

*Review*

# Vitreoretinal influences on lens function and cataract

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The lens is composed of a thin metabolically active outer layer, consisting of epithelial and superficial fibre cells. Lying within this outer shell are terminally differentiated, metabolically inactive fibre cells, which are divided into an outer cortex and central nucleus. Mature fibre cells contain a very high protein concentration, which is important for the transparency and refractive power of the lens. These proteins are protected from oxidation by reducing substances, like glutathione, and by the low-oxygen environment around the lens. Glutathione reaches the mature fibre cells by diffusing from the metabolically active cells at the lens surface. With age, the cytoplasm of the nucleus becomes stiffer, reducing the rate of diffusion and making nuclear proteins more susceptible to oxidation. Low pO<sub>2</sub> is maintained at the posterior surface of the lens by the physical and physiological properties of the vitreous body, the gel filling the space between the lens and the retina. Destruction or degeneration of the vitreous body increases exposure of the lens to oxygen from the retina. Oxygen reaches the lens nucleus, increasing protein oxidation and aggregation and leading to nuclear cataract. We suggest that maintaining low pO<sub>2</sub> around the lens should prevent the formation of nuclear cataracts.

**Keywords:** nuclear cataract; oxidation; oxygen; vitreous body; ascorbic acid; glutathione

## 1. LENS STRUCTURE AND METABOLISM

The lens is an unusual structure, composed entirely of specialized epithelial cells surrounded by a thick, flexible capsule. Thin fibrils, the zonules, suspend the lens in the eye. The zonules arise from the ciliary epithelium and insert into the capsule at the lens equator. A simple cuboidal epithelium lies beneath the capsule on the side of the lens that faces the cornea. At the equator, these epithelial cells withdraw from the cell cycle and then elongate greatly to form fibre cells. During their elongation, the basal ends of the fibre cells move along the inner surface of the posterior capsule and their apical ends extend beneath the anterior epithelium. Elongation brings the ends of the fibre cells towards the central axis of the lens, where they meet fibre cells extending from the opposite side. In humans, the apical and basal ends of the fibre cells meet along three or more suture planes [1]. In foetal life, the suture planes intersect to form an upright 'Y' at the anterior pole of the lens and an inverted 'Y' at the posterior pole. After birth, the tips of the sutures bifurcate to form a total of six suture planes. Later in life, the tip of each suture bifurcates again, forming 12 sutures in the adult lens. After

fibre cells reach the sutures, they stop elongating and are displaced from the surface of the lens by fibre cells that were formed immediately after them. This process, which continues throughout life, causes earlier born fibre cells to be buried deep within the lens, with elongating fibre cells forming a thin shell at the surface of the fibre mass. Soon after they detach from the capsule, fibre cells complete their differentiation by degrading all membrane-bound intracellular organelles, including mitochondria and nuclei [2]. They remain in this state for the rest of the life of the individual. By this process, fibre cells formed during foetal life lie in the middle of the lens, comprising a region known as the nucleus. Fibre cells that differentiate after birth form an outer shell in the adult lens, called the cortex.

Together with the epithelium, the thin layer of nucleated fibre cells near the surface of the adult lens is responsible for most of its metabolic activity. Once the nuclei and other membrane-bound organelles are lost from the fibre cells, their ability to synthesize proteins, carry out oxidative metabolism and synthesize new membranes ceases. It has been reported that activity of lactic dehydrogenase (LDH), the last enzyme in the glycolytic pathway, ceases soon after organelle degradation [3]. If this is correct, glycolytic metabolism also ceases soon after organelle degradation, since LDH is required to produce the NAD<sup>+</sup> required by 'upstream' steps in glycolysis. Even if

