Ocular oxygen measurement

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Oxygen is essential for the metabolism of retinal cells and thus for their functioning. Considered metabolically as a very active tissue, the retina is sensitive to hypoxic conditions. Even in health, the functional reserve of oxygen in the human retina lasts for only seconds.

The function of the blood is transportation of nutrients and oxygen to the tissue; that function will be compromised in vascular pathology. Blood flow measurement is an interesting tool for assessment of haemodynamic alterations, although at present few techniques are applied in the clinical setting. Even more challenging for progress in ophthalmology research is direct evaluation of the metabolic supply and needs of the ocular tissue. Apart from the importance of oxygen for retinal metabolism, a direct pathophysiological role of oxygen in ocular pathology also deserves attention. Retinal hypoxia has been implicated in the formation of pathological new vessels. This neovascularisation is a major cause of visual loss in ischaemic retinal diseases, of which diabetic retinopathy is the most common.

There is no current clinical method to quantitate ocular hypoxia. Most investigations of the role of oxygen in the eye have been performed in animals, predominantly in the retina. Based on such data, distribution and consumption of oxygen in retinal tissue have been estimated using mathematical models of diffusion. In humans, only indirect evidence of the presence of retinal hypoxia has been obtained so far, either by measuring the size of intercapillary areas or by measuring oxygen tension during intraocular surgery.

The recent developments in techniques to measure ocular oxygen justify an overview of this subject and of their contribution to our knowledge of the oxygenation in the normal eye. In addition, a link between oxygenation and disease is proposed at the end of this review, with the main focus on diabetic retinopathy, in which a pathophysiological role for oxygen seems more and more evident.

Variables and techniques in oxygen studies

MEASURED VARIABLES
Research on oxygenation can be classified in several ways. (The various approaches to induce experimentally in the eye a fixed degree of oxygenation to study the effects of hypoxia or hyperoxia, are not considered here. They are described by Linsenmeier.) According to the applied method, a subdivision can be made into invasive and non-invasive techniques as well as into local and global assessment of hypoxia. A distinction can also be made between more direct or indirect methods for assessing oxygen deficits in the tissues (Table 1).

The interest of clinical research lies in methods that can assess hypoxia locally, quantitatively, and as directly as possible and that are suitable for patient use (thus preferably non-invasive). Therefore, the following classification for oxygen studies is proposed.

Global and indirect
A global impression of oxygen deficit in the entire retina can be derived from indicators of normal retinal functioning. Hypoxia occurs if oxygen inspiration at hyperbaric pressures improves physiological factors, such as vision, or retinal electrophysiological recordings. However, a large sized electrophysiological response cannot on its own be assumed to imply better function, since electrical resistance in the eye may have changed as a result of retinal disease.

Another global method is the one suggested by Grunwald et al. They assumed that the magnitude of the vascular regulatory response to 100% oxygen breathing, measured as a decrease in blood flow, could serve as an index of retinal hypoxia in diabetics. If the diabetic retina is hypoxic as is supposed by many authors, a part of the additional oxygen is used to satisfy the metabolic demand. The rest causes a smaller rise in partial pressure and can explain the smaller regulatory response they found in diabetic eyes compared with normals. This regulatory method can be valid only if it is assumed that regulatory power is normal.

Indirect demonstration of hypoxia and, more specifically, the metabolic supply can also take place by defining the geometry (size, area) of intercapillary non-perfused areas, as performed in patients with diabetic retinopathy. Enlarged capillary-free zones result in increased oxygen diffusion time, which may cause chronic hypoxia. The relation between ischaemia and hypoxia is, however, unclear. Tissue oxygenation can be maintained through blood flow alterations. If blood supply is insufficient, atrophy of the ischaemic tissue may reduce its oxygen consumption.

