an increase in saturation, PO₂ and saturation can be interconverted if the parameters characterizing the hemoglobin saturation curve (P50 and cooperativity) are known.

The effort to explore noninvasive imaging of retinal hemoglobin oxygen saturation (sO₂) dates back several decades. Although functional MRI has been explored for quantifying ocular sO₂ (Berkowitz and Wilson, 1995; Duong, 2014), the optical methods have played a dominant role (Harris et al., 2003). The physical basis of almost all the optical methods is the distinctive molar extinction coefficients of oxygenated and deoxygenated hemoglobin across a wide optical spectral range (Friebel et al., 2006; Hammer et al., 1995), which leads to different optical attenuation in blood with different sO₂ levels. In laboratory settings, such a measurement in *ex vivo* blood samples can be implemented relatively straightforwardly using a spectrophotometer or other commercial instrument. However, the challenge is how to obtain the optical attenuation of blood in retinal vessels noninvasively *in vivo*.

Since 1963, several groups have tried to develop methods based on multi-wavelength fundus photography to measure retinal sO₂ noninvasively, which is now referred to as retinal oximetry (Delori, 1988; Hammer et al., 2001; Hammer et al., 2008; Hardarson, 2013; Hardarson et al., 2006; Harris et al., 2003; Hickam et al., 1963; Smith et al., 2000; Traustason et al., 2013; Vucea et al., 2011), and a commercial product is already available (Figure 3a) (Geirsdottir et al., 2012). In principle, all these reported methods have relied on analyzing pixelated intensity variations in diffusively back-scattered retinal fundus photos using multiple wavelengths of illumination. Different pixelated intensity extraction methods were developed to obtain wavelength-dependent optical attenuation of blood with different values of sO₂. Figure 3a shows a numerically simulated region of a fundus photograph containing a vessel segment (diameter: 100 μm) and an area without obvious vascular structures (often referred to as background). From the image, the mean intensity within the region highlighted by the white dashed box in the vessel segment is considered the vessel optical reflection intensity $I_v(\lambda)$ and the mean pixel intensity within the two neighboring areas (highlighted by the two dashed-boxes on both sides) is considered the background optical reflection intensity $I_b(\lambda)$. Then the optical attenuation (defined by optical density or OD(λ)) by the blood vessel is calculated by

$$OD(\lambda) = \log_{10} \left(\frac{I_b(\lambda)}{I_v(\lambda)} \right), \quad (8)$$

where the underlying assumption is that $I_b(\lambda)$ is minimally affected by the local retinal environment. Here, the optical attenuation caused by blood is considered to be mainly caused by optical absorption, and the influence from optical scattering is considered to be negligible. After OD(λ) is measured from multiple optical wavelengths, the vessel sO₂ can be estimated based on the molar extinction coefficient at corresponding wavelengths (Harris et al., 2003).

Almost all the optical illumination wavelengths are within the visible light spectral range, for example 510 nm and 640 nm (Hickam et al., 1963), to take advantage of the strong blood optical absorption. Hickam et al. (Hickam et al., 1963) used two wavelengths and estimated sO₂ in retinal arteries and veins, but the accuracy of the dual-wavelength method suffered severely from optical scattering. Additional optical wavelengths were later employed to compensate for optical scattering. For example, Delori (Delori, 1988) used three narrowly separated wavelengths and obtained improved results by compensating for the wavelength-independent scattering. Later on, more wavelengths were employed in retinal oximetry by other groups (Vucea et al., 2011) until hyperspectral oximetry was reported (Desjardins et al., 2016; Mordant et al., 2009), where over 50 optical wavelengths can be employed.

Despite the efforts to develop and improve retinal oximetry, many limitations still exist. The accuracy of retinal oximetry is affected by factors such as variations in vessel size and pigmentation, multiple light paths, and vessel wall thickness. Variations in vessel size and pigmentation are considered to have a major impact on the accuracy of sO₂ measurement mainly due to unknown photon propagation paths in diffusive fundus photography (Hammer et al., 2001). Optical scattering in the retina profoundly affects the accuracy of retinal

oximetry when the axial resolution is low. The major reason is believed to be inaccurately classifying photons coming from the “vessel” and “background,” which leads to inaccurate values for $I_v(\lambda)$ and $I_b(\lambda)$. Such inaccurate classification is caused by unpredictable optical path lengths as a result of multiple optical scattering. To better illustrate the effect of multiple scattering on classifying photon origins, several research groups (Hammer et al., 2001; Liu et al., 2013; Smith et al., 2000) categorized detected photons into three groups: backscattered, single-pass, and double-pass photons (for more details on the definitions of these photon groups, readers can refer to Fig. 2 in (Liu et al., 2013)). Among the three groups, backscattered photons contributed the most to both $I_v(\lambda)$ and $I_b(\lambda)$, and single-pass photons are the key to the accuracy of $I_b(\lambda)$ and, therefore, OD of vessels (Liu et al., 2013). More specifically, $I_v(\lambda)$ becomes less accurate when vessel diameter increases, due to stronger optical attenuation by the blood of single-pass photons. Also, when the RPE melanin concentration increases, $I_b(\lambda)$ becomes less accurate due to stronger optical absorption of backscattered photons. These combined inaccuracies in extracting $I_v(\lambda)$ and $I_b(\lambda)$ collectively lead to the inaccuracy in fundus imaging based retinal oximetry. Liu et al (Liu et al., 2013) found that when vessel diameter increased from 40 μm to 160 μm , the relative measurement error increased 15%; when the melanin concentration increased from 4 mmol/L to 8 mmol/L, the relative measurement error quadrupled. According to Liu’s numerical investigation (Liu et al., 2013), the optimal solution to improve the accuracy of oximetry is to increase the axial resolution so that photons with different path lengths can be better categorized.

Recently, two other modalities with much improved axial resolution in retinal imaging were explored to quantify retinal sO_2 , including photoacoustic ophthalmoscopy or PAOM (Hennen et al., 2015; Jiao et al., 2010; Song et al., 2012; Song et al., 2014) and visible-light optical coherence tomography or Vis-OCT (Chen et al., 2015b; Chong et al., 2015; Yi et al., 2015a; Yi et al., 2013).

2.4.1. Photoacoustic Ophthalmoscopy (PAOM)—PAOM is modified from photoacoustic microscopy to be suitable for retinal imaging (Jiao et al., 2010) based on the laser-excited ultrasound generated through transit optical absorption and thermoelastic expansion (Zhang et al., 2006). PAOM measures the optical energy deposition in the retina, which reflects directly the optical absorption properties of the retina. Two chromophores, hemoglobin and melanin, dominate optical absorption in the retina; hence, PAOM can image retinal blood vessels and the RPE with a much improved contrast-to-background ratio than any other intrinsic retinal imaging modality (Song et al., 2012). An *in vivo* PAOM image from a rat’s eye is shown in Figure 4a. In PAOM, the lateral resolution is identical to other retinal imaging modalities, which is limited by the ocular optical aberration; the axial resolution is determined by the bandwidth of ultrasonic detection. Using a 30 MHz ultrasonic detector with a 60% bandwidth, the axial resolution can reach 23 μm , which can separate the signals of retinal blood vessels from RPE melanin (Song et al., 2014). Owing to the improved axial resolution, PAOM can be shown to be insensitive to the RPE melanin concentration inhomogeneity in quantifying retinal sO_2 (Liu et al., 2013).

Figure 4b shows that PAOM measures the optical absorption properties from a circular cross section at three different laser illuminating wavelengths. Then the multi-wavelength

photoacoustic amplitudes of the vessels are used to estimate sO_2 in every vessel based on the molecular extinction coefficients of oxygenated and deoxygenated hemoglobin through a least squares inverse calculation. The detailed steps can be found from several good reviews of photoacoustic microscopy (Song et al., 2014; Wang and Hu, 2012; Yao et al., 2016). After inverse calculation, sO_2 values in single major retinal vessels are shown in Figure 4c.

Although the accuracy of *in vivo* retinal sO_2 imaging is improved in PAOM, there are several obstacles that need to be overcome in the future. First, there need to be better ultrasonic detection methodologies in PAOM. Ultrasonic signal detection requires physical contact, which is not preferred for retinal imaging in clinical settings. In addition, traditional piezoelectric ultrasonic detectors are opaque, posing additional challenges in designing optical illumination and co-axial ultrasonic detection (Li et al., 2014). Second, a high-speed tunable laser system is needed, because existing tunable nanosecond laser systems are often too slow in wavelength tuning for patient imaging.

2.4.2 Visible light optical coherence tomography (Vis-OCT)—Quantifying retinal sO_2 using Vis-OCT represents the latest technology development. Since it was developed in 1991 (Huang et al., 1991), optical coherence tomography (OCT) has gone through rapid technological improvements and is now considered the “gold standard” in nearly every area of ophthalmic research and clinical care. To minimize ocular discomfort during imaging, ophthalmic OCT systems from all manufacturers and research groups mainly use near-infrared light with center wavelengths from 830 nm to 1330 nm. Within the near infrared spectral range, OCT has already achieved great success in ultra-high resolution anatomic imaging (Wojtkowski et al., 2005) and microvascular imaging (Bojikian et al., 2016; Hong et al., 2014; Jia et al., 2012). Based on statistical photon-tissue interaction analysis and *in vivo* verification in rats, Chen et al. (Chen et al., 2015a) concluded that OCT can only quantify retinal sO_2 when it is used within the visible spectrum (520 nm – 610 nm). This conclusion has been verified by several groups in both rat and mouse using Vis-OCT (Chong et al., 2015; Soetikno et al., 2015; Yi et al., 2015b).

Reported Vis-OCT systems were developed based on wideband spectral-domain OCT design and used supercontinuum lasers, instead of super luminant diodes, as the probing sources. Due to the reduced center wavelength, the axial resolution of Vis-OCT in the retina approaches 1 μm (Yi et al., 2015b). Figure 5a shows a typical Vis-OCT fundus image acquired from a pigmented rat. When motion contrast induced by the flowing blood was extracted, Vis-OCT demonstrated retinal microangiogram capability comparable to OCT systems operating within the near infrared spectral range (Figure 5b and Figure 5c). To calculate sO_2 through Vis-OCT, a series of A-lines were reconstructed from the same scanning location with moving sub-spectral windows within the illuminating spectral range via short-time-Fourier-transforms (Chong et al., 2015; Soetikno et al., 2015; Yi et al., 2013). Such an operation provided quasi-spectroscopic back-scattered optical properties of retinal blood at the cost of reduced axial resolution (Yi et al., 2013). Then sophisticated analysis of this quasi-spectroscopic back-scattered information yielded the retinal sO_2 in individual retinal vessels (Yi et al., 2015b) as shown in Figure 5b. Vis-OCT also benefits from microangiographic gating in measuring sO_2 in smaller retinal vessels (Chen et al., 2015b).

Vis-OCT has shown clear advantages over other modalities in quantifying retinal sO_2 . It has the highest axial resolution in both anatomical and functional imaging as compared with retinal oximetry and PAOM. Since OCT is widely accepted in the clinic, translation of Vis-OCT from bench to bedside is much more straightforward than translating PAOM, and Yi et al recently demonstrated that Vis-OCT is safe for human imaging (Yi et al., 2015a). Another key advantage of Vis-OCT is its capability to measure the complete retinal metabolic rate of O_2 (IR-MRO₂) by combining retinal sO_2 and retinal blood flow imaging (Yi et al., 2015b). So far, *in vivo* imaging of IR-MRO₂ with a single instrument is not otherwise possible, and this new capability may bring profound impact to retinal research and clinical care. Yi et al. (Yi et al., 2015b) demonstrated that the IR-MRO₂ variation measured by Vis-OCT in rats subjected to reduced inspired O_2 agreed with the values estimated from the PO₂ distribution models measured by microelectrodes. The current limitation of Vis-OCT in quantifying retinal sO_2 is that back-scattered analytic models assumed continuous blood flow, which is incorrect in capillaries. Therefore, existing Vis-OCT cannot quantify sO_2 in capillaries accurately and a new analytic model is needed. In addition, Vis-OCT is more likely to suffer from phase wrapping errors than OCTs performed in the near infrared spectral range when quantifying blood flow in major retinal vessels.

Finally, one concern we need to address is whether the visible light used for vis-OCT imaging itself alters blood flow and metabolism, which could potentially bias MRO₂ measurement. Under steady state conditions, previous studies have shown no major difference in metabolism in the inner retina between light- and dark-adapted eyes, although as discussed in section 3.2.1, there may be a small change in blood flow (Braun et al., 1995; Riva et al., 2000). To induce substantial retinal hemodynamic alterations, however, strong flickering light for at least 30 seconds is employed (Mishra et al., 2011). Since the illumination intensity and duration in vis-OCT are negligible compared with those in flicker tests, we anticipate that vis-OCT imaging itself will not cause significant alterations in MRO₂. Hardarson (Hardarson, 2013) reported that $s_{ra}O_2$ and $s_{rv}O_2$ were both slightly higher during darkness than during bright light, but that the arteriovenous difference did not change, which suggests that IR-MRO₂ will not be affected by light.

3. Fundamentals of O_2 supply to the retina

As we have implied in section 2, the retina can be considered to have two domains from the point of view of vasculature and metabolism, inner and outer retina. The outer avascular retina consists entirely of photoreceptors and Muller cell processes, while the vascularized inner retina, beginning at the outer plexiform layer, consists of the two nuclear layers, the two plexiform layers, and the nerve fiber layer. Except in the fovea, where there is no inner retina, the inner and outer retina are each almost exactly half of the retinal thickness, as we will describe in a subsequent research note. Because the inner and outer retina have very different properties, we will continue to separate them in sections below.

Detailed comparative data on average values of PO₂ from microelectrode measurements in rat, cat, and monkey can be found in (Birol et al., 2007; Lau and Linsenmeier, 2012). Comparative measurements of QO₂ from microelectrodes and other methods can be found in the same references as well as in (Linsenmeier, 2012).