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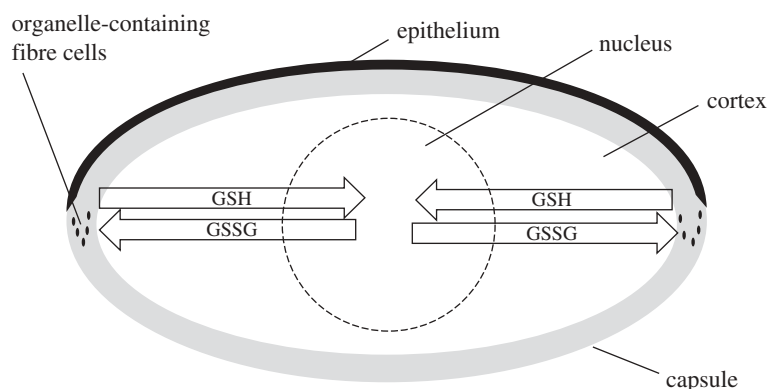


Figure 1. Diagram showing the location of the zone of active metabolism in the lens (shaded grey). Arrows indicate the direction of diffusion of glutathione (GSH) from these active cells to the metabolically inactive cells of the lens nucleus. When glutathione is oxidized to GSSG in the lens nucleus, it diffuses down its concentration gradient in the opposite direction, towards the lens surface.

this is not an accurate description of the fate of LDH activity, the concerted activity of 10 enzymes is required for glycolytic metabolism. Therefore, glycolytic activity would be expected to decay fairly quickly in the absence of continued protein synthesis. Most of the physiological needs of the mature cortical and nuclear fibre cells are, therefore, supplied by the thin shell of superficial, nucleated fibre cells. These needs are modest, but critical for the transparency of the lens. Mature fibre cells are intimately connected to the metabolically active superficial fibre cells by an extensive network of gap junctions. Small molecules produced in the superficial cells can reach the oldest nuclear fibre cells by diffusing from cell to cell across the gap junction network. One of the most important of these metabolites is the antioxidant glutathione.

Glutathione levels in the superficial fibre cells are remarkably high, reaching 10 mM or more in some species [4]. Experimental depletion of glutathione in the intact lens leads to rapid cataract formation [5]. A gradient of glutathione is present in the adult human lens, with the highest concentration at the lens surface and the lowest in the nucleus. When labelled cysteine, a precursor of glutathione, is added to cultured lenses, most of the labelled amino acid enters the lens near the equator and then gradually diffuses radially towards the nucleus [6]. With increasing age, glutathione levels decrease in the nucleus, but not at the lens periphery. Exposure of lenses to hyperbaric oxygen, an oxidative stress, has little effect on glutathione levels in the cortex, but markedly depletes glutathione levels in the nucleus. These observations indicate that glutathione is synthesized in the superficial fibre cells and reaches the nucleus by diffusion through the gap junctions that connect adjacent fibre cells (figure 1). Exposure of the lens to elevated oxygen depletes glutathione in the nucleus, which then must be replenished by diffusion from superficial cells.

The age-related decline in nuclear glutathione concentration correlates with a massive increase in the stiffness of the lens cytoplasm and is accompanied by a substantial decrease in the rate of diffusion from the surface to the lens nucleus [7–10]. It is also associated with an increase in oxidized glutathione (GSSG) and oxidized lens proteins in the nucleus. This trend is exacerbated in

nuclear cataracts, which have the lowest levels of glutathione and the highest levels of nuclear GSSG and oxidized proteins. Nuclear cataracts are also associated with a further increase in the stiffness of the nuclear cytoplasm, leading cataract surgeons to refer to them as ‘nuclear sclerotic cataracts’. The increased cytoplasmic stiffness in nuclear cataracts is likely to be due to increased protein disulphide cross-linking, although this remains to be directly demonstrated. Thus, with increasing age, the lens cytoplasm stiffens, decreasing the rate of diffusion of glutathione from the surface to the nucleus. This increases oxidation in the nucleus, further stiffening the cytoplasm. This positive feedback loop makes the nucleus of the ageing lens especially sensitive to oxidative insult.