Local and invasive
Electrode studies Until now most research on oxygenation of the retina has focused on measuring oxygen tension (Po2) in tissue using oxygen sensitive microelectrodes. It is believed that the perivascular Po2 controls in some way the supply of oxygen to tissues. The non-uniform distribution of vascular supply and consumption results in Po2 gradients across the retina. These gradients are assessed by withdrawing the electrode from the choroid to the vitreous

| Variables used to define and/or quantify oxygen (hypoxia) in the eye |
|--------------------------|-------------------------|
| General physiological ophthalmic variables (for example, vision) | Global |
| Size and other quantifiable characteristics of avascular zones | Global |
| PO2 | Local/invasive |
| Respiratory chain members (cytochrome oxidase, NADH) | Local/invasive |
| Oxygen saturation | Local/(non)invasive |
The steepness of the slope of recorded oxygen tension profiles is related to the amount of oxygen consumption. The oxygen consumption rate, which is an indicator of the rate of metabolism, can be calculated from the tissue Po$_2$ gradients by the application of the Krogh diffusion model.15

An important advantage of the microelectrode method is that it is possible to measure oxygen tension as a function of depth in the retina, providing detailed information of sources and sinks of oxygen in the retina. Disadvantages are initially linked to its invasive nature. Studies with this technique therefore have been limited almost exclusively to animal research. Clinical application is restricted to a selected group of patients undergoing intraocular surgery. The invasiveness can complicate the correctness of the measurements. The electrodes can thereby disturb the Po$_2$ gradients by compressing the capillaries in the tissue.12 The anaesthetics used for in vivo experiments reduce the blood flow in the central nervous system by reducing the activity of the tissue.12 Secondly, measurement is restricted to one location at a time and the complex three dimensional Po$_2$ gradients (through spatial distribution of the microvasculature) cannot be assessed directly.11 Difficulties may even exist in defining the precise electrode recording location.11 Furthermore, one of the major drawbacks of the use of oxygen sensitive electrodes is the fact that the electrode itself consumes oxygen.16 This does not apply to a fiberoptic sensor, as was used by Stefansson and coworkers.17

The considerable technical difficulties inherent in measuring retinal tissue Po$_2$ resulted in research in which point measurements of the vitreal partial pressure of oxygen close to the retina were made (that is, preretinal measurements).18-20 It was assumed that the results reflect the situation in the retina. Alder and Cringle,22 however, measured large Po$_2$ gradients in the vitreous near the internal limiting membrane. It is therefore doubtful whether such an extrapolation can be validated. These gradients depend on the Po$_2$ of the blood flowing in the retinal vessels and on their location.

**Quenching** Another technique that is well known in physiology, but relatively new in ophthalmology, uses boluses of chemicals which have the property of phosphorescence or fluorescence. This method is based on the theory of quenching.22 The molecules capable of, for example, phosphorescence can, if excited into the triplet state, emit phosphorescence or transfer their energy to another molecule (a quenching agent). The only significant quenching agent in biological materials is oxygen. The concentration of oxygen in the vicinity of the phosphorescent molecule determines the intensity changes of the phosphorescence emission. Two different approaches exist in optical imaging based on the quenching method. One uses intravascular23 and the other perivascular Po$_2$24 in the retina.

**Intravascular** measurements have been performed in cats by Shonat et al.,23 who used a metalloporphyrin bound to albumin. The optical procedure used had been developed by Vanderkooi et al.24 This method detects the phosphorescence emitted from all of the vasculature and the results are therefore dominated by the choroid that has a much larger blood volume compared with the retinal vessels. Recent indications for significant A-V shunting between retinal vessels25 imply that venous blood Po$_2$ may not be an adequate reflection of the surrounding tissue Po$_2$ at all locations in the retina, even in a steady state condition. Because retinal function is reflected more accurately by the Po$_2$ of the retinal tissue than that of the blood, attempts have been made to extend this technique to tissue measurements.