3.1. Outer retina

With the background on measurements and modeling, we can now address some of the important findings about retinal oxygenation. Figure 1 shows measurements in dark adaptation made in three animals, macaque, cat, and rat. Some of the features illustrated in these individual profiles are consistent across measurements, and others are quite variable. A principal result is that in the dark adapted retina one consistently observes a trough in PO_2 at 70 to 80% retinal depth corresponding to the O_2 sink caused by O_2 utilization of the inner segments. There are gradients toward this region from both sides in dark adaptation, indicating that both the choroidal circulation and retinal circulation participate in supplying the metabolic demand of the photoreceptors. In the light-adapted retina, there is no change in choroidal PO_2 (Braun and Linsenmeier, 1995; Linsenmeier and Braun, 1992), but there is lower OR- QO_2 , so the minimum PO_2 in the outer retina is higher, as shown in Figure 6. In some cases, as in Figure 6, the gradient through the outer nuclear layer (ONL – layer 3) shows that the retinal circulation still provides some of the photoreceptor supply during illumination, but in other profiles the gradient through the ONL is reversed, so that a small amount of O_2 diffuses from the choroid into the inner retina. While the general shape of the profiles in the outer retina is consistent, the PO_2 at the boundaries of the outer retina, P_c (or P_{cO_2}) for the choriocapillaris, and P_L at the inner border of the avascular layer (i.e. at the outer plexiform layer; see Figure 2), are variable. On average, the choroidal PO_2 is the same in all three animals we have studied, at about 47 mmHg when systemic arterial PO_2 (PaO_2) was 90 mmHg or above. A value of 53 to 56 mmHg has been reported for minipig (Pournaras et al., 1989), which is probably not significantly different given the variability in all the measures. (The profiles in Figure 1 were chosen to be high quality profiles, and P_c was higher than average in these profiles.)

This value of P_c is somewhat puzzling. While it is reasonably high, it is not nearly as high as expected from measurements of choroidal venous O_2 saturation. Measurements from the vortex vein indicated that the choroidal O_2 extraction was 0.4 to 1 volume % (i.e. $mLO_2/100$ ml blood) in dogs, pigs, and cats (Alm and Bill, 1970; Takahashi et al., 2008; Wang et al., 1997a; Wang et al., 1997b). Based on the hemoglobin saturation curve, this would make venous PO_2 in the choroidal circulation above 70 mmHg, so it is not clear how choriocapillaris PO_2 , which is approximately what our measurements of P_c represent, would be 47 mmHg, that is, substantially lower than choroidal venous PO_2 . There are at least three possibilities without resorting to the conclusion that either of these measurements is in error. First, our measurements of P_c are made in the posterior pole, and the vortex vein measurements were averages obtained from blood across the entire choroid, as well as anterior uvea. A higher extraction in the posterior pole and lower extraction toward the periphery or in the anterior uvea would make the average extraction from the choroid lower than it is at the posterior pole. If $s_{cv}O_2$ could be measured at the posterior pole, it might correspond better to measured values of P_c . Second, there could be some non-nutritive blood flow through the choroid, with some of the blood bypassing the choriocapillaris altogether, and making the venous value at the vortex vein unreflective of the blood that goes through the choriocapillaris. Third, it is conceivable that when the blood first enters the venous system of the choroid from the choriocapillaris the saturation is lower, but then rises due to O_2 diffusion from choroidal arteries to choroidal veins. That is, there might be shunting via

diffusion. If true, the arterial supply to the choriocapillaris would have lost O_2 to the veins and would also have a lower PO_2 than systemic arterial blood. The extraction would still be $s_{ca}O_2 - s_{cv}O_2$, but in the large vessels of the choroid $s_{ca}O_2$ and $s_{cv}O_2$ might be 20 vol% (full saturation) and 19 vol% (i.e. 1 vol% less), but for the arterioles and venules near the choriocapillaris, these values might both be lower, e.g. 15 and 14 vol%, so that extraction would still be 1 vol%, but the PO_2 s would be more consistent with the measured P_c . None of these possibilities is very easy to test, and there could be a combination of them. At any rate, at present the two types of measurements do not align.

Returning to Figure 1, the profile of PO_2 in the outer retina looks qualitatively similar in the three animals we have studied, and OR- QO_2 is about the same in all three, although there are some quantitative differences. First, the effect of light adaptation on OR- QO_2 is somewhat different, with metabolism being reduced to about half of the dark value in cats and rats, but by less in monkeys. Second, the minimum PO_2 around the inner segments in dark adaptation is substantially lower in cat and monkey than in rat. Our data are for pigmented rats, but the relatively high minimum PO_2 in darkness was observed earlier in albino rats (Yu and Cringle, 2002). Third, in darkness, the O_2 supply to the photoreceptors comes mainly from the choroid in all cases, but there are quantitative differences among animals. Analysis using equation 3 shows that the retinal circulation accounts for an average of 15% of OR- QO_2 in macaque (Birol et al., 2007), 10% in cat (Lau and Linsenmeier, 2012; Linsenmeier and Braun, 1992) and 18% (Lau and Linsenmeier, 2012) in rat, but across animals of the same species and within an animal there is also some variation, depending on the PO_2 at the border between the inner and outer retina, which in turn depends on the PO_2 in deep capillaries. The higher fraction of photoreceptor QO_2 contributed by the retinal circulation is one of the reasons that the minimum PO_2 in the outer retina is higher in rats (Lau and Linsenmeier, 2012). While the contribution of the retinal circulation to photoreceptor metabolism is modest, it is important, as discussed briefly in section 5.

3.1.1. Decreased outer retinal oxygen utilization in light adaptation—The decrease in oxygen utilization with light has been found in all vertebrates that have been studied, including frog (Sickel, 1972), toad (Haugh-Scheidt et al., 1995a; Kimble et al., 1980), and rabbit (Ames et al., 1992), animals which are not otherwise considered in this review. The time course of the change in metabolism can be studied with a microelectrode placed near the inner segments while the illumination is changed, as shown in Figure 7. In most of the retina OR- QO_2 is dominated by rods, and the decrease in metabolism, and consequent increase in PO_2 , is graded with illumination and saturates at the modest illumination corresponding to rod saturation in cats and monkeys, when the rods are maximally hyperpolarized (Linsenmeier, 1986; Wang et al., 2010b). The time constant for the increase in PO_2 during illumination, which directly reflects the time constant of the decrease in OR- QO_2 , varies across mammals from about 10 sec to 50 sec (Wang et al., 2010b). Metabolism reverses rapidly to the dark-adapted state for moderate illuminations, but the reduction in OR- QO_2 is prolonged well after the retina returns to darkness when the illumination is high and the photoreceptors remain hyperpolarized – a metabolic rod after-effect (e.g. (Steinberg, 1969)), as shown in Figure 7.

The overall decrease in OR-QO₂ with light masks the fact that there are two metabolic processes in the photoreceptors that are strongly light dependent. The first, which causes a *decrease* in QO₂ during illumination, is a reduction of sodium pumping by the Na⁺/K⁺ ATPase in the inner segment. This occurs because the cation channels in the outer segment close in the light e.g. (Lamb and Pugh, 2006), so less Na⁺ and Ca⁺² enter the photoreceptors, and therefore less Na⁺ needs to be pumped out. (The Ca⁺² is eliminated by a Na⁺/Ca⁺⁺ exchanger in the outer segment, which brings in more Na⁺, so the metabolic load due to Ca⁺² entry is all transferred to the need to pump Na⁺.) The second metabolic change is an *increase* in QO₂ during illumination (Ames et al., 1992; Haugh-Scheidt et al., 1995a; Kimble et al., 1980), because there is more turnover of cGMP in light (Dawis et al., 1988; Goldberg et al., 1983), which uses more GTP. It is difficult to measure the absolute magnitude of the pump and cyclic nucleotide components, partly because there are other illumination-independent processes that use energy, and partly because trying to block one process could influence the other. Nevertheless, by blocking the pump in the isolated rabbit retina, Ames et al. (Ames et al., 1992) calculated that the requirement for cGMP turnover was about 5% of the total metabolic demand of the two processes in the dark. Because cGMP turnover increased by about a factor of 4 in bright light, and Na⁺ pumping decreased by a factor of about 3, the energy demand of the two processes became about equal in the light. Okawa et al. (Okawa et al., 2008), calculated the magnitude of the two processes from experimental data obtained on several species, and also concluded that the two processes were of similar magnitude in the light, but calculated that cGMP turnover was only about 2% of the total in the dark. They calculated that Na⁺ pumping decreased in light by a factor of more than 20. The difference between the estimates in these two studies could partly be the maximum light intensity, since very bright light should close all the cation channels.

3.2. Inner retina

In the inner retina, the profiles (Fig 1, 6) generally show peaks corresponding to the presence of capillaries. These peaks are relative maxima, and do not necessarily mean that the electrode was exactly at a capillary. It has not been possible to know how far away the electrode is from the closest vessel. The deeper peak tends to be a few mmHg lower, and in cat this difference was significant in a paired t-test (Yancey and Linsenmeier, 1989). In general, because of the absence of a good mathematical model for the inner retina, we can only give average values of inner retinal PO₂. The range of values is quite large, as shown by the histograms for the normoxic inner retina of cat in Figure 8 (Linsenmeier and Braun, 1992). When one computes PO₂ from the more superficial (0–25% retinal depth) and deeper (25–50% retinal depth) parts of the inner retina, the average PO₂ in the deeper region is slightly lower in the dark-adapted cat (Braun and Linsenmeier, 1995) and rat (Linsenmeier, unpublished), which is consistent with the small difference in superficial and deep peak PO₂s. This difference between superficial and deep values disappears in light adaptation (Braun and Linsenmeier, 1995), as expected if some choroidal O₂ can diffuse to the inner retina during illumination.

3.2.1. Effect of light on inner retinal PO₂ and metabolism—In contrast to the large change in outer retinal PO₂ with illumination, steady light has a rather small effect on inner retinal PO₂, as can be appreciated from the histograms of Figure 8. A better way to evaluate

the effect of illumination is to leave an electrode stationary in the inner retina and illuminate the retina. Then only a very small decrease in PO_2 is observed (Lau and Linsenmeier, 2012; Linsenmeier, 1986). In rat this decrease was only about 0.5 mmHg. Steady light does not appear to cause a substantial change in IR-QO₂ relative to darkness in monkey, cat or rat (Bill and Sperber, 1990a; Braun et al., 1995; Medrano and Fox, 1995). One would not then expect a change in retinal blood flow between darkness and steady light, which was supported by results with an infrared laser Doppler method in humans. Riva et al. (Riva et al., 2000) found a transient increase in retinal blood flow at the onset of light, but no maintained change, and suggested that previous measurements (Feke et al., 1983; Riva et al., 1983) that found a dark-light difference were probably influenced by this transition. However, nearly simultaneously with the more recent study by Riva et al., Havelius et al. (Havelius et al., 1999), using an ultrasound (color Doppler) method in humans, reported a modest increase in central retinal artery velocity of about 20% during 25 min of darkness relative to steady light (120 to 1350 lux). There is little if any measurable dilation of the retinal vessels during darkness (Barcsay et al., 2003), but the increase in velocity alone would imply an increase in blood flow. Havelius et al. (Havelius et al., 1999) attributed this reasonably to an increased O₂ delivery from the retinal circulation to the photoreceptors in darkness, rather than an increase in IR-QO₂. Why these studies differed is not clear, but could relate to the sensitivity of the two methods.

In contrast to steady light, flickering light causes increases in retinal vascular diameter (Felder et al., 2015; Hammer et al., 2011; Palkovits et al., 2015; Palkovits et al., 2014b; Polak et al., 2002) and retinal and optic nerve blood flow (Kondo et al., 1997; Palkovits et al., 2015; Palkovits et al., 2014b; Shih et al., 2013; Toi and Riva, 1995) relative to steady light in rat, cat, and human. The mechanisms of this neurovascular coupling have been discussed by Newman (Newman, 2013) and Kur et al. (Kur et al., 2012), and will not be covered here. Our interest is to assess the impact of flicker on IR-MRO₂. For this purpose measurements of retinal arteriovenous O₂ differences are needed in combination with blood flow, and here the findings on rat and human conflict. Vascular oxygenation has been measured with phosphorescence methods in rat and show that the arteriovenous difference increases (Blair et al., 2009; Shakoore et al., 2006; Teng et al., 2014) during flicker. In contrast, the arteriovenous saturation difference in humans, measured with reflectance oximetry, decreases during flicker (Felder et al., 2015; Hammer et al., 2011; Palkovits et al., 2015). It is possible that the rat data were influenced by anesthesia, or by the very low retinal artery PO_2 of 41 to 51 mmHg in the phosphorescence studies. In the only study to combine blood flow and O₂ measurements, IR-MRO₂ increased by 34% relative to steady light in humans with light flickering at 12.5 Hz (Palkovits et al., 2015). This is consistent with earlier measurements in monkeys, which showed that accumulation of deoxyglucose in the inner retina was greater during flicker than steady light (Bill and Sperber, 1990a, b). The quantitative effect on metabolism is likely to depend on illumination and frequency, but this has not been fully investigated. At the level of tissue PO_2 , flickering light caused a significantly larger decrease in inner retinal PO_2 than maintained light in rats, but it was still a reduction of only 1.5 mmHg relative to darkness (Lau and Linsenmeier, 2012). Tissue PO_2 is likely to be the regulated variable, and the small change in PO_2 shows that increased blood flow does a very good job of keeping PO_2 nearly constant.

3.2.2 Spontaneous fluctuations—Measurements of average inner retinal PO₂ smooth out the interesting phenomenon of spontaneous PO₂ fluctuations, illustrated in Figure 9, which we studied primarily in cat (Braun et al., 1992). With an electrode placed in the inner retina, we frequently observed slow spontaneous changes in PO₂ of a few mmHg. The left side of Figure 9 shows a series of 60 sec traces recorded at progressively greater depths, showing that these irregular fluctuations only occurred in the inner retina. They were absent in the avascular outer retina, so they were not due to electrode noise, but to the presence of retinal vessels. In the choroidal trace, one can see regular fluctuations at the respiratory frequency, which are quite different. We computed power spectra for the spontaneous fluctuations in the inner retina, and found that the total power was variable from one penetration to another, so we computed the average power spectrum of the fluctuations after normalizing the spectrum from each episode to its own maximum. The resulting spectrum had a dominant peak at about 5 cpm and gradually falling power at higher frequencies. While this is the only demonstration of this in the retina, we and others have observed similar fluctuations at similar frequencies in the brain of anesthetized animals (Hudetz et al., 1998; Manil et al., 1984; Padnick et al., 1999) and awake rabbits (Linsenmeier et al., 2016a). Generally these fluctuations are believed to reflect ongoing local fluctuations of vascular resistance, termed vasomotion (Aalkjaer et al., 2011), but there is no direct proof of this in the retina.