It should be mentioned that there is an alternative view of lens physiology that is at odds with the previous description. Based on the presence of ion currents measured around the outside of the lens, a hypothesis has been formulated over the past 25 years suggesting that there is a fluid current that circulates through the lens [11]. This hypothesis suggests that ions diffuse through the extracellular space to the nucleus and enter the cytoplasm of deep fibre cells. The accumulation of ions creates an osmotic gradient that draws water into the deeper fibre cells. This fluid then flows out of the lens, from the nucleus to the equatorial surface, mostly through gap junctions. It is hypothesized that the lens fluid circulation removes metabolic products from, and delivers metabolic substrates to the deeper lens fibre cells. While the lens circulation may be an appealing concept, it has not been directly tested and its predictions appear to conflict with published studies. For example, it is not clear that there is glycolytic metabolism in the centre of an adult lens that requires this transport system. Most importantly, water flow from the centre to the periphery of the lens would prevent the diffusion of glutathione through gap junctions in the opposite direction. If the lens circulation model is correct, there must be sufficient glycolytic metabolism, glutathione synthesis and glutathione reduction to maintain the reducing environment in the nucleus. Testing of this model awaits evidence that sufficient metabolism exists in the nucleus to maintain its

reducing environment as well as direct demonstration that fluid flows from the lens centre to its periphery.

## 2. OXYGEN TOXICITY

In general, most of us tend to think of oxygen as a 'good thing' and lack of oxygen, or hypoxia, as a 'bad thing,' an opinion acquired early in life by just holding our breath. The many pathologic syndromes that involve systemic or local hypoxia, including asphyxiation, hypoxia–reperfusion injury, respiratory distress syndrome of prematurity, diabetic retinopathy and macular degeneration, add considerable medical and scientific weight to this point of view.

Evidence for the sensitivity of the older human lens to oxygen-induced damage was directly demonstrated in a study of patients treated with long-term hyperbaric oxygen therapy [12]. Patients with severe atherosclerosis often develop leg ulcers, owing to poor blood supply to their peripheral tissues. Breathing pure oxygen at increased pressure increases the level of dissolved oxygen in the blood, which supplies more oxygen to peripheral tissues and promotes the healing of leg ulcers. In this study, all subjects except one developed increased myopia during hyperbaric oxygen treatment. A 'myopic shift' typically precedes the onset of nuclear cataracts. Of the 15 patients with clear lenses at the beginning of treatment, seven developed frank nuclear cataracts and seven showed increased nuclear opacification. None of the patients in the untreated control group developed a myopic shift or nuclear cataract, even though these patients were somewhat older and had more severe peripheral ischaemia. The single treated subject who showed no increase in myopia or nuclear opacity was 23 years old, while all other subjects were over 40. The results of this study, which are consistent with results from subsequent animal trials [13] and shorter term treatment of human subjects [14,15], suggest that exposure of the lens to increased oxygen causes nuclear cataracts. The myopic shift seen in all of the older subjects is due to increased refractive power of the lens, most probably caused by hardening of the lens cytoplasm. These results are consistent with the consequences of the age-related stiffening of the lens cytoplasm, mentioned above, which would make the lenses from older subjects less able to withstand oxidative damage.

It is important to remember that humans and all other organisms on Earth depend on physiological mechanisms that are 'adapted' to an environment in which the partial pressure of oxygen is less than 21 per cent. Exposing organisms to oxygen at higher partial pressure than is typically encountered in their environment has pathological consequences, whether one is considering an air-breathing mammal or an obligate anaerobic bacterium. For mammals, the harmful effects of oxygen are called oxygen toxicity. Oxygen toxicity causes most noticeable damage to the lungs and nervous tissue. Harmful effects can be seen in the upper respiratory system in as little as 4 h after exposure to 95 per cent O<sub>2</sub> [16]. Experts in the therapeutic use of oxygen point out that 'the margin of safety between effective and potentially toxic doses

of oxygen is relatively narrow' [16, p. 205]. As illustrated above, long term, systemic exposure to hyperoxia can also cause cataracts.