Zuckerman and associates17 have developed a method to measure retinal tissue oxygen concentration using quenching of the fluorescence of the very lipid soluble fluorophore sodium pyrene butyrate. This technique is based on observations of Vaughan and Weber, who demonstrated, in 1970, the functional use of phenylbutyrate as an accurate and precise dynamic probe of the oxygen concentration in a microenvironment.22 After administration of the substance intravenously or intraperitoneally, it passes through the vessel wall because of its high lipid solubility, and accumulates within the lipid bilayers of the retinal tissue cells. With this technique an oxygen map can be made of the retinal perivascular oxygen tension, derived from intensity changes or fluorescence lifetime estimation. The distinct advantage of the latter method is that it is not necessary to make corrections for haemoglobin absorption. It is also not necessary to compare the results with an image of the same retinal region as referential 'zero line' (0 mm Hg, to be assessed by breathing 100% nitrogen). A disadvantage is that the model can only be applied for capillary-free zones around the retinal arterioles. Like other methods it is also not able to reflect the complex three-dimensional gradients in parts of the tissue traversed by a network of retinal capillaries. The technique can be improved by the use of better charge coupled device cameras to suppress noise and by slicing the retina optically with the use of confocal microscopy or digital procedures. Although a very promising technique aimed at future use for human investigations, it can at present only be used in animal experiments. The toxicological dangers have still to be assessed.

First reports of a study on simultaneous measurement of oxygenation and blood flow (in vitro) were presented at the ARVO meeting 1995.26 The techniques of phosphorescence quenching and laser Doppler velocimetry were combined.

**Spectrophotometry** Oxygen saturation can be assessed in the vasculature by spectrophotometry, which is mostly applied non-invasively (see below). For visual control of spatial location and to minimise noise, a micro light guide has been applied in the rabbit retina and anterior eye segment.27 28
Intermediates of oxidative phosphorylation At the molecular level, hypoxia can be assessed by measuring intermediary substances of oxidative phosphorylation process. The reduced forms accumulate during hypoxia because the oxidation process is stagnating. The phosphorescence (using UV-A) and spectrophotometric methods (using near infrared) are mainly used now in animal research of the brain. 29

Novack and coworkers measured cytochrome a,a3 in the eye (optic nerve head) by means of dual wavelength reflection spectrophotometry with an intraocular microprobe. 30 To maximize the collection of reflected light, the optical probe was inserted into the eye via a pars plana incision and positioned as close as possible to the optic nerve. Possibly this method can be developed for non-invasive measurements without the use of light guides. It must be realised, however, that only a weighted average of the phosphorescence from different optic nerve depths is achieved in this way. The technique can be used as an indicator for the metabolic state of the tissue, because it is very sensitive to small changes in oxygen availability.

Nuclear magnetic resonance (NMR) spectroscopy Wilson et al developed a relatively non-invasive method to measure preretinal oxygen tension in human eyes by means of 19F NMR spectroscopy. 31 This technique was previously used for the measurement of vitreal PO2 in the rabbit eye. 32 It makes use of small droplets of perfluorotributylamine that are retained in the eye after intraoperative retinal tamponade with a larger volume of this material during retinal detachment repair. The spin lattice relaxation rate (T1) of the fluorine nuclide is directly proportional to the partial pressure of dissolved oxygen (PO2) at a given temperature, which can be partly attributed to the high oxygen solubility of the perfluorocarbons relative to water. The results are comparable with the microelectrode studies without the disadvantage of limitation to per-operative measurement. The approach is, however, limited to vitrectomised eyes. Furthermore, the long time constants for oxygen uptake and clearance, particularly in perfluorotributylamine volumes of the order of 10 μl and greater, may represent a practical limitation of this method for determining rapid oxygen flux in the preretinal vault site.

Local and non-invasive Spectrophotometry Oxygen saturation measurements as performed by spectrophotometry were used for the first time in the 1970s in rabbit experiments 33 and in human retinal blood. 34 35 The laborious method employed was not suitable for clinical use or for the study of rapid temporal changes. The disadvantages were lessen by Delori who developed a three wavelength method. 36 The principle of this method is reflection oximetry. A fundus camera is used to project monochromatic light of several wavelengths on the retina. The reflected light is enhanced, digitised, and interpreted on line. The grey values are proportional to the optical density of the tissue. By making use of the differences in absorption characteristics of haemoglobin and oxyhaemoglobin, the oxygen saturation of the blood can be assessed. Arterial oxygen saturation varies between 96% and 98% in subjects and is a factor that depends on the function of the lung. 37 For the retinal arterial oxygen saturation a fixed value of 97% can therefore be taken in experiments. 38 When venous oxygen saturation is assessed, the oxygen extraction by the tissue (that is, oxygen demand or consumption in a steady state situation) can be calculated. In combination with assessment of blood flow, the oxygen availability/demand ratio can be calculated which can be used as an index of hypoxia. 3 De Kock et al modified a standard haptic contact lens for continuous monitoring of retinal arterial oxygen saturation with a pulse oximeter in anaesthetised patients undergoing surgery 39