3.3 Vitreal PO₂

Many measurements of PO₂ have been made in the vitreous humor near the retina. These are less invasive and less complicated, and can be made in humans as discussed in section 6.1. The assumptions in such measurements are that 1) the O₂ comes from the retinal circulation and 2) the O₂ consumption of the vitreous is low (about 1 $\mu\text{l}\cdot\text{ml}^{-1}\cdot\text{hr}^{-1}$ (Filas et al., 2013), or $1.6 \times 10^{-3} \text{ ml}\cdot 100\text{g}^{-1}\cdot\text{min}^{-1}$, which is more than 3 orders of magnitude lower than IR-QO₂ (Braun et al., 1995)). For these reasons, vitreal measurements reflect the average PO₂ in the inner retina, which is 15 to 25 mmHg during air breathing (e.g. (Wangsa-Wirawan and Linsenmeier, 2003)). However, the preretinal vitreous PO₂ is not always sufficient for understanding inner retinal PO₂. Alder and Cringle (Alder and Cringle, 1985, 1990) and others (Buerk et al., 1993; Petropoulos et al., 2013) have shown that gradients of PO₂ extend for several hundred μm into the vitreous and they depend on the proximity to retinal vessels. PO₂ is higher at the retina near arterioles, and decreases with distance into the vitreous from the retina. The opposite gradients are found near venules and intravascular zones, with higher PO₂ in the vitreous than at the retina. Filas et al (2013) captured this variation with a computational model shown in Figure 10. The flow arrows make it clear that O₂ circulates very locally near the retina, away from more arterial regions, through the vitreous, and back into intravascular or venous regions. (It has not been carefully studied, but such vitreal gradients could provide part of the supply of O₂ to the fovea from the richly vascularized tissue in the parafovea.) While the values that one measures with a larger electrode (e.g. Sakaue et al., 1989; Maeda and Tano, 1996) or a fiberoptic probe (e.g. Stefansson et al, 1989; Lange et al, 2011) are similar to average values in the inner retina, they lack detail about these local gradients because these probes average over a larger area than microelectrodes, and cannot be placed near enough to the retina. In particular, vitreal

electrodes could miss changes that may occur after photocoagulation or in diabetic retinopathy or in any situation where there is focal damage.

3.4. Oxidative metabolism and glycolysis

In considering retinal O_2 and oxidative metabolism, we should keep in mind that the photoreceptors also do a great deal of anaerobic glycolysis, and as a result they produce a considerable amount of lactate (Ames et al., 1992; Winkler, 1981, 1995). The relative amounts of glycolytic metabolism in the outer and inner retina *in vivo* are difficult to compare to measurements of oxidative metabolism obtained from microelectrodes, but in one series of measurements and Fick principle calculations it was possible to obtain inner and outer retinal values for oxidative and glycolytic metabolism. Wang et al. (Wang et al., 1997b, c) sampled oxygen, lactate and glucose from a systemic artery as well as from the vortex vein to characterize the outer retina, and a vessel in the venous plexus draining the retina to characterize the inner retina. They then multiplied these arteriovenous differences by the blood flow rates in the choroid and the retinal circulation respectively, which were obtained with microspheres. Their results are shown in Figure 11. The conclusions from their work on the pig retina largely agree with the results on oxidative metabolism obtained with microelectrodes, although these are measurements of OR-MRO₂ and IR-MRO₂ rather than OR-QO₂ and IR-QO₂. Outer retinal O_2 utilization was about 50% higher in darkness than in light, but there was no difference between dark and light in the inner retina. The inner and outer retina used about the same amount of O_2 in light, which is similar to the conclusion of Medrano and Fox (Medrano and Fox, 1995) using a very different method *in vitro*. We had found that OR-QO₂ and IR-QO₂ were about the same in darkness, rather than light (Braun et al., 1995), so there is some discrepancy. However, each of these methods for measuring IR-QO₂ or IR-MRO₂ is limited in a different way, and a match in all respects is not to be expected. What is clear from all the measurements is that the photoreceptors do not use tremendously more O_2 than the inner retina, and in fact, because there are so many photoreceptors compared to second and third order neurons, the metabolic rate of individual photoreceptors is probably less than a typical inner retinal neuron. What makes the photoreceptors unique among retinal neurons is the very unusual localization of almost all the mitochondria in one location, the inner segments which are further from any blood vessels than mitochondria typically are.

The new information from Figure 11, however, is about glucose and lactate. It shows the extraordinary utilization of glucose in the outer retina to produce lactate, which is almost missing in the inner retina. Winkler calculated that 90% of the glucose used by the retina as a whole was converted to lactate (Winkler, 1995), and Figure 11 shows that this occurs primarily in the outer retina. Like oxidative metabolism, anaerobic glycolysis in the outer retina decreases with illumination, but this does not occur in the inner retina. Notably, the very large amount of lactate production in the outer retina is not nearly the maximum possible. During hypoxia, when oxidative metabolism is compromised, the lactate production in the outer retina can increase a great deal, based both on direct measurements of lactate production during hypoxia (Ames, 1992; Winkler, 1981, 1995), and increases in retinal acidity (Padnick-Silver and Linsenmeier, 2005; Yamamoto and Steinberg, 1992).

While the present article focuses on retinas with a full retinal circulation (holangiatic), it should be noted that there are both similarities and differences with the merangiatic retinas of rabbits and guinea pigs, for example, which are missing a retinal circulation except for a peripapillary circulation in wings extending from the optic disc. This abbreviated retinal circulation probably nourishes primarily the nerve fiber layer as it becomes thicker near the disc. In general, it is thinner retinas that lack a full retinal circulation (Buttery et al. 1991). In these retinas, the outer retina is metabolically similar to the outer retina of holangiatic animals, but the inner retina is quite different. It has little O_2 , and very low $IR-QO_2$ (Yu and Cringle, 2001). The inner retina of merangiatic animals appears to rely heavily on glycolysis (Ames and Li, 1992). There are striking differences in the laminar distribution of glycolytic and oxidative enzymes in the inner retina of monkeys and rabbits (Lowry et al., 1961), with the activity of the rate limiting enzyme in glycolysis, phosphofructokinase, being much greater in rabbits.

4. Retinal PO_2 in hypoxia and hyperoxia

4.1. Hypoxia

Experimental hypoxia is usually produced by lowering the inspired oxygen fraction. Because the retinal and choroidal circulations behave differently in response to systemic hypoxia, PO_2 in the outer and inner retina behaves differently as well. The choroidal circulation does not appear to regulate according to the metabolic needs of the outer retina, and its resistance is not thought to change during hypoxia (Bill, 1962), although there has been very little work on this. Choroidal blood flow could increase as a result of increased arterial pressure during hypoxia, but the choroidal circulation is regulated to some extent during moderate pressure changes (Kiel, 2012; Kiel and van Heuven, 1995), so this is not a major effect. As a result, when PaO_2 falls, there is no mechanism to compensate and deliver more blood to the choroid. As a result, choroidal PO_2 falls (Linsenmeier and Braun, 1992; Moret et al., 1992; Pournaras, 1995), as shown in Figure 12. Because the PO_2 around the inner segments is already quite low in the dark-adapted retina of cats during normoxia, the decrease in choroidal PO_2 reduces the PO_2 gradient between the choriocapillaris and the inner segments, which in turn reduces the flux of O_2 and therefore also $OR-QO_2$ (Linsenmeier and Braun, 1992). There is a similar set of changes when intraocular pressure is elevated and reduces choroidal blood flow (Yancey and Linsenmeier, 1989). In both cases, $OR-QO_2$ decreases essentially linearly with the decrease in PaO_2 or increase in IOP. Choroidal PO_2 decreases during hypoxia in the light-adapted retina as well, but because the PO_2 around the inner segments is higher during normoxia, there is much less change in O_2 flux or in $OR-QO_2$ (Linsenmeier and Braun, 1992).

The decrease in $OR-QO_2$ in darkness leads to some functional changes, detectable primarily in the ERG c-wave and the light peak of the DC EOG (Linsenmeier et al., 1983; Linsenmeier and Steinberg, 1986; Niemeyer et al., 1982; Yancey and Linsenmeier, 1988). Most of these can be attributed, directly or indirectly, to changes in Na^+/K^+ pumping that alter subretinal $[K^+]_o$ (Linsenmeier and Steinberg, 1984; Steinberg, 1987). There is very little effect on the a-wave of the ERG until hypoxia is very severe (Kang Derwent and Linsenmeier, 2000; Kang Derwent and Linsenmeier, 2001), consistent with the preservation

of the a-wave in ischemia and anoxia (Mittelman et al., 1973; Noell, 1951). Presumably the a-wave is protected because anaerobic glycolysis increases, replacing production of ATP by oxidative metabolism, at least in part. As further evidence of the importance of this switch to glycolysis, hypoglycemia prevents this and makes the a-wave more sensitive to hypoxia (Kang Derwent and Linsenmeier, 2001). However, if the increased glycolysis allowed the Na^+/K^+ pump to work as well as it does in normoxia, the changes in $[\text{K}^+]_o$ would not occur and the slow potentials would not be affected, so this story is still not quite complete.

In contrast to the choroid, the retinal circulation does regulate in response to metabolic demand, and blood flow increases during hypoxia (Cheng et al., 2016; Eperon et al., 1975; Hickam and Frayser, 1966; Palkovits et al., 2014c; Wanek et al., 2013; Yi et al., 2015b). As a result, inner retinal PO_2 is quite resistant to hypoxia, which can be appreciated from Figure 12. In anesthetized cats, average inner retinal PO_2 does not decrease until PaO_2 is below about 40 mmHg (Enroth-Cugell et al., 1980; Linsenmeier and Braun, 1992). Limited evidence, discussed further in section 5, indicates that IR- QO_2 is also maintained to relatively low PaO_2 (Yi et al., 2015a; Palkovits et al., 2014a). Because of the regulation, the b-wave is also relatively resistant to hypoxia, but it begins to change before the a-wave (e.g. (Brown et al., 1957; Kang Derwent and Linsenmeier, 2000; Tinjust et al., 2002)).

It is reasonable, in light of issues related to neovascularization in disease, to ask whether hypoxia itself can lead to neovascularization in the retina. In young animals the retina shows increased HIF-1 α and VEGF before development of the retinal vasculature, and this is presumably the main stimulus for the normal development of the retinal circulation (Caprara et al., 2011; Nakamura-Ishizu et al., 2012; Stone et al., 1995). Interestingly, elimination of HIF-1 α and VEGF in astrocytes does not alter this (Weidemann et al., 2010), so other cells or other compensatory mechanisms must be involved. In the hypoxic conditions of oxygen-induced retinopathy, HIF-1 α and VEGF are also important to abnormal neovascularization (Ozaki et al., 1999; Weidemann et al., 2010). There is less information about whether hypoxia per se can lead to angiogenesis in adult animals. Several hours of hypoxia in rats led to upregulation of HIF-1 α protein and VEGF expression (Crosson et al., 2009). In addition, VEGF was upregulated in primate retina after venous occlusion (Pournaras et al., 1997). This might have been due to a variety of the changes occurring in ischemia, but systemic hyperoxia reduced VEGF mRNA, showing that hypoxia had been the main reason for VEGF elevation (Pournaras et al., 1997). However, the only evidence directly linking pure systemic hypoxia and angiogenesis is from work by Shortt et al. (Shortt et al., 2004), who showed that normal rats made hypoxic for two weeks exhibited histological signs of angiogenesis. As many authors have suggested, neovascularization in ischemic diseases, such as diabetes and vein occlusion, which do not simply destroy the whole inner retina, may be due to hypoxia, but retinal disease always involves a complex mixture of events besides hypoxia, so the role of hypoxia itself is rarely certain.

4.2. Hyperoxia

When arterial PO_2 increases as a result of increased inspired O_2 , the changes in PO_2 are largely opposite to those in hypoxia. The choroidal circulation does not constrict during hyperoxia (Friedman and Chandra, 1972; Geiser et al., 2000), so Pc increases

proportionately with the increase in PaO_2 as illustrated in Figure 13A, a compilation from several studies in rat, cat, pig, and monkey. The amount of O_2 carried by the choroid does not change much beyond the point of full hemoglobin saturation (i.e. when PaO_2 is about 100 mmHg), but the increase in Pc is quite important, because this is the driving force for O_2 diffusion into the retina. If the increase in Pc is large enough, O_2 will diffuse all the way to the inner retina, as described further below. The value of Pc will increase still further with hyperbaric oxygen. This is the basis on which hyperoxic and hyperbaric treatments for vascular occlusive disease have been suggested, but there is a misconception that hyperbaric O_2 would be much better than 100% O_2 at atmospheric pressure, a topic that will be considered further in section 7.

Because the influence on of hyperoxia on Pc is large, the PO_2 in the outer retina increases dramatically, as shown in Figure 13B (Yu and Cringle, 2001). These data are for light adapted rat retina, but similar results are seen in dark adaptation in cat (Chung and Linsenmeier, 2007; Linsenmeier and Yancey, 1989) and monkey (Yu et al., 2005b). There is no measurable change in OR-QO_2 during hyperoxia (Braun and Linsenmeier, 1995; Chung and Linsenmeier, 2007; Linsenmeier and Yancey, 1989; Yu et al., 2005b), but there could be a small change that is masked by the increased difficulty of obtaining unique model fits to the data during hyperoxia and the normal variation of OR-QO_2 in both air breathing and hyperoxia.

The retinal circulation constricts during hyperoxia (Cheng et al., 2016; Gilmore et al., 2005; Grunwald et al., 1984a; Pournaras et al., 2008; Riva et al., 1986; Werkmeister et al., 2015). This tends to keep inner retinal PO_2 close to levels during air breathing, but the regulation is not perfect in cats, at least partly because the diffusion of O_2 from the choroid is unavoidable. Still, as illustrated in Figure 13B, the increase in inner retinal PO_2 in a normal animal is considerably less than the increase in PO_2 in the outer retina. In cat, the average increase in PO_2 during inspiration of 100% O_2 was 39 ± 25 mmHg in proximal retina, and 126 ± 96 mmHg distal to the photoreceptor inner segments (Linsenmeier and Yancey, 1989). At the retinal surface in minipigs, PO_2 can be unchanged during hyperoxia (Pournaras et al., 1989; Riva et al., 1986).

Hypercapnia superimposed on hyperoxia can reduce or eliminate the hyperoxic vasoconstriction of the retinal circulation (Deutsch et al., 1983; Frayser and Hickam, 1964; Sponsel et al., 1992; Tsacopoulos and David, 1973) and lead to much larger increases in inner retinal PO_2 (Berkowitz, 1996; Berkowitz et al., 2001; Chung and Linsenmeier, 2007; Pakola and Grunwald, 1993; Pournaras et al., 2004; Yu and Cringle, 2001).