Given its potential toxicity, it is worth considering the ways in which exposure to excess oxygen can damage cells. It is generally thought that most of the harmful effects of oxygen are due to excess production of reactive oxygen species (ROS) in a setting in which defences against ROS are insufficient. The ROS that are typically encountered in and around cells are due to the gain of one, two or three electrons by oxygen on its way to being reduced to water. These intermediates are superoxide anion (O<sub>2</sub><sup>−</sup>), which is a by-product of normal mitochondrial activity, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is produced from O<sub>2</sub><sup>−</sup> by the action of superoxide dismutase and as a by-product of a number of other oxidative reactions, and hydroxyl radical (HO•) [17]. Cells also encounter nitric oxide, peroxynitrite, hypochlorite and other oxidative species, which may be generated by metabolic or pathological processes. Protection against ROS is afforded by the enzymes superoxide dismutase, catalase and several peroxidases, as well as by reducing agents in the cytoplasm, such as ascorbate and glutathione. Exposure of cells to oxygen at higher partial pressure than is 'normal' for that cell leads to the excess production of ROS [17]. If the antioxidant protections of the cell are not sufficient to cope with the excess ROS, they can oxidize lipids, proteins and nucleic acids, leading to transient or permanent damage. When the accumulated damage is sufficient, cells may undergo apoptosis or necrosis [18].

An important organizing principle in thinking about oxidative damage is that cells that live in different environments or in different parts of the body may be 'adapted' to different levels of oxygen. This concept is especially applicable to the eye. The cells in the basal layer of the corneal epithelium are normally exposed to high levels of oxygen, close to the pO<sub>2</sub> in air (160 mmHg or 21% O<sub>2</sub>). Arterial pO<sub>2</sub> is typically 100 mmHg. In inner retina tissue, close to inner retinal vessels, or in the adjacent vitreous body, the pO<sub>2</sub> is approximately 20 mmHg (approx. 3% O<sub>2</sub>) [19,20], a value that is close to the normal level of oxygen in most tissues. In the vitreous near the posterior of the human lens, the pO<sub>2</sub> drops to approximately 7 mmHg (approx. 1% O<sub>2</sub>) [21] and our recent measurements in human patients revealed that the pO<sub>2</sub> near the anterior and lateral surfaces of the lens is typically about 3 mmHg (less than 0.4%) [22]. The low oxygen near the surfaces of the lens and the consumption of oxygen by lens cells results in even lower pO<sub>2</sub> in the lens nucleus [23,24]. Therefore, cells in the eye are exposed to conditions that, for typical cells, would range from hyperoxic to severely hypoxic. Corneal epithelial cells, which normally are exposed to high oxygen, must have defences that reduce the production of ROS or that protect against ROS [25]. Conversely, cells that are never normally exposed to more than 1 per cent O<sub>2</sub> are less likely to withstand the harmful effects of high oxygen and ROS.

While this scenario is likely to apply to most cells in the eye, lens cells are not typical. As indicated above, cells near the lens surface have very high levels of

glutathione, an important protection against oxidative damage, and lens cells have abundant antioxidant enzymes [26]. Therefore, although the lens appears to be exposed to relatively low levels of oxygen, it has strong antioxidant protection. Mature fibre cells are even more atypical. These cells have no mitochondria, peroxisomes, endoplasmic reticulum, or other organelles known to generate ROS. They are also in a very low-oxygen environment [24,27]. With their high levels of glutathione, superficial fibre cells are probably the least likely cells in the body to suffer oxidative damage.

If the lens is exposed to particularly low levels of oxidative stress, it is appropriate to ask why lens cells are so well-protected against oxidative damage. The answer is probably related to the unique structure of the lens. Unlike typical cells, mature fibre cells exist for decades with little ability to repair damage to their lipids or proteins and these constituents are never replaced [28–30]. Therefore, it makes sense that mature fibre cells are heavily fortified against oxidative damage. Since mature fibre cells derive most or all antioxidant protection from the metabolically active cells near the lens surface, it also makes sense that surface cells must have high levels of small antioxidants, such as ascorbate and glutathione, since surface cells supply these antioxidants to mature fibre cells by simple diffusion through gap junctions. Thus, cells in the lens nucleus depend on two factors for their transparency, diffusion of antioxidants from the cells near the lens surface and protection from oxidants such as molecular oxygen and ROS.