The technical difficulties of retinal oximetry are to be found principally in the signal to noise ratio: how much light can be projected without retinal damage and how much light is needed for proper detection of the information? Technical improvements in light sources and more sensitive cameras for detection can boost the signal to noise ratio and promise clinical use. While measuring oxygen within veins, as with oxygen tension measurements, arteriovenous shunts have to be reckoned with. Tissue PO2 can be derived through modelling. Then, the small fraction of the ‘delivered’ oxygen that is dissolved in the tissue and later used up (a non-steady state condition) 40 is also taken into account.

Derived variables Important derived variables in oxygen physiology are oxygen supply and oxygen consumption, which can be assessed in combination with the blood flow and by mathematical models.

The basic aspects of oxygen supply and usage can be derived from the shapes of the oxygen tension profiles as measured in electrode studies. To analyse and interpret the experimental data on oxygen supply in a quantitative way, many attempts have been made to fit these data into mathematical models. There are numerous investigators who have contributed by mathematical modelling to our understanding of oxygen transport in the microcirculation (the phases of release, diffusion, and consumption). 41 42 43 August Krogh launched the first important theory with his famous oxygen diffusion model, which still is the basis of all such studies. 44 This model is based on the first law of Fick; the oxygen extracted from a (cylindrical) capillary is equal to that supplied to a cylinder of tissue concentric around the vessel. It has been used to estimate the oxygen consumption rate in the rat retina from tissue PO2 gradients. 45 Kreuzer reviewed this model and its assumptions and stated that, after decades of research in the theoretical field rather than the experimental field, a linkage and comparison between them is urgently needed. 46 Attempts in this direction give different opinions about the experimental usefulness of the Krogh model. 47 To date the heterogeneity of the microvascular system seems to be the challenge to handle, with the most novel and promising approach being the Bruley-Williford-Kang (B-W-K) technique to calculate three dimensional, time dependent solutions in heterogeneous, convection, diffusion, and reaction systems. 48

Mathematical modelling of retinal oxygen diffusion and consumption gives attention to the stratified structure of the retina and the heterogeneous distribution of blood vessels and mitochondria. The heterogeneity of flow in different regions has also to be reckoned with, especially in pathology. Ben-Nun et al demonstrated with a trichrome technique in diabetic rats the loss of patency of red cells in some retinal vessels resulting in mainly plasma perfusion. 49 Models of steady state oxygen diffusion have been developed for the outer avascular retina, where oxygen consumption and supply are spatially separated. 50 Data from retinal PO2 profiles are fitted to the models to calculate the consumption of the tissue. So far, they cannot be applied to the vascularised inner retina. The application is useful currently for the study of photoreceptor oxygen consumption under different experimental circumstances, such as occlusion studies as performed by Alder et al and Braun and Linsenmeier. 51 Bohne and associates described a model to assess the influence of pressure induced ischaemia on oxygen transport in the optic nerve head. 52

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Oxygenation in the normal eye

Oxygen is delivered to retinal tissue via two vessel systems. The retinal circulation shows autoregulation, which helps to maintain oxygen homeostasis,54 55 while the choroidal bed has no56 or only limited capacity for autoregulation.52 53

The choroidal circulation has a high blood flow, 20 times larger than the retinal circulation. Nevertheless, about the same amount of oxygen extraction takes place from both circulations. Therefore, in the choroidal blood the arteriovenous oxygen saturation difference is small and the PO2 in the choriocapillaris, which lies adjacent to the retinal pigment epithelium, is high.54 One explanation frequently stated is that this is needed to move enough oxygen by diffusion into the outer avascular retina to supply the photoreceptors in particular.55 Bill postulates that the choroid has other purposes than feeding the retina, and suggests a function as a thermostat.66