5. Integrating oximetry and microelectrode measurements

With the advances allowed by phosphorescence imaging, PAOM or vis-OCT, more accurate measurements of retinal sO_2 have become possible, leading to measurements of IR-MRO_2 in animals (Wanek et al., 2011; Wanek et al., 2013; Yi et al., 2015b) and paving the way for measurements in humans. There have been measurements of IR-MRO_2 using reflectance oximetry in humans as well (Palkovits et al., 2014a; Palkovits et al., 2014c; Werkmeister et al., 2015). Nevertheless, important challenges remain. One is that so far oximetry cannot be

used to derive $OR-MRO_2$ because the choroidal input and output vessels cannot be clearly identified. A second challenge is that even for the inner retina the measurements are so far essentially global rather than regional, because they sum amounts of O_2 entering or leaving the retina in all of the retinal vessels near the optic nerve head. A third challenge is that currently there is a tacit assumption that the values of $IR-MRO_2$ one derives from oximetry will be equal to the metabolic demand of the inner retina, $IR-QO_2$. Unfortunately, this is not true under normal dark-adapted conditions and may not be true in disease. This can be appreciated with reference to the schematic in Figure 14. Here the blue arrow represents the retinal circulation, and the Fick Principle input-output analysis of flow and saturation in this circulation yields $IR-MRO_2$. Small green arrows show that much of the oxygen delivered by the retinal circulation is used in the inner retina ($IR-QO_2$), but, as noted above, analysis of O_2 gradients in the outer retina indicates that the retinal circulation delivers some of its oxygen to the photoreceptors. We can calculate how much of the photoreceptor demand is met by the retinal circulation (10 to 18% in darkness, see Section 3.1), but do not know the fraction of O_2 extracted from the retinal circulation that is delivered to the photoreceptors rather than the inner retina. However, because the metabolic rate of photoreceptors is about the same as the metabolic demand of the inner retina, a rough estimate is that about 15% of the O_2 extracted from the retinal circulation contributes to outer retinal metabolism. Thus, $IR-MRO_2$ is expected to significantly overestimate $IR-QO_2$, or, expressed differently,

$$IR-MRO_2 = IR-QO_2 + R-OR-QO_2 \quad (9)$$

where the last term is the part of $OR-QO_2$ supplied by the retinal circulation. There is some evidence that this contribution of the retinal circulation to photoreceptor metabolism is critical. When the deep layer of retinal capillaries is lost in diabetic humans, the photoreceptors in those regions are abnormal on OCT scans (Scarinci et al., 2015).

The contribution of the retinal circulation to $OR-QO_2$ is not constant. During hypoxia in rats, $IR-MRO_2$ increased (Wanek et al., 2013; Yi et al., 2015b), which was surprising. With less O_2 available in the circulation, it was difficult to believe that there could be an increase in $IR-QO_2$. Inner retinal PO_2 tends to be stable during mild hypoxia (Section 4.1) and when it falls, one would certainly expect that $IR-QO_2$ would decrease, if anything. The resolution of this paradox involved recognizing that choroidal PO_2 was probably decreasing during hypoxia in rats as it does in cat (Linsenmeier and Braun, 1992) (Figure 12). This would reduce PO_2 in the outer retina, leading to increased flux of O_2 from the retinal circulation to the photoreceptors, so $R-OR-QO_2$ increases. A mathematical model of this situation, based on outer retinal metabolism in rat (Lau and Linsenmeier, 2012) and known changes in choroidal PO_2 (Linsenmeier and Braun, 1992) in cat (used because there was no appropriate information for rat) showed that this exactly explained the increase in $IR-MRO_2$ (Yi et al., 2015b). That is, the increase in $IR-MRO_2$ in hypoxia was not due to an increase in $IR-QO_2$, but an increased contribution of the retinal circulation to $OR-QO_2$. This was a striking case of $IR-MRO_2$ overestimating $IR-QO_2$. $IR-MRO_2$ has also been measured in humans during hypoxia (Palkovits et al., 2014c), and in contrast, no change in $IR-MRO_2$ was found, at least down to 12% inspired O_2 , which corresponded to P_aO_2 of 45.5 mmHg. The difference between rats and humans may reflect the fact that in humans, less of the O_2 from the retinal

circulation diffuses to the outer retina than in rats. This is consistent with the microelectrode work referred to earlier, which showed that QO_2 of the outer retina depended less on the retinal circulation in monkeys than in rats.

The opposite situation, in which IR-MRO₂ *underestimates* IR-QO₂, also occurs. This probably accounts for the apparent *decrease* in IR-MRO₂ that has been measured during brief episodes of systemic hyperoxia (Palkovits et al., 2014a; Werkmeister et al., 2015), when no decrease in IR-QO₂ would be expected. This situation is not shown in Figure 14, but it will occur any time O₂ from the choroid helps supply the inner retina. The value of $s_{vT}O_2$ increases, and the arteriovenous difference decreases, because part of the inner retinal demand is met by diffusion from the choroid (Palkovits et al., 2014a).

Measures of IR-MRO₂ are very likely to reach the clinic, probably with vis-OCT, and possibly with other methods. However, the considerations above show that without understanding how it relates to actual QO₂, it will be very easy to misinterpret IR-MRO₂ and draw erroneous conclusions about inner retinal metabolism.

6. Oxygen and diabetes

Attempts to understand the role of O₂ in diabetic retinopathy go back several decades and there are many hypotheses about mechanisms (Frank, 2004; Stefansson, 1990; Stitt et al., 2015), but our understanding is still not complete. There are several issues to be considered with respect to O₂: 1) whether the retina is hypoxic in diabetes, and if so, when it begins; 2) how to interpret recent work on the protective effects of illumination; and 3) how panretinal photocoagulation and vitrectomy influence retinal O₂.

6.1. Retinal oxygen in human diabetics

The first question is whether the inner retina becomes hypoxic in diabetes, and if so, when this occurs, and whether or not O₂ plays a central causative role in pathogenesis. Tied up with this is the question of whether hypoxia or some other factor primarily explains the elevation of VEGF that is associated with vascular leakage and proliferative diabetic retinopathy.

While there are many similarities between the effects of diabetes on the retinas of humans and other mammals, there are enough uncertainties about how experimental diabetes in animals applies to the human disease that we consider humans and animals separately. In humans there is clear evidence that retinal VEGF is elevated in both background retinopathy (Amin et al., 1997; Kunz Mathews et al., 1997) and proliferative retinopathy (Adamis et al., 1994; Aiello et al., 1994). This appears to be a primary cause of vascular leakage, microaneurysms, and vasoproliferation, and the success of anti-VEGF treatments speaks to the importance of VEGF (Miller et al., 2013). The increase in VEGF could be caused by hypoxia, which would elevate VEGF by first increasing hypoxia inducible factor 1 α (HIF1 α). There are changes in the diabetic retina that could cause hypoxia at some stage, including leukostasis and slowed circulation (Chibber et al., 2007; Kaji et al., 2007; Kern, 2007), excitotoxic damage to glial cells (Lau et al., 2013), which could lead to impaired neurovascular coupling and hypoperfusion (Stitt et al., 2015), and endothelial cell and

pericyte loss that destroys capillaries (Kern and Engerman, 1996; Stitt et al., 2015). However, VEGF upregulation can be stimulated by many factors (Birk et al., 2008; Neufeld et al., 1999; Xie et al., 2004), including inflammation and acidosis, so the upregulation of VEGF alone cannot be taken as evidence of hypoxia. In addition, several stressors could occur together, so isolating the effect of hypoxia is difficult.

Direct evidence of retinal hypoxia in human diabetics is quite limited and is only available for advanced diabetic retinopathy, when the PO_2 in the vitreous has been measured just before vitrectomy. Holekamp et al. (Holekamp et al., 2006) measured PO_2 with a fiberoptic probe at two locations, in the center of the vitreous and close to the lens, before vitrectomy. They found that diabetics with ischemic disease (most of whom had proliferative retinopathy and/or vitreous hemorrhage) had significantly lower PO_2 (5.7 ± 0.7 mmHg) than non-diabetic controls (8.5 ± 0.6) in the midvitreous, and also lower PO_2 than normal subjects near the lens. Lange et al. (Lange et al., 2011) confirmed this result for the midvitreous of diabetics with macular retinal detachment (about 7 mmHg) compared to non-diabetics (about 11 mmHg). Much of the O_2 in the vitreous comes from the retina, according to animal studies (Alder and Cringle, 1985, 1990; Alder et al., 1991; Linsenmeier et al., 1981; Petropoulos et al., 2013), but in the midvitreous there is a great deal of spatial averaging, so midvitreal measurements are not good for resolving details at the retina, as noted in section 3.3. Consequently, Lange et al. (Lange et al., 2011) also made two measurements “at the retinal surface,” one near the macula and one superior to the superior artery, and averaged them, because they were reported to be similar. At this location, diabetics had significantly higher PO_2 s (about 16 mmHg) than controls (less than 10 mmHg). They also reported a positive correlation between PO_2 and vitreal VEGF levels, so it is possible that these patients were beyond a hypoxic phase and into a compensatory phase, and this is why PO_2 was higher. Presumably the lower vitreal PO_2 s in their diabetics then reflected the influence of spatial averaging across regions where the PO_2 at the retina was normal or high, with regions where neovascularization had not yet occurred, or where vessels had collapsed. The earliest measurements in diabetics were by Maeda et al. (Maeda and Tano, 1996; Maeda et al., 1992), who did not have a control group, but did make measurements with electrodes in multiple locations. Their electrode probably approached the retina more closely than the optical probes used in the studies above, but the cathode was still more than 100 μ m away. Consistent with the findings of Lange et al. (Lange et al., 2011), the PO_2 over a neovascular area was 32.2 ± 9.9 mmHg, higher than over the macula (17.4 ± 4.0 mmHg), photocoagulated retina (16.5 ± 5.5 mmHg) or non-photocoagulated retina (18.6 ± 4.9 mmHg). None of the measurements made by Maeda et al. would be regarded as hypoxic, however. These measurements, while valuable, illustrate the difficulty of studying the role of O_2 in diabetic retinopathy when measurements are made only late in the disease, only in the vitreous, and only with probes that average PO_2 over a large area.

In addition to the limited direct evidence of hypoxia in proliferative retinopathy, there are several lines of indirect evidence. First, as noted above, endothelial cells and pericytes are lost from the retinal circulation in animals and humans (Gardiner et al., 1994; Hammes, 2005; Kern and Engerman, 1996; Mizutani et al., 1996) and lead to capillary loss. The retinal circulation is generally not redundant, so loss of capillaries would be expected to leave areas of hypoxic tissue. It is possible that there is some compensation for this if there

is increased blood flow through nearby capillaries, which would extend the tissue volume that those capillaries typically supply, and minimize the hypoxia that would otherwise occur. Increases in blood flow can occur in diabetes relative to controls and can occur as diabetes progresses (Clermont and Bursell, 2007; Konno et al., 1996; Pemp and Schmetterer, 2008), but there is insufficient information about the degree of capillary loss, or about very local changes in blood flow, to know if this leads to a functional O₂ supply in areas without capillaries.

Second, it has been shown that diabetics with no apparent retinopathy have reduced contrast sensitivity, and that inspiration of 100% O₂ can normalize it (Harris et al., 1996). This does not occur in normal subjects, suggesting that the diabetic retina must have been hypoxic, possibly because of subtle retinal capillary non-perfusion. However, an alternative explanation is that this sensitivity change could be a consequence of changes in the outer retina (Bavinger et al., 2016; Boynton et al., 2015; Kern and Berkowitz, 2015; Kurtenbach et al., 2006), resulting from decreased choroidal blood flow (Nagaoka et al., 2004), or some other factor, and not hypoxia in the inner retina.

A third indirect line of evidence about hypoxia is that two treatments that can reduce or halt neovascularization in diabetic retinopathy, vitrectomy and panretinal photocoagulation, are most easily understood in terms of their ability to increase O₂ in the inner retina (Grunwald et al., 1989; Grunwald et al., 1986; Molnar et al., 1985; Petropoulos et al., 2013; Pournaras et al., 2008; Stefansson, 2006; Stefansson et al., 1981; Stefansson et al., 1992). The evidence for panretinal photocoagulation and vitrectomy is complicated and relies on both human and animal measurements, so these topics will be discussed further in sections 6.3 and section 6.4.

Fourth, some indirect evidence of hypoxia comes from investigating the regulation of the circulation in response to hyperoxia, which normally constricts the retinal circulation and decreases blood flow, as discussed in Section 4.2. These effects of hyperoxia are reduced in diabetics (Grunwald et al., 1984b; Justesen et al., 2010; Trick et al., 2006), and the usual argument is that the tissue was hypoxic during air breathing, so hyperoxia corrected this, and a constriction would have been inappropriate. Increases in blood flow (Garhofer et al., 2004) and vasodilation (Lim et al., 2014) in response to flickering stimuli are also reduced, possibly because the vasculature was already dilated to relieve tissue hypoxia. However, in both cases the situation is complicated by the finding that hyperglycemia itself blunts the response to hyperoxia (Gilmore et al., 2008) and flicker (Dorner et al., 2003), so hypoxia may not be the reason for changes in these vascular responses in diabetics.

Finally, Arden and colleagues first postulated (Arden et al., 2005; Arden et al., 1998), and subsequently found evidence (Arden et al., 2010; Arden et al., 2011) that preventing complete dark adaptation at night slows the progression of retinopathy [reviewed in (Ramsey and Arden, 2015; Sivaprasad and Arden, 2016)]. The rationale was that if the PO₂s in the inner retina are low, O₂ from the choroid would be helpful in compensating for this deficit. It is clear from the difference in PO₂ profiles in light and darkness that there is no “leftover” O₂ from the choroid to assist the inner retina in the dark, but during moderate illumination the reduced OR-QO₂ could allow choroidal O₂ to supply the deeper part of the inner retina,

even during air breathing. While the concept of preventing dark adaptation makes sense qualitatively, there have not been enough measurements of PO_2 in primates to predict the quantitative benefit. One piece of evidence cited by Arden and coworkers in support of the concept of light adaptation is that individuals who have both retinitis pigmentosa (RP) and diabetes rarely have diabetic retinopathy, and Arden argues that this is because choroidal O_2 reaches the inner retina in the absence of photoreceptors. This is true in animal models of RP (Padnick-Silver et al., 2006b; Yu et al., 2004; Yu and Cringle, 2005; Yu et al., 2000), but the conclusion from diabetics with RP cannot necessarily be generalized to all diabetics. When photoreceptors are lost in RP, the change in $OR-QO_2$ is much larger than it is during illumination, and the inner retina also moves physically closer to the choroid, so the flux of choroidal O_2 to the inner retina is much larger than during illumination (Padnick-Silver et al., 2006b; Yu et al., 2004; Yu and Cringle, 2005; Yu et al., 2000). The diffusion of O_2 from the choroid is so great in advanced RP that the retinal vasculature is strongly attenuated (Grunwald et al., 1996; Padnick-Silver et al., 2006b). While choroidal O_2 may provide an explanation for the lack of retinopathy in diabetics with RP, it does not necessarily follow that light would do the same thing. A larger clinical trial of the effect of light is currently in progress (Ramsey and Arden, 2015; Sivaprasad and Arden, 2016). If successful, it could provide further indirect evidence of inner retinal hypoxia.