### 3. THE MECHANISMS THAT MAINTAIN THE LOW-OXYGEN ENVIRONMENT AROUND AND WITHIN THE LENS

#### (a) *Oxygen distribution around the lens*

A thin, fibreoptic oxygen sensor (optode) was used to map  $pO_2$  in several regions of the anterior chamber and in the posterior and vitreous chambers of patients undergoing vitrectomy, cataract or glaucoma surgery [21,22]. Oxygen diffused from the superficial retinal vessels into the gel vitreous, reaching a partial pressure there of approximately 20 mmHg [20,31]. However, the  $pO_2$  at the posterior of the human lens is low, approximately 1 per cent (7 mmHg) [21]. Measurements in animals and in patients undergoing vitrectomy surgery suggest that oxygen consumption by the lens, the gel structure of the vitreous body and the reaction of ascorbate (vitamin C) with oxygen help to maintain this low value [32].

A steep oxygen gradient is also present across the fluid-filled anterior chamber, from  $\approx 24$  mmHg at the inner surface of the cornea to  $\approx 3$  mmHg near the anterior lens capsule [22]. As the cornea is avascular, the  $pO_2$  at its inner surface is set by oxygen diffusion from the air at the outer surface of the cornea and oxygen consumption by corneal cells [33]. Similarly, the oxygen gradient across the anterior chamber must be due to oxygen consumption by lens epithelial cells and the underlying superficial fibre cells. It is difficult to know whether oxygen levels anterior to the lens are higher during daily activity, when the aqueous may be stirred by eye movements

or convective mixing. Initial attempts to promote convective mixing by altering the orientation of the rabbit eye caused no detectable changes in intraocular oxygen distribution (C. J. Siegfried 2010, unpublished data). In any case, oxidative metabolism by superficial lens cells should assure that oxygen levels in the deeper fibre cells are much lower than near the lens surface. This prediction is supported by measurements of oxygen gradients across the lens in rabbits [27] and in the human lens nucleus [24], as well as by much higher  $pO_2$  anterior to the lens after cataract surgery and intraocular lens implantation, when the superficial tissues at the anterior of the lens have been removed [22].

#### (b) *Oxygen and the gel state of the vitreous*

In a normal eye, the space between the posterior surface of the lens and the inner surface of the retina is filled with a gel, the vitreous body. The gel state of the vitreous body assures that the movement of molecules within it occurs only by diffusion [34,35]. Measurements made with oxygen microelectrodes or nuclear magnetic resonance spectroscopy showed that oxygen diffuses into the vitreous from the retinal vessels [19,36]. Microelectrode studies demonstrated that much of this oxygen was consumed by the nearby retina, thereby creating an oxygen gradient in the vitreous near the surface of the retina (figure 2a) [19]. Measurements in human and rabbit eyes showed that oxygen was low in the central vitreous and near the lens [21,27,37].

The first step in nearly all retinal surgery is to remove the vitreous gel and replace it with balanced saline solution. Since the vitreous gel does not regenerate, vitrectomy permanently alters the physical state of the material in the vitreous chamber. When the vitreous gel is removed, movement of the head or eyes will cause circulation and mixing of the fluid in the vitreous chamber [38,39]. Mixing of the vitreous fluid carries oxygen away from the surface of the retina and distributes it throughout the vitreous chamber (figure 2b). Consistent with this view, eyes that had previously had vitrectomy surgery had higher  $pO_2$  near the posterior surface of the lens than eyes with an intact vitreous gel [21].

The vitreous gel tends to degenerate in older individuals. With increasing age, the randomly oriented collagen fibrils in the centre of the vitreous body aggregate, creating fluid-filled lacunae. As this process continues, the lacunae coalesce to form larger, fluid-filled spaces surrounded by strands of aggregated collagen fibrils [40,41]. Advanced vitreous degeneration is associated with increased traction of the remaining vitreous on the surface of the retina. This traction can damage the retina, causing retinal detachment, macular traction syndrome or macular hole. Collapse of the vitreous also increases the tendency for the vitreous gel to separate from the retinal surface, a process called posterior vitreous detachment (PVD). After PVD, the remaining vitreous gel contracts, leaving a fluid-filled space at the surface of the retina. Therefore, vitreous degeneration is a kind of 'slow vitrectomy'.



