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Iron homeostasis and eye disease

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Summary

Iron is necessary for life, but excess iron can be toxic to tissues. Iron is thought to damage tissues primarily by generating oxygen free radicals through the Fenton reaction. We present an overview of the evidence supporting iron's potential contribution to a broad range of eye disease using an anatomical approach. Firstly, iron can be visualized in the cornea as iron lines in the normal aging cornea as well as in diseases like keratoconus and pterygium. In the lens, we present the evidence for the role of oxidative damage in cataractogenesis. Also, we review the evidence that iron may play a role in the pathogenesis of the retinal disease age-related macular degeneration. Although currently there is no direct link between excess iron and development of optic neuropathies, ferrous iron's ability to form highly reactive oxygen species may play a role in optic nerve pathology. Lastly, we discuss recent advances in prevention and therapeutics for eye disease with antioxidants and iron chelators,.

Keywords

Iron; Retina; Cornea; Lens; Chelator; Oxidative stress

1. Iron Metabolism in Humans

Iron is an essential component of cellular metabolism; however, excess iron can be damaging to tissues. Dietary iron is absorbed through the small intestine and is lost in sweat, shed skin and intestinal cells, and menstruation. However, the body is unable to actively excrete excess iron[1]. As a result, iron stores in certain tissues increase with age. It is thought that excess iron may be toxic due to the release of reactive oxygen species via the Fenton reaction. In this reaction, ferrous iron (Fe^{2+}) is oxidized to ferric iron (Fe^{3+}) by hydrogen peroxide (H_20_2) producing an hydroxyl ion (OH-) and the dangerous hydroxyl radical (OH·). The hydroxyl radical is very reactive and can cause oxidative damage to lipids, DNA, and proteins. It is hypothesized that iron may contribute to the pathogenesis of ocular diseases through oxidative damage[2].

In order to understand the evidence linking iron metabolism to ophthalmologic diseases, it is important to review the proteins involved in iron homeostasis. Both He et al [3] and Wong et al[2] describe iron homeostasis in detail in their reviews of iron toxicity and the retina. The following is a summary of relevant aspects of iron homeostasis. Dietary iron is reduced from

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the ferric to the ferrous state in the lumen of the duodenum and proximal jejunum. The free ferrous iron is transported across the luminal surface into the enterocytes by a proton symporter, divalent metal transporter-1 (DMT1). Mice with a mutated Nramp-2/DMT1 gene have decreased iron absorption across the gut and severe microcytic anemia[4]. In order for iron to reach the bloodstream, it is transported across the basolateral membrane of the enterocyte by the transporter protein, ferroportin (Fpn). Ferroportin is also expressed in many other tissues including placenta, tissue macrophages in the liver, lung, brain and retina[5-9]. Ferroportin works in conjunction with ferroxidases ceruloplasmin (Cp) and hephaestin (Heph) that oxidize ferrous iron to its ferric state facilitating iron export. Additionally, a recently-discovered serum peptide called hepcidin regulates iron absorption[10] by triggering degradation of ferroportin. Hepcidin knockout mice have severe iron overload[11] and mice over expressing hepcidin have iron deficiency[12]. Ultimately, once iron is absorbed by the enterocytes and exported from the cells, the majority of the non-heme iron in the circulation is bound to the serum protein transferrin.

The circulating iron is carried by transferrin, but requires a special mechanism to cross the blood brain barrier (BBB) since transferrin alone cannot cross. Transferrin carrying two molecules of iron binds to a transferrin receptor on a cell of the BBB. After endocytosis, the ferric iron is released from transferrin within acidified endosomes[3]. Transferrin and transferrin receptor complex are then returned to the cell surface.

The intracellular iron can be stored by ferritin, a 450 kDa multimeric protein complex capable of carrying approximately 4500 ferric iron atoms[13]. Ferritin exists as a combination of heavy, or H-ferritin (21kDa), and light, or L-ferritin (19.5kDa) polypeptides. In addition to size, H-ferritin differs from L-ferritin in that it contains a ferroxidase allowing it to rapidly convert ferrous iron to ferric iron. Interestingly, it has been shown that increased expression of H-ferritin in lens epithelial cells decreases the amount of intracellular free iron and improves cellular defenses against oxidative stress[14].

Balanced iron homeostasis is necessary to provide adequate iron for cellular functions and simultaneously avoid the toxicities from excess iron. As a result, iron-handling proteins are exquisitely responsive to vacillating iron levels. This iron-responsive mechanism is mediated by iron regulatory proteins (IRPs) that control the levels of the iron related proteins. This is accomplished by IRP binding to iron-responsive elements (IREs) on mRNAs when intracellular labile iron levels are low. For example, a state of relative iron deficiency would require decreased expression of iron storage proteins like ferritin and increased expression of transferrin receptor to augment uptake. To accomplish this, the IRPs bind to the IRE on the 5′ end of ferritin mRNA and obstruct translation; however, in the case of transferrin regulation in iron deficiency, the IRP binds to the IRE on the 3′ end of transferrin mRNA protecting the mRNA from degradation leading to increased protein levels[3]. Also, the various iron-handling proteins are differentially regulated depending on their location in different cell types. For example, iron depletion results in down regulation of ferroportin in the liver, but increased production in the duodenum[5] .