The oximetry measurements in diabetes do not, so far, point directly to hypoxia, but interpretation is complicated here as well. Oximetry shows an increase in $svrO_2$ in diabetics relative to controls (Hammer et al., 2009; Hardarson and Stefansson, 2012). Without simultaneous measurements of blood flow it is difficult to know exactly how to interpret this, but if the blood flow in diabetics were similar to or lower than that in controls, which is often the case (Clermont and Bursell, 2007; Pemp and Schmetterer, 2008), then an increase in $svrO_2$ would imply a reduced $IR-MRO_2$, and most probably decreased $IR-QO_2$. Such a reduction in metabolism would be consistent with neural loss and/or dysfunction that is seen in humans and animals (Abcouwer et al., 2014; Antonetti et al., 2012). Increased $svrO_2$ would also imply increased tissue PO_2 , rather than tissue hypoxia, although this is very uncertain because the $svrO_2$ measurements are global and could reflect redistribution of blood flow or simply be too coarse to detect patches of hypoxia. A further observation is that when diabetics were made hyperglycemic, their $svrO_2$ decreased, which was suggested to mean that $IR-MRO_2$ increased (Tiedeman et al., 1998).

6.2. Oxygen in animal models of diabetes

In animals there is evidence of hypoxia in diabetes, but it is not uniform or entirely compelling, partially because rodents, which have been studied most, only exhibit some of the signs of retinopathy observed in humans. Diabetic rats do experience at least some degree of capillary loss (Hammes, 2005; Kern and Engerman, 1994, 1996) and have been found in many studies to have greater vascular leakage than age-matched controls (Brucklacher et al., 2008; Lu et al., 2007; Quam et al., 2001; Xu et al., 2001). However, rodents do not exhibit the later stages of retinopathy (Engerman and Kern, 1995), where a strong link to hypoxia would be expected. The best evidence to date comes from a small number of cats studied after 6.67 to more than 8 years of diabetes, which had vascular leakage and microaneurysms (Hatchell et al., 1995; Linsenmeier et al., 1998), essentially

background retinopathy similar to that in humans, as shown in Figure 15A. Other diabetic cats prepared in the same way went on to extensive capillary dropout (Figure 15B), but we elected to investigate intraretinal pH in those animals (Budzynski et al., 2005). In animals like those in Figure 15A there was a striking degree of hypoxia, indeed anoxia, in some profiles in the inner retina (Figure 15C), particularly in regions where some vascular disturbance could be identified. As a result of these extremely hypoxic profiles, the histogram of average inner retinal PO₂ was shifted to lower values compared to that in normal cats (Figure 15D). These remain the only intraretinal measurements in a large animal.

There have been relatively few measurements of intraretinal PO₂ in diabetic rats. Yu et al. (Yu et al., 2009) reported that the inner retina of Sprague-Dawley rats was hypoxic at 5 weeks, but there was very little detail in that report. We found that diabetic Long-Evans rats had higher inner retinal PO₂ after 3 months than age-matched controls (Lau and Linsenmeier, 2014), which appears to be consistent with the increased svrO₂ in humans in background retinopathy. However, at later times, up to a year of diabetes, we were unable to detect differences in inner retinal PO₂ between diabetics and age matched controls (Linsenmeier et al., 2016b), and found no profiles similar to those in Figure 15C. These animals were not immune to retinal changes, however. There were ERG changes and ganglion cell loss in these animals, and in comparable cohorts of rats there were reductions in the mRNA for ionotropic glutamate receptors and glutamate transporters (Lau et al., 2013) as well as retinal pH changes (Dmitriev et al., 2016a, b). With microelectrodes one cannot sample the entire retina, so it is possible that there were hypoxic regions that were missed, but the lack of hypoxia could explain why rats do not develop proliferative retinopathy. There could still be a decrease in IR-QO₂ in diabetic rats that is accompanied by a decrease in blood flow, so that PO₂ remains unchanged, and this needs to be tested.

The fluorescent tag pimonidazole and oximetry have also been used in diabetic rodents. Pimonidazole was developed for use in tumors, and is said to label regions where the PO₂ is less than 10 mmHg (Arteel et al., 1995; Raleigh and Koch, 1990), although this value has never been properly validated for the retina. Nevertheless, in agreement with our finding of increased PO₂ at 3 months, Wright et al. (Wright et al., 2010) found less pimonidazole labeling in diabetic Long-Evans rats than in control animals at this time. In another study, long term (5 months) diabetic mice showed a small but significant increase in labeling over age-matched controls (de Gooyer et al., 2006).

Non-invasive imaging methods have also been used to measure O₂ in the inner retina of rodents. Wanek et al. (Wanek et al., 2014) measured retinal artery and vein PO₂ in 4 and 6 week diabetic rats with phosphorescence imaging, and measured retinal blood flow by a particle tracking method. They found that IR-MRO₂ in rats was not different in diabetics and controls. We used Akita/+ TSP^{-/-} mice as a model for diabetes (Sorenson et al., 2013), because the absence of thrombospondin makes them develop retinal complications sooner than Akita/+ mice, which spontaneously become diabetic. Vis-OCT was used to evaluate retinal arterial and venous saturation and blood flow at several time points up to 13 weeks of diabetes, and IR-MRO₂ gradually increased, relative to control, non-diabetic TSP^{-/-} mice, mainly as a result of an increased arterial-venous saturation difference (Liu, 2016). This is

an intriguing result that currently has no counterpart, except the human study by Tiedeman et al. (Tiedeman et al., 1998), where the A-V saturation difference increased in diabetics during hyperglycemia. These studies currently need to be interpreted with caution, because it is possible that in both cases the A-V difference increased not because of a change in IR-QO₂, but because more of the O₂ extracted from the retinal circulation supplied the outer retina. This could happen if there was some change in the outer retina, for instance, a decrease in choroidal PO₂. We found such a change in acute hyperglycemia in cats (Padnick-Silver and Linsenmeier, 2003), where choroidal PO₂ decreased, and the fraction of OR-QO₂ supplied by the retinal circulation increased as a result. There was a suggestion of this in diabetic cats as well (Linsenmeier et al., 1998), but there was not enough information for a statistical test.

No measurements of changes in retinal blood flow during flicker have been made in animals with diabetes, but flicker usually evokes a small increase in the arteriovenous O₂ difference, and this was missing in rats with four weeks of diabetes (Blair et al., 2009). Blair et al suggested that the increase in inner retinal metabolism that usually accompanies flicker did not occur, but this was not tested directly. There has been little work in diabetic animals on investigating blood flow or PO₂ in response to hypoxia or hyperoxia, which could provide useful information about vascular regulation.

While an increase in VEGF in diabetes does not prove that the retina is hypoxic, the absence of an increase can be taken to mean that the retina must not be hypoxic. In rats, VEGF is elevated about 1 month of diabetes (Kirwin et al., 2009; Lau and Linsenmeier, 2014; Schrufer et al., 2010), when hypoxia is not suspected, but it is not increased at 3 months. Hammes et al. (Hammes et al., 1998) found increased expression of VEGF after 6 months of diabetes in Wistar rats, but suggested that this was due to increased reactive oxygen species rather than hypoxia (Kuroki et al., 1996), because the number of acellular capillaries at this time was small (although 2.7 times higher in diabetics than controls). Hammes et al. used immunohistochemistry to identify VEGF; in our measurements using a commercial ELISA, we have not found an increase in VEGF at 6 months in Long Evans, Brown Norway, or Sprague Dawley rats (Linsenmeier and Henderson, unpublished).

6.3. Panretinal photocoagulation and oxygen in animals and humans

We need to address the mechanism of outer panretinal photocoagulation (PRP) in stopping the progression of proliferative diabetic retinopathy. While the anti-VEGF agents have become valuable in diabetes, PRP is still a mainstay of treatment (Ferraz et al., 2015; Writing_Committee_for_the_Diabetic_Retinopathy_Clinical_Research_Network. et al., 2015). The rationale for PRP is entirely based on relieving hypoxia, by some combination of destroying the photoreceptors, the outer retinal sink for oxygen, which allows oxygen to diffuse across the scar tissue into the hypoxic inner retina from the choroid, and by reducing the thickness of the outer retina and bringing the inner retina physically closer to the choroid. Both human and animal studies have contributed to understanding the changes caused by photocoagulation of the outer retina. Decreases in retinal blood flow in humans after photocoagulation (Grunwald et al., 1989; Grunwald et al., 1986; Pournaras et al., 2008), which are consistent with relief of hypoxia, and vitreal PO₂ measurements in animals

(Funatsu et al., 1997; Molnar et al., 1985; Pournaras et al., 1985; Pournaras et al., 1990; Stefansson et al., 1986; Stefansson et al., 1981) supported the idea that PRP increased inner retinal PO_2 . However, what actually happens to the PO_2 gradients in the retina following photocoagulation was examined only recently in normal cats (Budzynski et al., 2008) and rats (Yu et al., 2005a). The results of both studies were similar. In cat, after making lesions that destroyed the outer retina but spared the inner retina, and allowing the lesion to heal, the inner retinal PO_2 was elevated relative to adjacent unlesioned areas (Budzynski et al., 2008), as shown in Figure 16A, supporting the hypothesis that photocoagulation in DR relieves inner retinal hypoxia. This is very similar to the situation in retinitis pigmentosa when photoreceptors are lost. In that case, enough oxygen diffuses from the choroid into the inner retina to nearly obliterate the retinal circulation (Padnick-Silver et al., 2006b). In cat, our photocoagulation was confined to the outer retina, but had to extend further across the retina than in humans for the practical reason that the microelectrodes entered the retina at an angle of about 45 degrees to the surface and the lesion needed to be large enough that the microelectrode would stay within the photocoagulated region. The surprise in this work was not the main result, but the decrease in PO_2 at the choroid that almost always occurred in the lesioned area (Figure 16B). In subsequent work (Lee et al., 2011), we showed that the choriocapillaris had been destroyed by the lesion as well, and severe damage is likely in humans, although little attention is paid to this. This happened in small lesions (Figure 16C) as well as large ones, which was verified by the absence of intraarterially delivered fluorescent microspheres in those regions (Figure 16D). An interesting aspect of this in terms of O_2 supply was that the loss of the choriocapillaris only reduced the PO_2 at the back of the retina by about 10%, so evidently the larger vessels of the choroid were capable of supplying O_2 almost as well as the choriocapillaris.

Because the photocoagulation lesions were large, we could not fully mimic the situation of panretinal photocoagulation. The experiments showed that the PO_2 in inner retinal layers directly over the lesioned outer retina would be higher, but it was not clear if the lesion had an effect on the PO_2 of inner retinal layers lateral to the lesion, a question that is relevant for the number and spacing of lesions. To understand this we turned to a mathematical simulation of the PO_2 distribution in and around a lesion (Budzynski, 2005; Budzynski and Linsenmeier, 2006). The geometry we investigated and two of these simulations are shown in Figure 17. Panel A shows a cat fundus with several small lesions. Panels B and C are cross sections through the retina cutting through the center of a lesion. Since the lesions were circular, a cylindrical region could be used to represent the lesion, and finite element methods could be used to calculate PO_2 gradients through the depth of the retina (z) and at different distances from the center of the lesion (r). The parameters for the outer retina were those from previous work. The inner retina was assumed to have oxygen utilization but no oxygen supply, mimicking the situation of complete capillary loss, but the PO_2 at the vitreal surface of the retina was taken to be 12 mmHg, because there is likely to be diffusion through the vitreous from undamaged regions of the retina. Choroidal PO_2 was assumed to be 62 mmHg in normal parts of the retina, but lower in the center of the lesion. In histological sections, the photoreceptors were sometimes replaced with scar tissue, and Figure 17B represents this geometry. The scar allowed diffusion, but did not consume oxygen. At other times, there was no scar tissue and the inner retina was adjacent to the

choroid. This geometry is represented in Figure 17C. For that condition, a transition region was created between the lesioned area and the adjacent normal retina, where the outer retina gradually thickened. At the right side of Figures 17B and 17C are the PO_2 maps in and around the lesion for the adjacent geometries, and a lesion radius of 250 μm . Above the lesioned area itself, the inner retinal PO_2 s are higher than in adjacent retina (lighter blue). It is also clear that the increased PO_2 made possible by the lesion extended only a small distance outside the cylinder of the lesion. It is difficult to tell from the dark colors of Figure 17, but the increase in PO_2 caused by the lesion only extended about 50 μm past the edge of the lesion in Figure 17B, and barely past the transition region in Figure 17C. Reducing the metabolism of the inner retina to half of normal allowed the effect to extend to 40 to 90 μm past the transition layer (not shown). The limited lateral effect of the lesions, which could only be assessed through modeling, indicates why so many closely spaced photocoagulation spots must be placed in the retina in order to achieve a clinical effect.