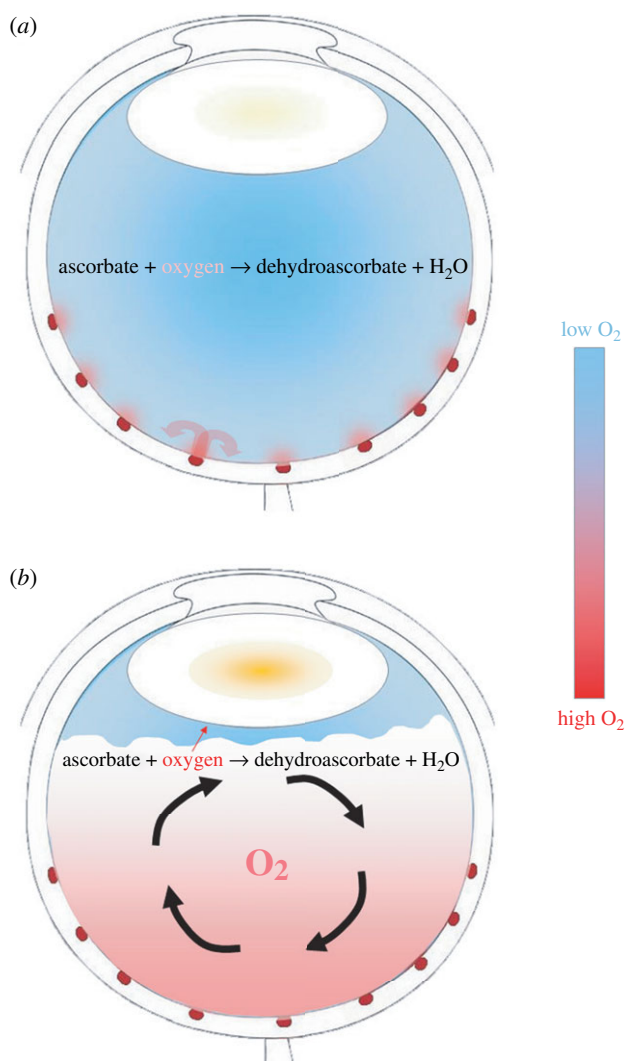


Figure 2. The distribution of oxygen (a) in the normal eye and (b) after degeneration or removal of the vitreous body. (a) In an eye with an intact vitreous body, oxygen diffuses into the vitreous gel from vessels near the surface of the retina. Much of this oxygen is consumed by retinal tissue farther from the vessel (curved red arrows). Oxygen levels are lowest in the centre of the vitreous, owing to the reaction between ascorbate and oxygen. (b) After the vitreous gel is removed during vitrectomy or degenerates and detaches from the retinal surface, much of the vitreous cavity is filled with liquid. This fluid mixes readily (curved black arrows), carrying oxygen away from the retina and distributing it throughout the vitreous cavity. Mixing increases exposure of the ascorbate in the vitreous to oxygen, promoting its oxidation to dehydroascorbate. Mixing also delivers more oxygen to the posterior surface of the lens, where it diffuses into the lens, causing nuclear cataract. The high concentration of antioxidant molecules, such as glutathione, in the lens cortex is likely to account for the relative resistance of this part of the lens to oxidative damage.

Previous studies found that degeneration of the vitreous body was associated with increased nuclear opacification [38,42]. The state of the vitreous gel was not related to the occurrence of either cortical or posterior subcapsular opacities, the other two main types of age-related cataracts. In eyes from subjects between 50 and 70, the state of the vitreous gel was a more significant predictor of nuclear opacity than was age. On the basis of these results, we predicted

that preserving or replacing the vitreous gel would delay the formation of nuclear cataracts.

The collagen fibrils in the vitreous gel are formed by a core of type II collagen wrapped with an outer layer of type IX and type XI collagen [40]. Type IX collagen has a terminal proteoglycan side chain that protrudes from the surface of the fibrils. Paul Bishop's group showed that, with increasing age, the amount of the proteoglycan side chains decreased exponentially. As this occurred, more of the inner collagen II became accessible to antibodies [43]. On the basis of these results, they suggested that the gradual loss of the proteoglycan groups from vitreous type IX collagen permitted the collagen fibrils to aggregate, contributing to the age-related collapse of the vitreous gel.