In contrast, the retinal circulation has a relatively low blood flow and a high arteriovenous oxygen saturation difference. Venous saturation here is 20–28% lower than in veins elsewhere.55 57 The retinal circulation can only supply enough oxygen to support the aerobic metabolism of 15–35% of the retina.54 55 The choroidal circulation takes care of the rest; however, in darkness (see below) it can supply only about a quarter of the retinal thickness.67 Next to respiration, aerobic lactate production seems to take place in the retina.44 Major glycogen supplies are found in the inner nuclear layer at the location where retinal oxygen content is lowest. This suggests that oxygen consumption should be used with care as an indicator of metabolism.

Influence of physiological factors

To gain insight into the dynamics of retinal oxygenation, (pre)retinal PO2 assessment has been done in response to occlusion of the retinal circulation,15 48 54 52 changes in intraocular pressure,37 45 44 and adaptation to light or dark.40 47 54 55

As argued by Steflansson, it is difficult to relate blood flow studies directly to the presence or absence of retinal hypoxia, not knowing what is cause and effect.68 It seems possible under occlusion of the retinal circulation to oxygenate the whole retina when the choroidal circulation is made hyperoxic.69 An acute rise in intraocular pressure lowered the PO2 in the choriocapillaris, and an anoxic region developed in the middle retinal layers. The inner layers of the retina were relatively resistant to rises in intraocular pressure.34

Several authors conclude that retinal oxygen consumption is considerably increased in the dark,9 49 suggesting a greater risk of hypoxic injury in the dark than in the light. This is probably due to increased metabolism in the rods, as reflected by the greater magnitude of the dark current. Rods are quite numerous in mammalian retinas. One might expect that if measurements are limited to cones, as in the macula, oxygen consumption would remain as high in the light as in darkness. However, the literature so far does not confirm this.65 Dark adaptation experiments can therefore provide a means for early detection of hypoxia in ocular tissue. With the electrode method this is possible to do accurately because no illumination is needed.

Animal studies

Extensive measurements of oxygen distribution have been made in the retina of the cat,15 47 60 and in the miniature pig or piglet.60 70 Recently such measurements have also been reported for the rat,71 72 and in primates.65 It is still difficult to compare those studies with results obtained in humans, although the cat retina may provide a relatively good model for oxygen distribution and consumption in the parafoveal retina of primates and, presumably, humans; the fovea of primates is comparable to the human fovea.15

Microelectrode measurements have shown in mammals that there is a relatively uniform distribution of oxygen tension in the inner retina, which reaches a minimum at some point near the middle of the retina and rises steeply to a maximum value at the tips of the outer segments, near the choroid (see Fig 1). The exact location of the minimum is at present unknown. Bruch's membrane between choroid and retina has a high electrical resistance and forms a diffusion barrier to oxygen.15 71 Local peaks in oxygen tension have been found in the fovea of primates,55 indicating diffusion from vascularised regions around the fovea and from the vitreous. In the cat it has been shown that when oxygen inhalation was raised to 100%, arterial and venous tissue PO2 increased and a steep PO2 gradient developed preterminally in the vitreous.76 The results of Linsemeier and coworkers confirm this.66 On the other hand, in miniature pigs no change in preretinal PO2 was found at hyperoxia,64 which may be explained by species differences, or a difference in response of the vascular autoregulation to the anaesthetics used.54 Oxygen tension gradients have also been measured across the wall of the large retinal blood vessels and arterioles, and even in the bloodstream near the vessel wall.79 Considering the observed gradients, oxygen seems to be already diffusing out of these vessels before reaching the capillaries.49 70 71 One can postulate that this diffused oxygen is not lost, but supplies the capillary-free zone of the retina. Buerg and associates have tried to quantify these '02 losses' by measuring the oxygen tension gradients in the vitreous near the cat retina.75 All arterioles had outward oxygen flux with an overall mean of 2.58×10−6 ml O2/sec/cm².