In sum, iron is necessary for cellular metabolism, but excess iron can be toxic through the formation of oxygen free radicals. Iron levels are delicately regulated and perturbation of iron homeostasis is increasingly being investigated as a potential cause of eye disease. Following an overview of basic ocular anatomy, this paper will explore the evidence for iron's potential role in the development of eye diseases.

2. Anatomy of the Eye

The anatomy of the eye is organized to optimize its functions: refract light and serve as a portal to the nervous system. In this section the anatomy of the eye will be explored following the

path of light: cornea, iris, lens, retina, optic nerve. Similarly, our discussion of iron's potential role in the development of eye disease will be organized anatomically beginning with disease of the cornea, followed by the lens and retina, and, lastly, the optic nerve.

2.1 Cornea

The cornea is the transparent dome that covers the anterior aspect of the eye. The cornea functions as the predominant refractive surface as well as protects the inner eye against infections and structural damage. The human cornea is a multi-layered tissue composed of five main layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium. The outermost aspect of the cornea is composed of 4-7 cells of nonkeratinized, stratified squamous epithelium that is covered by a tear film. Without tears, the corneal epithelium dries out and becomes damaged. Bowman's layer consists of collagen fibers beneath the basement membrane of the epithelium. The stroma is the thickest layer of the cornea and the arrangement of its fibers retains water and allows for transparency. Descemet's membrane is a thin layer lying superior to the endothelium. The endothelium is important in maintaining fluid balance within the eye. The single cell layer pumps excess fluid from the stroma into the anterior chamber to prevent stromal distortion.

A number of eye diseases affect the cornea and some involve perturbations of the epithelium. Evidence for iron's potential participation in corneal disease is demonstrated in the development of iron lines in the cornea. Iron lines develop in the normal aging cornea (Hudson-Stahli line [48,53]) as well as in keratoconus (Fleischer's Ring [41,48,62]) and in pterygium (Stocker's line [66-72]). It can also be seen near filtering blebs following surgery for glaucoma (Ferry's line[73]).

2.2 Lens

The lens is a transparent structure behind the iris, suspended in the aqueous humor by the zonular fibers. The zonular fibers are connected to the donut-shaped ciliary body surrounding the circumference of the lens. The ciliary body has a muscular as well as vascular component with blood vessels extending from the choroid layer in the posterior of the eye. The muscles within the ciliary body alter the tension on the zonular fibers changing the shape of the lens. The ability of the lens to change shape alters its refractive power focusing light for high acuity in near and distant vision. In addition to serving the needs of the lens, the ciliary body secretes aqueous humor that fills the space between the cornea and the iris, the anterior chamber, as well as the space posterior to the iris and ciliary body, the posterior chamber. The aqueous humor supplies nutrients to the avascular cornea and lens.

The fiber cells that comprise the lens are uniquely organized and develop from lens epithelial cells. Young fiber cells develop at the expanding periphery of the lens creating new layers throughout the lifetime. As a result, the nucleus of the lens consists of the embryonic cells and the cortex remains metabolically active generating new cells.[16]

The most common pathology of the lens is the development of opacities of the normally transparent lens called cataracts. Cataracts can result in vision loss and eventually blindness. Increasing evidence suggests that oxidative damage may play a role in the development of cataracts[16,17]. In his review, Spector offers a detailed description of the development of lens fiber cells as well as presents evidence for oxidative damage's role in cataract development [16]. Iron is implicated in the pathogenesis of cataracts because of its participation in the formation of oxygen free radicals [14] [18] [19] as well as the fact that iron foreign bodies in the eye cause cataracts[20].

2.3 Retina

The retina is the photoreceptor-containing layer in the posterior segment of the eye that ultimately transduces light into neural impulses. The majority of the cells of the retina can be organized into two layers: the neural layer and the pigment cell layer. The neural layer processes light and neuronal cell axons carry sensory information to the visual processing centers in the brain; the pigmented layer absorbs excess light and supports the maintenance of the photoreceptors in the neural layer. Interestingly, the posterior neural layer receives light and the information is processed as it moves anteriorly through the five types of neurons arranged in alternating layers in the retina. The retinal neurons consist of photoreceptors, bipolar cells, ganglion cells, horizontal cells and amacrine cells.