While the loss of type IX collagen is an important observation that helps to explain the tendency of the vitreous gel to collapse in older individuals, it does not explain why the gel state of the vitreous varies greatly in patients of similar age. Studies that measured the extent of vitreous degeneration in the eyes of older subjects found that some eyes in a given age group had extensive vitreous liquefaction, while others had a nearly intact vitreous gel [32,38,44]. If the gel state of the vitreous is important to protect the lens from nuclear cataract by minimizing its exposure to oxygen, the factors responsible for the liquefaction of the vitreous are important risk factors for nuclear cataract formation.

The increased traction on the retina caused by vitreous degeneration can cause retinal detachment or macular hole. For this reason, vitreoretinal surgeons have explored the use of enzymes to destroy the vitreous gel or separate it from the retinal surface. Recent studies in animals have shown that enzymatic destruction of the vitreous gel leads to increased transfer of oxygen from the retinal vasculature to the vitreous cavity and the lens nucleus [23,45]. Therefore, degeneration of the vitreous gel, like vitrectomy, exposes the posterior of the lens and the lens nucleus to increased oxygen and increases the risk of nuclear cataract formation.

### (c) Vitreous oxygen levels and ascorbate

Our previous measurement of oxygen distribution in the vitreous prior to vitrectomy revealed that oxygen levels were lower in the central vitreous than behind the lens [21]. After vitrectomy, oxygen levels increased in both locations and the oxygen gradient disappeared. Loss of the oxygen gradient is consistent with mixing of the vitreous after vitrectomy.

One explanation for the oxygen gradient in the intact vitreous gel is that the human vitreous consumes oxygen. This would make oxygen levels lowest at the greatest distance from the source of oxygen, the retina. To test this possibility, we constructed a micro-respirometer to measure oxygen metabolism in small amounts of vitreous fluid. Vitreous fluid removed from cadaver eyes or collected from patients at the time of vitrectomy consumed oxygen at a rate that depleted all of the oxygen in the sample within 1–2 h [32]. We hypothesized that oxygen might be removed by reacting with the abundant ascorbate (vitamin C)

normally present in human vitreous [46]. Exposure of vitreous fluid to 5 per cent oxygen depleted ascorbate from the vitreous. Removal of ascorbate from the vitreous, using ascorbate oxidase, prevented oxygen consumption by the vitreous. These studies showed that ascorbate is an essential component of the reaction in the vitreous fluid that consumed oxygen. Boiling the vitreous or treating it with agents to chelate copper or iron slowed the rate of oxygen consumption, but by less than 50 per cent. This suggested that there is a heat-stable catalyst in the vitreous that is responsible for promoting the reaction between ascorbate and oxygen [32].

We then determined ascorbate levels and the rate of oxygen consumption in vitreous samples collected from a series of patients who were undergoing vitrectomy. To assess whether vitreous liquefaction affected the ascorbate concentration or the reaction between ascorbate and oxygen, a subjective system was developed to score the gel state of the vitreous body. Patients with firm gel vitreous had higher vitreous ascorbate levels and higher oxygen consumption than those with more liquid vitreous or those who had previous vitrectomy. We hypothesized that destruction or degeneration of the vitreous gel permitted increased mixing of the vitreous fluid, causing oxygen that diffused from the retinal vessels to mix with the vitreous fluid. This would increase the reaction between ascorbate and oxygen, deplete ascorbate in the vitreous and permit more oxygen to reach the lens. Thus, the relatively high level of ascorbate in human vitreous (2 mM) may help to protect the lens from exposure to oxygen.