Section 6.4. Vitrectomy and oxygen in animals and humans

Vitrectomy has been promoted as a treatment for diabetic retinopathy, and like panretinal photocoagulation, the rationale is largely based on O_2 . This is an area where there has been confusion about what the data show. The concept is that with a less viscous vitreous, O_2 can move more readily from regions of higher PO_2 to lower PO_2 , and to some extent relieve hypoxia in areas where there is ischemia. Stefansson (Stefansson, 2006) reviewed the strong clinical evidence that vitrectomy is effective, and in that paper and an earlier one (Stefansson, 1990) described the hypothesis that the effect is due to convection of oxygen. Measurements of PO_2 have been made in several human studies to investigate whether vitrectomy in fact changes O_2 delivery to the retina. Maeda and Tano (Maeda and Tano, 1996) measured PO_2 at several sites in the eye in patients just before vitrectomy, and in another population that had previously had a vitrectomy. PO_2 was not different in most locations, including locations close to the retina (macula and non-macula), but the PO_2 was lower in vitrectomized eyes in regions near the optic disk and in neovascularized areas. The oxygen electrode was a 100 μm platinum wire in a 25g needle, so it could not record very locally near the retina, and it was not possible to do measurements in the same individuals before and after vitrectomy. Holekamp et al. (Holekamp et al., 2005) did make measurements before and after vitrectomy, but only in the mid-vitreous and behind the lens. They showed that PO_2 s increased shortly after vitrectomy, but they recognized that this was likely due to the perfusion of the eye with oxygenated fluid, so they also made measurements in previously vitrectomized eyes, and, in contrast to Maeda and Tano (Maeda and Tano, 1996), Holekamp et al. (Holekamp et al., 2005) did find increased PO_2 in the vitreous in vitrectomized eyes compared to eyes that were about to have vitrectomies, possibly suggesting an increase in PO_2 at the retina as well as in the mid-vitreous. A noninvasive magnetic resonance imaging study of vitreal PO_2 in subjects before and at least 3 months after vitrectomy supports this result (Simpson et al., 2013). However, it seems possible that with less viscous intraocular fluid oxygen from the ciliary body rather than the retina might mix into the vitreous and elevate PO_2 in the mid-vitreous and near the lens, but not change PO_2 substantially at the retina. Williamson et al. (Williamson et al., 2009) used electrodes to make measurements closer to the retina than Holekamp et al. (Holekamp et al., 2005) before and after vitrectomy following central retinal vein occlusion rather than

diabetic retinopathy. They found that preretinal PO₂ increased from 8 to 75 mmHg after vitrectomy, but unfortunately, their measurements were made at an unknown time after introducing the perfusion fluid used in the surgery and were undoubtedly contaminated by the O₂ in that fluid. The electrodes in that study were also not small enough to obtain localized preretinal measurements and have been criticized on that basis (Park et al., 2011). Thus, the human measurements are all inconclusive. If vitrectomy helps, it is likely to move O₂ very locally through vitreous adjacent to the arterial side of the retinal circulation to locations in a more venous area or one without circulation, and the important changes are likely to occur very close to the retina, as Figure 10 shows, so measurements in mid-vitreous or with larger electrodes that cannot approach the retina would not be expected to resolve the matter.

Because adequate measurements in the human eye are so difficult, two sets of microelectrode measurements in animals are relevant. In cats, Stefansson et al (Stefansson et al., 1990) showed that a prior vitrectomy allowed preretinal PO₂ to remain close to normal when a branch retinal vein was occluded, whereas average PO₂ fell dramatically following venous occlusion if no vitrectomy had been done. Petropoulos et al (Petropoulos et al., 2013) recorded gradients in the vitreous near arterioles, venules, and intravascular zones in normal eyes of minipigs. While vitrectomy initially increased the PO₂s in all areas, this was attributed to the oxygenated perfusion fluid, and over the next 2.5 hours, the PO₂s and the gradients returned to initial levels. Stefansson et al (Stefansson et al., 1990) and others have postulated that it is convection in the far less viscous fluid in the vitrectomized eye that moves O₂. It has not been possible to directly investigate the influence of convection on O₂ transport in the vitreous, because the eye needs to be stationary during measurements, but there is strong theoretical support that vitreal convection can occur, and would enhance the movement small molecules including O₂ (Balachandran and Barocas, 2011; Filas et al., 2013; Repetto et al., 2010). However, changes in diffusion in the vitrectomized eye would not be expected, because the O₂ diffusion coefficient across tissues is strongly dependent on percent water content (Vaupel, 1976). The gelled vitreous is 99% water, so after vitrectomy, when the vitreous is 100% water, would not matter. Consistent with this, Petropoulos et al (Petropoulos et al., 2013) convincingly showed experimentally that O₂ gradients do not change after vitrectomy. Arguments that it does change (Gisladdottir et al., 2009; Stefansson and Loftsson, 2006) are based on considering the vitreous as a viscous fluid rather than a gel and are not valid (Barton et al., 2007). Thus, the results of Stefansson et al (Stefansson et al., 1990) that PO₂s are higher over occluded regions after vitrectomy are the ones that are puzzling, because presumably convection did not occur in the cat experiments. A close examination of the data shows that vitrectomy did not increase PO₂ in the ischemic region in every eye, and it seems possible that in some animals movement of the probe could have caused convection that enhanced transport.

An additional factor is that ascorbic acid in the vitreous consumes O₂, converting ascorbate to dehydroascorbic acid. Vitreal ascorbate concentration is close to 2 mM, whereas Henry's law predicts that at a PO₂ of 20 mmHg, O₂ concentration would be only about 0.03 mM. Thus, there is an excess of ascorbic acid, and the vitreous does have the capacity to degrade O₂ in this way. This is an important effect (Filas et al., 2013; Shui et al., 2009), and the capacity of vitreous to metabolize O₂ decreases after vitrectomy, so vitreal PO₂ could

increase as a result, although this is probably more important for the lens than the retina. At this time it is safe to conclude that the beneficial effect of vitrectomy is not due to changes in diffusion, but could well be due to convection, as Stefansson et al (Stefansson et al., 1990) proposed and theoretical analyses show, combined with the ascorbate mechanism proposed by Beebe and colleagues.

7. Oxygen in retinal artery occlusion

Central or branch retinal artery occlusion is a condition for which there is no accepted treatment (Hayreh, 2011), but where O₂ could probably play a larger role. It has been demonstrated several times that if the retinal circulation is occluded, which makes the entire thickness of the inner retina anoxic, then inspiration of 100% oxygen can bring inner retinal or vitreal PO₂ back to nearly normal values in cat and monkey retina (Alder et al., 1990; Braun and Linsenmeier, 1995; Landers, 1978). One study in rat showed that part of the inner retina remained anoxic, but hyperoxia elevated PO₂ through most of the inner retina (Yu et al., 2007). The beneficial effect of O₂ inspiration is due to O₂ diffusion from the choroid as illustrated in Figure 18A. The retina can survive occlusion for at least 100 min (Hayreh et al., 1980; Hayreh et al., 2004) without hyperoxia, and it can undoubtedly survive longer occlusions in an animal breathing 100% O₂. Even when PO₂ is restored to normal, hyperoxia does not completely normalize the retina, because there is still a reduced b-wave (Braun and Linsenmeier, 1995) and acidosis (Birol et al., 2005), but if metabolism is maintained until reperfusion, which often occurs, e.g. (Duker and Brown, 1988), the retina has a good chance of recovery. There is a concern that O₂ would lead to excess reactive oxygen species, but this is not certain and some damage is better than a dead retina. Of course, if there is a normal part of the retina, as in a branch occlusion, it is important that hyperoxia does not damage this part of the retina. Relevant to this point is the finding that in cats with a retinal detachment, 3 days of 70% oxygen did not lead to increased apoptosis in the normal part of the retina (Mervin et al., 1999). There is also a fear that excess O₂ will constrict the retinal circulation, but if the inner retina is severely hypoxic, this is not likely to occur.

Ischemia is a complex topic, and many facets have been reviewed elsewhere (McLeod and Beatty, 2015; Osborne et al., 2004; Pournaras, 1995). We can offer some ways to think about the problem with respect to O₂ in arterial occlusion. The primary parameters that are expected to play a role in the efficacy of hyperoxia are the delay between occlusion and treatment, and the total amount of time that O₂ is administered. The shorter the time between occlusion and treatment, the better (Augsburger and Magargal, 1980), and the delay that would still allow some benefit is not currently known and almost certainly varies with the severity of the lesion. In cats a delay of an hour before starting 100% O₂ still allowed recovery of the b-wave to about 50% of normal (Birol et al., 2004), but some benefit is likely with delays of several hours as described below. In animals it is possible to use 100% O₂ for long periods, but oxygen toxicity to the lungs limits this in humans. There may also be retinal toxicity with 100% administration for 18 or more hours per day (Beehler et al., 1964), but the duration that leads to retinal toxicity is not currently known. It would only be relevant if retinal toxicity occurs at shorter durations than lung toxicity.

The clinical experience with hyperoxia during arterial occlusion is mixed. The duration of hyperoxic episodes and the time between them appears to vary between successful and unsuccessful clinical trials, with more successful trials delivering more oxygen sooner, more often, and at hyperbaric pressures (Butler et al., 2008; Oguz and Sobaci, 2008). For instance, in one relatively successful trial that included patients with arterial occlusions of 8 hours or less (Beiran et al., 2001), O₂ was administered at 2.8 atmospheres (ATA), twice per day for 90 min each time. Acuity improved in 83% of treated patients compared to 30% of controls, which had some adjunct treatments but no O₂. Another successful trial was done with patients having 12 hours or less of occlusion (Menzel-Severing et al., 2012). These patients received 2.4 ATA for 3 times on the first day, for 90 min each, as well as hemodilution to reduce blood viscosity. These patients had a mean acuity improvement of 3 lines, while control patients receiving only hemodilution averaged one line. In contrast to these studies, Atebara et al. (Atebara et al., 1995) found that carbogen (95% O₂–5% CO₂) plus paracentesis, had no benefit over paracentesis alone, with only 15% of the patients improving in either case. However, the average duration of the occlusion before treatment was 15 hours, and patients were accepted into this study for up to 24 hr. Carbogen was only given for 10 minutes during each of 12 hours per day. This was very likely too little and too late.

A further complication in retinal artery occlusion is whether it is actually complete, and whether there is some potentially useful plasma flow even if a blood clot stops erythrocyte flow. In their review of the value of fibrinolytic therapy, McLeod and Beatty (McLeod and Beatty, 2015) suggested that even in a serious occlusion, there is a penumbra around the infarct that is much like the penumbra after a stroke in the brain. This region has hypoxic but not anoxic silent tissue that can be recovered if blood flow is restored within a few days, as it is in a large number of cases. Hyperoxia may assist this region, and McLeod and Beatty (McLeod and Beatty, 2015) have argued that the function of this region needs more attention.

It is important to realize that the issue of whether to treat arterial occlusion with hyperoxia is not yet settled. The questions that can be addressed with animal data and modeling are whether hyperbaric oxygen is substantially better than hyperoxia with 100% O₂, and what duration of O₂ makes sense.

7.1 Simulation of retinal oxygen gradients during occlusion

In order to simulate gradients of O₂ across the retina during occlusion and hyperoxia (Figure 18B), we assumed values for OR-QO₂ and IR-QO₂ from dark adapted cat retina, which is the worst case, since light will reduce OR-QO₂ and allow more O₂ to reach the inner retina. The entire inner half of the retina can be considered to have uniform IR-QO₂, consistent with measurements (Braun et al., 1995). We further assumed that breathing 100% O₂ will result in the choroidal PO₂ established in animal work (Figure 13) and that the only O₂ reaching the inner retina does so by diffusion from the choroid. That is, we took the PO₂ at the vitreal surface of the retina to be zero, which is again a worst case. Then we used the simulation to determine what fraction of the normal IR-QO₂ would allow PO₂ to be above zero throughout the retina. (Alternatively, one could determine the fraction of the inner

retina that could have normal IR-QO₂, and the part that would have zero IR-QO₂.) Given these assumptions, the inner retina could have a value of IR-QO₂ that is 80% of normal during 100% O₂ inspiration at 1 ATA. If the choroidal PO₂ were 20% higher (i.e. if the inspired O₂ was 1.2 ATA), then IR-QO₂ could be maintained at its normal value. In Figure 18B the asterisk near a choroidal PO₂ of 100 is the value expected in a person breathing O₂ through a nasal cannula, which is reported to result in the equivalent of about 35% O₂ inspired. This would not elevate intraretinal PO₂ enough to be useful. Now, because Pc is linear with inspired PO₂, we can also ask what the gradient would look like at 2 ATA of 100% O₂, and this is shown in Figure 18C. This will make the PO₂ very high across the whole retina, but this is not necessarily beneficial. The modeling suggests that hyperoxia at slightly elevated total pressure is expected to be adequate, and even at 1 ATA hyperoxia is expected to maintain almost normal inner retinal metabolism.

The next question is how rapidly PO₂ would rise and fall at the onset and end of hyperoxia, so that we can judge whether hyperoxia has any beneficial effect beyond when it is actually administered. For 100% O₂ breathing, where the oxygen is all used within the retina on a continuous basis, the time constants are short, less than 1 min for the increase or decrease of intraretinal PO₂ at the onset and end of an episode of O₂ breathing (Linsenmeier and Yancey, 1989). Thus, the only value of hyperoxia would be during the actual administration. Hyperbaric O₂ might have a somewhat longer lasting effect, because during administration, O₂ diffuses into the vitreous, and then it diffuses back to the retina following hyperoxia. Unfortunately this is quite slow. If hyperbaric oxygen produces a PO₂ of 150 mmHg at the vitreoretinal border, then just 5 mm into the vitreous the PO₂ would only reach about half of that value after an hour. In addition, the flux from the vitreous at the end of hyperoxia is slow compared to the metabolism of the retina. The contribution of the vitreal reservoir is therefore expected to be small. Further modeling is required to completely analyze this situation, but it is likely that hyperoxia at 1 ATA, which is available at any hospital, would be almost as good as hyperbaric oxygen.

Because O₂ is not stored, the duration of hyperoxia should be as long as possible and the frequency of episodes should be as high as possible without causing lung or brain damage. While a detailed recommendation is beyond the scope of this report, the duration could be at least as long as the episodes of hyperbaric oxygen that have been used successfully, i.e. 90 min. One can calculate that only a 2% (and reversible) decrease in vital capacity is likely to occur after 10 hours of hyperoxia at 1 ATA (van Ooij et al., 2013), and no lung or neural toxicity was detected in a study that used 3 hours of hyperoxia at 1 ATA (Rockswold et al., 2010). Hyperoxia with anything like this duration has never been attempted as a treatment for arterial occlusion.

8. Oxygen and retinal detachment

In retinal detachment the gap between the choroidal circulation and the photoreceptors increases dramatically, and, while there is no tissue that consumes O₂ under the detachment, the distance alone is a problem, because it reduces the flux of O₂. As a consequence of the lack of O₂ and possibly other factors, photoreceptors undergo a number of changes, and also die (Delolme et al., 2012; Fisher et al., 2005; Nork et al., 1995) and there is an activation of

Muller cells (Lewis et al., 1999). Remarkably, photoreceptors have some protective mechanisms during detachment which undoubtedly limit cell death. The photoreceptors deconstruct their outer segments (Fisher et al., 2005), and they also reduce their synaptic contacts with second order neurons (Fisher et al., 2005). Both of these changes, but particularly the loss of outer segments, are protective, because they reduce the O_2 demand dramatically. As discussed in section 3.1.1., the two largest metabolic demands in photoreceptors are for ATP to power the Na^+/K^+ ATPase in the inner segment, and for GTP to serve as the substrate for the cGMP that regulates the light-dependent cation channels. By losing the outer segment, the photoreceptor eliminates the demand for cGMP and therefore GTP, and it dramatically reduces Na^+ influx and Na^+ pumping, and therefore the demand for ATP. The photoreceptor no longer needs to make new outer segment membrane either, which is an additional small saving of energy. At some point, if left untreated, there should be a balance between the available O_2 and the number of remaining photoreceptors, which is likely to vary with the height of the detachment.