Human studies

During cataract surgery, the PO2 distribution has been measured in the ocular chambers.15 49 It shows a gradient from chamber angle to the centre of the pupil. This indicates that aqueous humour oxygenation occurs along the iris anterior surface.15 The lens is an extremely low oxygen compartment.15 10 During vitreous surgery values of vitreous oxygen tension have been found to range from 16 mm Hg in the posterior vitreous to 20 mm Hg in the anterior peripheral vitreous body.79 Saturation measurements in human retinal blood have been done by spectrophotometry.35 36 Delori measured, in a group of 22 normal subjects, an average oxygen saturation of 98% for arteries and 45% for veins.80 Thus, little more than half of the oxygen carried by the arterial haemoglobin is extracted in the retinal tissue.

Oxygenation and ocular disease

Insight into tissue oxygenation might improve our understanding of ocular diseases in which hypoxia may play a role—for example, glaucoma,50 neurogenic optic atrophy,86 and pseudoxefoliation syndrome.80 In addition, it may help to evaluate treatments aimed at improvement of ocular oxygenation—for example, with gaseous oxygen,61 66 with oxygen carriers like perfluorocarbons,87 or with other drugs.69 88 The issue of tissue oxygenation has been studied particularly in diabetic retinopathy,67 72 81-86 and in the neovascularisation process which characterises this disease, as well as in the photocoagulation used to treat it.71

Diabetic retinopathy

Release of angiogenic factors in response to an ischaemic retina is currently the most important hypothesis for the neovascularisation seen in proliferative retinopathy.98 One
of the molecules identified is vascular endothelial growth factor (VEGF), which seems to be regulated by the oxygen level in the tissue.\(^1\) The production of VEGF in the eye is increased by hypoxia in retinal pigment epithelial cells,\(^1\) \(^2\) in retinal pericytes\(^3\) and in ischaemia induced neovascularisation of the iris in primates.\(^4\) It has been shown in vitro that this production is reversible by establishing a normal oxygen supply.\(^5\) \(^6\) The relevance of this phenomenon for human disease is indicated by a significant increase in the VEGF levels in the ocular fluids of patients with progressive diabetic retinopathy, sampled during intraocular surgery, compared with normals.\(^7\)

Neovascularisation of the iris is explained by diffusion of growth factors from a hypoxic retina to the iris.\(^8\) Another explanation is that a hypoxic retina steals oxygen from the iris and anterior chamber, thereby causing localised areas of iris hypoxia which produce new vessels. This in turn may lead to a facilitated oxygen flow to the hypoxic retina.\(^9\) Furthermore, iris neovascularisation is most prominent where there is contact between iris tissue and aqueous humour, such as along the angle of an iridectomy.

Many animal and theoretical studies concerning hypoxia and neovascularisation have been related to photoocoagulation therapy.\(^10\) \(^11\) \(^12\) \(^13\) \(^14\) \(^15\) \(^16\) Both procedures are thought to relieve hypoxia. The suggestion that the inner retinal oxygen supply is improved by laser treatment because the outer retinal oxygen consumption has been decreased, is strengthened by the results of several investigators.\(^17\) \(^18\) \(^19\) \(^20\) \(^21\) Stefansson confirmed these animal results in humans by measuring the preretinal oxygen tension over laser treated and untreated parts of the retina in diabetic patients undergoing vitreous operations for proliferative diabetic retinopathy (Fig 2).\(^22\) Gottfredsdottir et al found vasoconstriction after macular photoocoagulation and postulated that this resulted from improved oxygenation.\(^23\) Further measurements of retinal oxygenation could verify the concepts of photoocoagulation and provide a rationale for adequate therapy.

**Conclusion**

A large gap still exists in our knowledge concerning oxygen distribution in the human retina because of the invasive nature and technical incompleteness of the methods currently used. Literature discussing the differences between species and the merits of extrapolation of the extensive animal research data to human beings is still scarce. Further discussions on this point are needed. Suggestions about pathophysiological processes in several retinal diseases are derived from animal studies and a few human data. These give rise to curiosity about the human situation. The methods to quantify oxygen in the human eye are gradually improving towards clinical application. Future prospects of such techniques combined with blood flow measurements may have important implications for developments in the management of ischaemic ocular disorders.

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