#### 4. WOULD LOWERING THE OXYGEN IN THE VITREOUS PROTECT THE LENS FROM NUCLEAR CATARACT?

Measurement of oxygen in the vitreous of patients about to have vitrectomy revealed that diabetics had significantly lower oxygen in their vitreous than non-diabetics [47]. Based on this observation, we suggested that diabetics would be protected from nuclear cataract following vitrectomy. To test this possibility, we used Scheimpflug photography to measure light scattering in the lenses of consenting diabetic and non-diabetic patients before, and six and 12 months after vitrectomy [48]. Light scattering in the lens nucleus of the operated eye was compared with that in the fellow, unoperated eye. Diabetics in this study could be divided into two groups. Many were having vitrectomy owing to complications of ischaemic diabetic retinopathy. Ischaemic diabetic retinopathy results from diabetes-induced damage to retinal capillaries, leading to tissue ischaemia and the growth of retinal blood vessels into the vitreous. These abnormal vessels often haemorrhage and cause traction on the retina, requiring vitrectomy. Other diabetics in the study did not have ischaemic retinopathy; they were having vitrectomy for reasons unrelated to their diabetes. Diabetics with no evidence of ischaemic diabetic retinopathy developed nuclear cataracts after vitrectomy at the same, rapid rate as non-diabetics. Those with ischaemic retinopathy

showed no significant progression of nuclear opacity during the 1 year follow up after vitrectomy. These data suggest that ischaemic retinopathy, which is caused by reduced oxygen delivery to the retina, lowers oxygen in the vitreous, protecting the lens from post-vitrectomy nuclear cataract. More direct tests of this hypothesis require a means to protect the lens from oxygen exposure after vitrectomy in eyes that would otherwise be susceptible to post-vitrectomy cataracts. Interventions that accomplish this goal are being developed in our laboratory.

In the study described above, ischaemic diabetic retinopathy was associated with lower nuclear opacity at baseline in the operated and in the fellow, unoperated eyes. One explanation for this result is that the lower oxygen in the eye of ischaemic diabetics protects the lens from the normal, age-related development of nuclear opacity. If this interpretation was correct, it would suggest that therapies that lowered oxygen in the vitreous would slow or prevent the opacification of the nucleus that occurs in everyone as they age. While this is an exciting possibility, at least one alternative explanation should be considered.

We previously showed that degeneration of the vitreous body is an important risk factor for nuclear opacification [38]. Degeneration of the vitreous body is also an underlying cause of retinal detachment, macular hole, vitreomacular traction syndrome and other retinal problems. Most of the non-diabetic and non-ischaemic diabetic patients in our study had vitrectomy for one of the retinal conditions associated with vitreous degeneration. Therefore, some of the nuclear opacity detected at baseline in this group was likely to be the consequence of increased oxygen exposure secondary to age-related vitreous degeneration. It has been suggested that diabetes may cause early vitreous degeneration [49], but other studies have suggested the opposite [50]. If ischaemic diabetic retinopathy is not associated with early degeneration of the vitreous body, ischaemic diabetics may have had less vitreous degeneration than those who underwent surgery for other retinal conditions. Their more intact vitreous gel may have contributed to the lower nuclear opacity seen in their lenses at baseline. In either case, our results suggest that preservation of the structure of the vitreous body protects the lens from nuclear cataract and, in the absence of the vitreous gel, lower oxygen in the vitreous fluid may protect against the formation of nuclear cataracts.

#### 5. SUMMARY

The lens is an unusual structure in an unusual environment. Metabolically active cells in a thin zone at the lens surface provide essential reducing substances, particularly glutathione, to the metabolically inert mature fibre cells in the deeper layers of the lens. With age, the cytoplasm in the deeper layers of the human lens becomes stiffer. This slows the diffusion of glutathione from the surface cells to the nucleus of the lens and of GSSG from the nucleus to the superficial cells. These changes make the nucleus of the older human lens more susceptible to oxidative damage. The lens is protected from oxidative damage

because it has low oxidative metabolism and exists in a low-oxygen environment. In addition, the superficial cells, especially the epithelial cells, consume most of the oxygen to which the lens is exposed. The vitreous gel protects the posterior of the lens from exposure to oxygen from the retinal vessels. Loss of the gel structure of the vitreous body owing to age-related degeneration or vitrectomy increases the mixing of the vitreous fluid. Mixing delivers more oxygen to the lens, leading to the formation of nuclear cataracts. Patients with ischaemic diabetic retinopathy have lower oxygen in their vitreous and are at least partially protected from nuclear cataract within the first year after vitrectomy. Therapeutic strategies that lower oxygen around the lens or protect the lens from exposure to increased oxygen from the retina could slow or prevent the formation of nuclear cataracts.

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