There is very strong evidence from animals that hyperoxia would be beneficial in limiting photoreceptor deconstruction and saving photoreceptors. The most compelling evidence is from studies in which retinal detachments were made in cats (rod-dominated retinas) (Mervin et al., 1999) and ground squirrels (cone-dominated retinas) (Sakai et al., 2001). In both studies, some animals were maintained in air, while others were kept in a 70% oxygen environment for 3 days. This level of hyperoxia was chosen to be safe for an extended period. At the end of this time, there was much less apoptosis of photoreceptors in the animals treated with hyperoxia. In order to explain these results, we did both mathematical modeling and experiments. Figure 19 shows the results of modeling (Linsenmeier and Padnick-Silver, 2000), in which we assumed that the normal complement of photoreceptors was always maintained. Figure 19A shows the gradient through the outer half of the dark-adapted retina when the retina is attached, and then when it is detached by 100 μm and 500 μm . Even a 100 μm detachment is expected to reduce OR- QO_2 to 34% of normal, and a 500 μm detachment reduces it to 15% of normal. The decrease is not linear with detachment height because the retinal circulation is assumed to ride along with the detachment, providing its normal contribution to the photoreceptors. Nevertheless, the O_2 supply is dramatically reduced even in small detachments, and the retina would either have to share this reduced supply among photoreceptors, or prune some. However, in Figure 19B the simulated animal breathes 100% O_2 and OR- QO_2 is restored to 78% of normal. Photoreceptor loss would be expected to be much smaller, as observed experimentally (Mervin et al., 1999; Sakai et al., 2001). Figure 19C shows the percentage of normal OR- QO_2 that can be maintained as a function of detachment height, assuming either air breathing (lower curve), hyperoxia with an increase in choroidal PO_2 but not the PO_2 in the retinal circulation (middle), and hyperoxia with an increase in PO_2 in both circulations (upper curve), which is probably the real situation, as discussed in section 4.2. These curves show that increased PO_2 in the choroid is helpful, but that adding the effect of increasing P_L (PO_2 at the inner-outer retinal boundary) is a key contributor to improved retinal oxygenation. These curves are for dark adaptation, and if one assumes the metabolic rate for light-adaptation, then the combination of increased P_c and P_L can maintain normal OR- QO_2 for any detachment height. The photoreceptors may naturally tend toward light adaptation in

the detached region, because once the retinal detaches from opsin after being activated by light, it will be much harder (at least slower) for the retinal to move to the RPE and back to the photoreceptors in a detached retina.

We verified the theoretical results of Figure 19 with measurements of PO₂ profiles in the detached cat retina during air and 100% O₂ breathing (Figure 20A,B) (Wang and Linsenmeier, 2007). With detachments calculated to be about 500 μm high in the center (where we were not always able to measure profiles), OR-QO₂ was reduced to an average of 47±18% of the control value, as shown in Figure 20C, which is a little more than expected from Figure 19C. Hyperoxia was beneficial, and restored OR-QO₂ to 68±17% of normal. Also, as predicted from the modeling, hyperoxia both held the PO₂ at the outer border of the photoreceptors at a relatively normal level (Figure 20D) and increased in PO₂ at the inner border of the outer retina (Figure 20E). Figure 20B shows two features that we did not include in the modeling of Figure 19. First, the profiles are not quite linear in the fluid layer, as would have been expected for a region with no consumption. This could have occurred if there were some convection in the fluid layer; in our case possibly caused by the movement of the electrode. The second factor is that there is a bend in the profile where the outer segments (os) meet the fluid layer (fl), but there is no consumption in either of these layers. This bend is a consequence of the difference in O₂ diffusion coefficient (D) between the fluid layer and the retina. The flux has to be the same on both sides of the boundary between the fluid and the outer segments, that is:

$$J = D_{fl} k_{fl} dP/dx|_{fl} = D_{os} k_{ok} dP/dx|_{os} \quad (10)$$

However, D in the fluid is essentially D for water, whereas D in the retina is 70% of that value (Roh et al., 1990). For equation 10 to be true then, dP/dx in the fluid should be about 70% of dP/dx in the outer segments, and it is. The implications of both of these deviations from our theory is that in reality more O₂ would reach the retina through the fluid than our model had predicted.

These results suggest that hyperoxia could be beneficial in saving photoreceptors, particularly in a macula-off retinal detachment, which has the most severe consequences for vision, but this has never been evaluated clinically. We recommend that once a macula-off detachment is identified, patients be admitted to the hospital and put on a regimen of hyperoxia until the retina is reattached. While 100% O₂ could be somewhat beneficial if given episodically, the experimental results (Mervin et al., 1999; Sakai et al., 2001) suggest that 70% O₂ would be beneficial as well, and this can be given nearly continuously. Hyperoxia should not only preserve photoreceptors, but reduce glial activation, enhancing the speed and extent of vision restoration. Clinically one could either provide 70% O₂ from a premixed gas cylinder, or mix 100% O₂ with air to achieve 70%. The latter would require monitoring the patient's arterial PO₂ to ensure that arterial PO₂ is in the right range, which cannot be done with a pulse oximeter because it will read full saturation for any inspired O₂ fraction above that in normal air. Catheter-based arterial PO₂ measurements would be useful, but are too invasive, and are not continuous. However, one could monitor end tidal PO₂,

which is very close to arterial PO_2 , with certain instruments designed for anesthesia, e.g. the Capnomax Ultima (Datex Ohmeda).

9. Oxygen and AMD

Age-related macular degeneration (AMD) takes two forms, one characterized by drusen and loss of photoreceptors and RPE cells (dry AMD progressing to geographic atrophy), and the other characterized by choroidal neovascularization (wet AMD), and while potentially related, these need to be considered separately here. No PO_2 measurements have been made in either type of AMD, which do not naturally occur in animals. Both forms are complicated, with a number of simultaneous changes (Bhutto and Luty, 2012; Spaide et al., 2003), and while hypoxia probably plays a role in both forms, we do not wish to suggest that it could explain everything.

For wet AMD, as in diabetic retinopathy, the presence of neovascularization and elevated VEGF, and the success of anti-VEGF therapies, has directed attention toward some role for hypoxia. It has been somewhat puzzling, however, that the RPE, the source of VEGF in the outer retina, could be hypoxic when it is adjacent to the choroid. Stefansson et al. (Stefansson et al., 2011) reviewed the potential role of O_2 and of metabolism in AMD, and concluded that at the root of the problem in wet AMD is decreased choroidal blood flow. This would very likely lead to increased O_2 extraction from the choroid, and reduce the PO_2 in the choriocapillaris, and as a result, reduce PO_2 at the RPE. A similar argument, starting with reduced perfusion, but emphasizing reactive O_2 species and activation of complement, was made by Feigl (Feigl, 2007, 2009). In fact, if the RPE is separated by drusen or even an altered Bruch's membrane, from the choroid, the VEGF produced by the RPE, which is believed to be vasoprotective to the choroid (Adamis and Shima, 2005), would have a more difficult time reaching the choroid, leading to choroidal damage and decreased flow. Both filling defects (Chen et al., 1992; Ciulla et al., 2002; Pauleikhoff et al., 1999) and reductions in choroidal blood flow rate (Ciulla et al., 1999; Grunwald et al., 1998; Metelitsina et al., 2008) are correlated with, and to some extent precede, AMD pathology.

In dry AMD, Stefansson et al. (Stefansson et al., 2011) suggested that the diffusion of O_2 to photoreceptors would be impaired by drusen, and that merely the increased distance of the photoreceptors from the choroid could be significant in causing retinal hypoxia. This conclusion was based largely on the analysis of retinal detachment, in which we showed that even very small detachments could impair O_2 transport. While it is not easy to know the causal relationship, photoreceptor loss is localized to areas of large drusen (Johnson et al., 2003; Sadigh et al., 2013), so this appears to be a reasonable suggestion.

In order to investigate the effect of drusen on the metabolism of photoreceptors, we turned again to a simulation, this time with a different geometry. The model for detachment used the one-dimensional geometry referred to in section 2 because the lateral extent of the detachment is large relative to retinal thickness, and the model should be valid except near the edges of the detachment. However, because drusen are small mounds underneath the RPE, we turned to a more appropriate geometry and used finite element modeling. The assumption was that choroidal PO_2 under the druse was normal. Any choroidal ischemia

would make the situation worse than shown in the simulation. We analyzed drusen of several sizes, roughly hemispherical in shape. The simulation again assumed dark adaptation, because this set the constraint that the minimum PO_2 was zero in the inner segments. The main question, as in detachment, was what level of photoreceptor oxidative metabolism could be supported in the presence of drusen? Figure 21 shows one of these simulations, for a druse that is 200 μm in diameter and 70 μm high. The PO_2 profile through a normal region has the familiar shape, but in the center of the druse, just as in a detachment, the gradient is shallower, meaning that less O_2 reaches the inner segments. One difficulty with these simulations is that the diffusion coefficient through drusen (D_{dr}) is not known, and it may be different than in retinal tissue. Drusen are also not all the same, but have varying lipid and protein compositions (Wang et al., 2010a). We addressed this by allowing D_{dr} to vary from half of D in the retina to twice that value, and Figure 22 shows how OR- QO_2 is expected to vary with both drusen height and the relative value of D_{dr} . The result is that any druse higher than about 40 μm is expected to compromise photoreceptor metabolism, and this does not depend greatly on D_{dr} . In order to come into balance with the available supply of O_2 , either each photoreceptor would have to have a lower metabolic rate, or chronically derive more of its total metabolic demand from anaerobic glycolysis, or else a variable fraction of the photoreceptors would have to be lost, depending on drusen height. There is a good correlation between thickness of the photoreceptor layers and drusen thickness (Sadigh et al., 2013), suggesting that the latter situation may obtain.

10. Challenges

The understanding of PO_2 and metabolism of the retina has progressed tremendously in the last 30 years, but there is still much more to do. Limitations of our current knowledge have been mentioned throughout and only a few of these will be highlighted here.

10.1. Longitudinal and spatially resolved O_2 imaging in humans

Animal experiments can and will provide guidance, but the role of oxygenation in slowly progressing human diseases, particularly diabetes, AMD, and glaucoma, is still unclear. The rodent animal models of retinal oxygenation and metabolism in these particular diseases are of somewhat limited value, because the rodents do not develop exactly the same phenotypes as humans. The new non-invasive imaging methods, which can not only measure saturation in vessels, but measure inner retinal metabolism by combining saturation with blood flow, will be helpful in tracking progression of human disease longitudinally, and in evaluating therapies. The imaging methods are still limited in several ways, however, and some technical challenges need to be addressed. First, the imaging measurements are still largely global, representing the entire inner retina, but the diseases of interest do not affect the whole retina equally, so the development of regional and capillary level measures will be important. Second, measuring IR-MRO₂ has become possible, but it is difficult to see how to obtain OR-MRO₂ from measurements on the choroid. The Fick Principle requires sO_2 in the inflow and outflow along with blood flow, and it is not apparent how to obtain these in the choroid. In addition, the influence of light on the outer retina is much larger than on the inner retina, and the imaging measurements all require light, making it more difficult to evaluate human OR-MRO₂ in darkness. Changes due to light in OR-MRO₂ are relatively

slow, however, so if measurements can be made quickly, an essentially dark-adapted value might be obtainable.

It has also been known for a very long time that retinal O₂ plays an important role in retinopathy of prematurity (Ashton, 1954; Caprara and Grimm, 2012; Hartnett and Penn, 2013; Patz, 1984; Smith, 2008). There is still a question of how much O₂ to give to very low birth weight infants, and whether treatments are safe and effective, so it would be helpful to be able to measure retinal O₂ in some way. Here the problem with PO₂ differences across the retinal depth and eccentricity are compounded by the small size of the eye, fragile health of the neonates, and changes in the vasculature over a short period of time.

10.2 Matching microelectrode and imaging measurements

As described in section 5, microelectrodes measure PO₂ in tissue with high spatial resolution, and imaging measures PO₂ intravascularly and more globally (except in a few cases, which do not yet have high spatial resolution (Shahidi et al., 2010)). The metabolic measures derived from the two methods are not the same, but should be complimentary, and the detailed knowledge from microelectrodes should be able to help with interpretation of imaging. We need to understand how MRO₂ and QO₂ are related, which will require combining microelectrode and imaging measurements in animals to derive a model for what the imaging methods tell us.

10.3 Effective use of hyperoxia in clinical practice

We have argued, on the basis of strong theoretical and animal studies, that hyperoxia needs to be revisited as a therapy for both arterial occlusion and retinal detachment. For occlusions, there is also supportive clinical data. What appears to limit the use of O₂ therapy is a misunderstanding of O₂ diffusion and storage, a fear of vasoconstriction, and a fear of oxygen toxicity. Prior trials of hyperoxia in arterial occlusion have not used hyperoxia in a way that is consistent with our understanding of retinal oxygen diffusion and consumption, and revisiting hyperoxia could potentially save or improve the sight of many individuals.

Whether hyperoxia could also play a role in diabetes is an interesting and unsolved question. Oxygen is easily available in all medical centers and has been used successfully in a small trial of diabetic macular edema (Nguyen et al., 2004). Hyperbaric O₂ has been used in cystoid macular edema (Pfoff and Thom, 1987). Godman et al. (Godman et al., 2010) have argued that hyperoxia induces antioxidant protection, and are using hyperbaric O₂ to treat chronic diabetic foot ulcers (G. Perdrizet, personal communication).

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Appendix. Abbreviations

Abbreviation	Definition	Typical units
ChBF	Choroidal blood flow	ml/min
D	Oxygen diffusion coefficient. Dr = Dos: in retina or outer segments. Ddr = in drusen. Dfl = in fluid under detached retina.	cm ² /sec
Fr	Fraction of oxygen used by the outer retina that is supplied by the retinal circulation	unitless
I (λ)	Light reflected at wavelength λ . I _v (λ) = from blood vessel. I _b (λ) = from background.	quanta or quanta/sec
k	Oxygen solubility	mlO ₂ -ml tissue ⁻¹ -mmHg ⁻¹
L	Thickness of outer retina in diffusion model	μ m
L ₁	Thickness of layer 1 (outer segments) in diffusion model	μ m
L ₂	Border between layer 2 and layer 3 in model. (L ₂ - L ₁) is thickness of layer 2 (inner segments)	μ m
MRO ₂	Metabolic rate of O ₂ . OR-MRO ₂ = O ₂ extracted from the choroidal circulation. IR-MRO ₂ = O ₂ extracted from the retinal circulation.	mlO ₂ -100g ⁻¹ -min ⁻¹
OD (λ)	Optical density at wavelength λ	unitless
PO ₂	Oxygen tension. PaO ₂ = systemic arterial PO ₂	mmHg
Pc	Choriocapillaris PO ₂	mmHg
P _L	PO ₂ at the boundary between inner and outer retina	mmHg
QO ₂	Oxygen consumption. Q ₂ = QO ₂ of layer 2 in diffusion model. Q ₄ = QO ₂ of layer 4 (inner retina) in diffusion model. OR-QO ₂ = O ₂ consumption of the outer retina (= Q _{av} = Q _{OR}). R-OR-QO ₂ = portion of OR-QO ₂ supplied by retinal circulation. IR-QO ₂ = O ₂ consumption of the inner retina (= Q _{IR}).	ml O ₂ -100g ⁻¹ -min ⁻¹
RBF	Retinal blood flow rate	ml/min
sO ₂	Oxygen concentration in vessels. Subscript r = retinal; subscript c = choroidal; subscript a = artery; subscript v = vein	ml O ₂ /100 ml blood

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Figure 1.

Representative O_2 profiles through the dark-adapted retina of rhesus monkey (top), cat (middle) and Long-Evans rat (bottom). A retinal depth of zero is the vitreo-retinal border, and 100% is the RPE-choroid border. An approximate scale of retinal layers is shown for cat. Modified from (Linsenmeier, 2012).

Figure 2.

Three-layer one-dimensional mathematical model of outer retinal oxygen diffusion and consumption. Parameters of the model are indicated: P_c : choriocapillaris PO_2 , P_L : PO_2 at the border between the avascular outer retina and the vascularized inner retina; L_1 and L_2 : borders of the consuming region, layer 2; L : border between outer and inner retina; Q_2 : Oxygen utilization of layer 2. Oxygen utilization in layers 1 and 3 is zero. The solid curve is an illustration for dark adaptation in which Q_2 is $21.6 \text{ ml O}_2\text{-}100\text{g}^{-1}\text{-min}^{-1}$ (so $OR\text{-}QO_2$ is $4.5 \text{ ml O}_2\text{-}100\text{g}^{-1}\text{-min}^{-1}$) and the dashed line is a simulation for light adaptation, in which Q_2 is half the value for the dark-adapted case. From (Linsenmeier and Pournaras, 2008).

Figure 3.

Principle of fundus photography-based retinal oximetry. (a) Illustration on how to extract pixelated intensity values to estimate optical attenuation by blood vessels. Modified from Figure 4(c) in (Liu et al., 2013). BK: background; (b) A typical result of human retinal oximetry. Figure 3 in (Geirsdottir et al., 2012).

Figure 4.

Retinal sO₂ imaging using multi-wavelength PAOM. (a) Fundus image generated by max-amplitude-projection of the volumetric photoacoustic data from an adult Sprague Dawley rat. Bar: 200 μ m; (b) PAOM cross-sectional images at three optical wavelengths (570 nm, 578 nm, and 588 nm) from the scanning trajectory highlighted by the white circle in panel a; (c) Upper: pseudo-colored vessels as imaged in the circular B-scan based on their measured sO₂ values; lower: comparison of sO₂ values in all the major vessels (numbered from 1 to

12) along the circular scanning trajectory. Figures are modified from Figure 1 and Figure 2 in (Song et al., 2014).

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Figure 5. Retinal sO₂ imaging using Vis-OCT. (a) *En face* mean-amplitude-projection image of the Vis-OCT volumetric data acquired from a Long Evans rat *in vivo*; (b) *En face* mean-amplitude-projection of the motion enhanced microangiography. Each major retinal arterioles and venules are color-encoded based on the measured sO₂ values; (c) A three dimensional rendering of retinal microvasculature imaged by Vis-OCT. Figures modified from Figure 3 in (Chen et al., 2015b).

Figure 6.
Dark-adapted (blue) and light-adapted (red) PO_2 profiles from a Long-Evans rat.

Figure 7.

PO₂ changes during 2 min steps of illumination in distal retina of a monkey. The retina was in darkness except between 30 and 150 sec, when it was illuminated with the levels shown at the right (in log quanta-deg⁻²-sec⁻¹). The transition from darkness to light is fitted with single exponentials. At and above 8.9 log quanta-deg⁻²-sec⁻¹, there was a rod after-effect, and the PO₂ took many minutes to return to baseline after illumination. From (Wang et al., 2010b).

Figure 8.

Histograms of inner retinal PO₂ in cat retina obtained from dark-adapted profiles (left) and light-adapted profiles (right) when arterial PO₂ was above 85 mmHg. Modified from (Linsenmeier and Braun, 1992).

Figure 9.

Spontaneous fluctuations in PO_2 in the cat retina. Left: Sixty second samples of PO_2 obtained at the depths shown in one cat retina. In the inner retina (top two traces), the PO_2 varies in a sporadic way. In the outer retina, fluctuations disappear and only electrode noise is apparent. Near the choroid (bottom trace) the dominant fluctuations are at the respiratory frequency. Right top: Total power in spontaneous fluctuations in the inner retina in 43 records from 13 cats. Right bottom: Frequency distribution of the fluctuations from the same records, with power normalized to its own total power before averaging. Modified from (Linsenmeier and Braun, 1992).

Figure 10.

Computational model of O_2 diffusion in the vitreous. A. the modeled region and PO_2 scale, which applies to A and B. The average PO_2 at the retinal surface was set to 20 mmHg, and random fluctuations of ± 15 mmHg were added to simulate the locations of arterioles or venules in the retina. B. Enlargement of the boxed region in A. Arrows show the direction and magnitude of local O_2 flux, illustrating the non-uniformity of PO_2 within about 250 μm from the retinal surface, and the diffusion of O_2 from regions of higher to lower PO_2 through the vitreous. The region in B is about 1200 μm by 1200 μm . C. PO_2 as a function of distance from the retina along the red and blue dashed lines in B. By approximately 250 μm into the vitreous the gradient becomes essentially independent of the PO_2 at the retinal surface, but this distance depends on the distribution of sources and sinks as suggested by panel A. (From (Filas et al., 2013)).

Figure 11.

Lactate production and O₂ and glucose consumption in the outer retina (top) and inner retina (bottom) in darkness (orange bars) and light (blue bars) derived from arteriovenous differences and blood flow measurements in the pig retina. From (Linsenmeier, 2010), modified from (Wang et al., 1997c).

Figure 12.

Effect of hypoxia on retinal PO₂ in cat. A. Profiles obtained during dark adaptation at three values of arterial PO₂, given at the right of traces. Profiles are superimposed in the lower part of the panel. B. Profiles obtained during light adaptation at three values of arterial PO₂. C. Choriocapillaris PO₂ as a function of arterial PO₂ in normoxia and hypoxia. Individual cats are represented by different symbols, with filled symbols representing measurements during dark adaptation, and open symbols representing measurements in light adaptation. From (Linsenmeier and Braun, 1992).

Figure 13.

Effects of hyperoxia on PO₂. A. Relationship of choriocapillaris PO₂, measured with microelectrodes, to inspired PO₂. Data from (Braun and Linsenmeier, 1995; Linsenmeier and Yancey, 1989; Pournaras et al., 1989; Yu et al., 1999; Yu et al., 2005b; Yu et al., 2007). B. Profiles of PO₂ in rat retina at graded levels of hypoxia. Modified from (Yu et al., 1999).

Figure 14.

Schematic representation of O_2 supply from the retinal and choroidal circulations to the inner and outer retina, showing the relationship between O_2 extraction from the retinal circulation ($IR-MRO_2$) and the O_2 consumption of the inner and outer retina ($IR-QO_2$ and $OR-QO_2$).

Figure 15.

Effect of long-term diabetes on oxygenation of cat retina. A. Fluorescein angiogram at the stage when O₂ measurements were made (7 to 9 years diabetic). B. Fluorescein angiogram from a cat at a later stage of retinopathy. C. PO₂ profiles in a normal cat (top), and two diabetic cats (middle and lower). D. Histograms of average inner retinal PO₂ in each profile from 3 diabetic cats (top) and 21 diabetic cats (bottom). A, C, D modified from (Linsenmeier et al., 1998); B from (Budzynski et al., 2005).

Figure 16.

Effects of photocoagulation on the cat retina. A. Average inner retinal PO₂ in cats in normal areas of retina and adjacent areas with argon laser photocoagulation of the outer retina. Recordings were at least 4 weeks after the photocoagulation. Each line represents data from one animal. Grand averages are shown to the left and right of data points. B. Choroidal PO₂ in control and photocoagulated areas in the same animals, normalized to PO₂ in the control area of each animal. C. Small outer retinal lesions made to investigate effect of photocoagulation on choroidal blood flow. D. Flat mounted choroid from animal shown in C, revealing the distribution of fluorescent microspheres (colored dots) embedded in the choriocapillaris after arterial injection. There were rarely any microspheres in the choriocapillaris under a lesion, indicating lack of choriocapillaris blood flow. A and B: From (Budzynski et al., 2008). C and D: (Lee et al., 2011).

Figure 17.

Simulations of PO₂ distribution in and around photocoagulation lesions. A. Photograph of lesions in a cat retina. B: Left: Cylindrical geometry for simulations of lesions. The lesioned area has scar tissue in place of the outer retina. The inner retina (light gray) is in its normal location. Outside the lesion the retina has the usual layers. For the simulations, layer 2 was assumed to have normal QO₂ outside the lesion, and no QO₂ in the scar tissue. The inner retina was assumed to have normal metabolism, but no circulation, so at all radial positions, the simulation represents an area with severe capillary loss. Right: PO₂ distribution resulting from the finite element simulation from the center of the lesion ($r=0 \mu\text{m}$) to normal retina at $r=500 \mu\text{m}$. The color scale for PO₂ is shown at the far right. C: Similar to B, but showing the other frequently observed lesion geometry, in which the outer retina disappears, and the inner retina (light gray) becomes adjacent to the choroid. In these simulations a transition region is included, in which the thickness of the outer retinal layers gradually increases from the lesioned area to normal retina. In both geometries, the PO₂ in the inner retina over the lesion is elevated (lighter blue) relative to adjacent areas, consistent with the data of Figure 16, but the effect of photocoagulation only extends a very small way lateral to the lesion. Modified and reanalyzed from (Budzynski, 2005).

Figure 18.

Effect of hyperoxia during arterial occlusion in the cat retina. A. PO₂ profiles in dark-adapted cat retina before occlusion, during occlusion of a branch retinal artery with a glass probe and air breathing, and during occlusion and oxygen breathing. B. Simulated profiles with a four-layer PO₂ model (Braun et al., 1995) during occlusion and air breathing, occlusion and 100% O₂ breathing (assuming a choroidal PO₂ that would be expected from Figure 13A, and occlusion with a choroidal PO₂ just high enough to sustain inner retinal metabolism (Q₄ or IR-QO₂) at the normal level. The asterisk shows the choroidal PO₂

expected for inspiration of O₂ through a typical nasal cannula. C. Similar to B, but including a simulation for O₂ delivery at 2 ATA.

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Figure 19.

Effect of retinal detachment on retinal PO_2 and outer retinal O_2 consumption. A. Simulation of outer retinal metabolism in a cat breathing air, when the outer retina (horizontal bar) is attached, or detached by 100 and 500 μm . The dashed line is the model for the attached retina in light adaptation. The boundary PO_2 s are 62 mmHg at the choroid, and 15 mmHg at the inner border of the avascular layer. Detachment is expected to severely reduce photoreceptor metabolism. B. Simulation of a 500 μm detachment during air and O_2 breathing, with the boundary values as noted on the figure. Inspiration of O_2 is expected to

elevate outer retinal QO_2 . C. Effect of detachment on OR- QO_2 for different detachment heights under three assumptions: air breathing (bottom curve), O_2 breathing when P_C but not P_L is elevated (middle curve), and O_2 breathing when both P_C and P_L are elevated (top curve). The retinal circulation makes only a small contribution to photoreceptor metabolism ordinarily, but in the setting of detachment, the modest hyperoxic increase in inner retinal PO_2 represented by P_L here, is of great benefit to the photoreceptors, as shown by comparing the middle and top curves. From (Linsenmeier and Padnick-Silver, 2000).

Figure 20.

Experimental results of using hyperoxia during retinal detachment in cat. A. A PO₂ profile through a dark-adapted cat retina during air breathing. P_C is at the left of the fluid layer (FL) under the retina, and P₀ represents the PO₂ at the border between the fluid and the retina. B. A PO₂ profile during hyperoxia and detachment. C. OR-QO₂ in detachment during hyperoxia, and detachment during air breathing, normalized to the value in the attached retina during air breathing in 9 cats. D. P₀ in detachment during hyperoxia, and detachment during air breathing, normalized to the value in the attached retina during air breathing. E. P_L in detachment during hyperoxia, and detachment during air breathing, normalized to the value in the attached retina during air breathing. In C-E, asterisks show significant differences at p<0.05. From (Wang and Linsenmeier, 2007).

Figure 21.

The effect of a druse on PO₂ in the outer retina. Top: Cylindrical geometry to simulate a druse (here 70 μm high and 200 μm in diameter) under the retina, which pushes the outer retina forward. The O₂ consumption of layer 2 (Q₂) was decreased in the area over the druse just enough to keep PO₂ from going below zero. For this simulation the druse had the same O₂ diffusion coefficient as the retina. Vertical lines represent the position of the PO₂ slices in the lower part of the figure. PO₂ color scale at right. Bottom left: PO₂ vs. distance from the simulation through normal retina. Bottom right: PO₂ vs. distance in the center of the druse. Modified and reanalyzed from (Peddada and Linsenmeier, 2005).

Figure 22.

Effect of a druse on outer retinal O_2 consumption as a function of the O_2 diffusion coefficient in drusen and drusen height. This graph was constructed from data obtained from simulations like those in Figure 21, in which the relative diffusion coefficient of drusen was varied from half of the value in the retina to twice the value in retina. As expected, if the diffusion coefficient is higher, more O_2 can get through the druse, so the impact on photoreceptor metabolism is lower, and the lines have small positive slopes. However, no matter what the diffusion coefficient, drusen of 40 μm or more in height are expected to compromise O_2 delivery and oxidative metabolism in the photoreceptors, and this is progressively worse for larger drusen.