The pigmented cell layer is called the retinal pigmented epithelium (RPE) because it contains melanin which decreases light scatter. The RPE is crucial to the maintenance of the photoreceptors in the neural layer of the retina. The photoreceptors contain a stack of discs comprised of the photopigment rhodopsin necessary for transduction. The inner segment of the photoreceptor is responsible for generating new discs which get pushed toward the peripheral outer segment as they age. Because of its intimate location adjacent to the outer segment, the RPE is responsible for phagocytosing the older discs to allow space for the new discs. A consequence of this phagocytosis is the transfer of iron from the outer segment to the RPE. The RPE cells contain melanosome granules which are melanin-containing organelles enclosed by a lipid membrane. The melanin sequesters iron ions and protects against oxidative damage [21].

After passing through the neural circuitry of the retina, the information from the light exits the retina through the axons of the retinal ganglion cells in the optic nerve. The posterior aspect of the eye contains the optic disc where the optic nerve exits the eye. The central portion of the retina also contains the macula and fovea, specialized areas of the retina with densely packed photoreceptors for high acuity vision.

A common pathology of the retina is age-related macular degeneration (AMD), a progressive degenerative disease of the central retina leading to central vision loss. The earliest sign of AMD are drusen which appear as small yellow-ish white spots in the macula that can be visualized by ophthalmoscopy. In the late stages, AMD presents with either geographic atrophy (“dry” AMD) or with chorodial neovascularization (“wet” AMD)[22]. One proposed contributor to the destruction of the RPE and evolution of age-related macular degeneration through oxidative damage is excessive iron in the RPE and photoreceptors[23].

The choroid exists between the retina and the sclera and consists of many vascular beds that supply nutrients and oxygen to the RPE and photoreceptors. The smallest vessels lie below the retina and are called the capillary lamina of the choroid or the choriocapillaris. The choroid is pigmented and also serves to absorb light.

The inner portion of the neural retina is perfused by branches of the central retinal artery, which run close to the inner retinal surface. Also playing a supportive role in the retina are Müller glial cells, whose functions may include facilitating iron transport. It has been demonstrated that Müller glial cells from transgenic mice expressing human transferrin are protected against iron-mediated stress[24].

2.4 Optic nerve

The axons of the ganglion cells of the retina exit the retina through the optic disc and gather together to form the optic nerve (cranial nerve II). The ganglion cell axons in the optic nerve run straight to the optic chiasm at the base of the brain where some cross to the opposite side of the brain and some continue ipsilaterally toward the visual processing centers [15]. There

is increasing evidence that oxidative damage may play a role in the development of four major diseases of the optic nerve: optic neuritis [25], ischemic optic neuropathy[26],traumatic optic neuropathy[27,28], and glaucoma[29]. The levels of iron related proteins are disturbed in some optic neuropathies raising the possibility of a potential role for iron in the pathogenesis of optic nerve disease.

3. Diseases of the Cornea

3.1 Iron in the Cornea

Iron is necessary for many functions within a healthy cornea and disruptions of iron homeostasis may contribute to the etiology of disease of the cornea. In the cornea as well as throughout the body, iron is necessary for the completion of the citric acid cycle, production of ATP as well as an essential component of the rate-limiting enzyme in DNA synthesis[3]. However, excess iron may contribute to disease by the formation of oxygen free radicals through the Fenton reaction.

Although iron's role in corneal biology is not fully elucidated, understanding functions of iron-related proteins including lactoferrin and ferritin has produced some clues to iron's function. Iron is present extracellularly in the tear film on the surface of the cornea and is carried by lactoferrin which is secreted by acinar epithelial cells in the lacrimal gland. Lactoferrin is an iron-binding glycoprotein found in many mucosal fluids including tears as well as most highly concentrated in breast milk[30,31]. In tears, more than of 90% of lactoferrin is not bound to iron and available as a chelator[32]. Transferrin exists in tears in lower concentrations[33] and likely participates in iron-transport in human tears as well. As an iron-binding protein, iron-unsaturated lactoferrin has been shown to suppress oxidative damage in rabbit corneal epithelial cells, while iron-saturated lactoferrin did not attenuate oxidative damage[34]. These data raise the possibility that dysregulation of lactoferrin may increase oxygen free radicals within the cornea. Additionally, lactoferrin has unique anti-microbial properties which may be important to host defense. Lactoferrin is known to be bactericidal at high concentrations by binding to and disrupting bacterial membranes[35], but is now thought to have a distinct anti-biofilm property related to iron chelation. By sequestering iron, lactoferrin inhibited *Pseudomonas aeruginosa* biofilm growth at a lower concentration than required for bactericidal effects[36]. Thus, lactoferrin seems to be involved in regulating iron levels, preventing oxidative damage, and strengthening the cornea's antibacterial defenses by binding to iron. Understanding the role of lactoferrin in ocular biology including its potential benefit as a chelator to decrease oxidative stress will hopefully be beneficial in treating eye diseases.

Similarly, recent studies characterizing ferritin, the iron storage protein, have revealed new insights into ferritin's potential antioxidant role in the cornea. Ferritin was initially thought to be only a cytoplasmic protein, but Cai et al found ferritin in the nuclei of avian corneal epithelial cells[37]. Their studies suggest that nuclear ferritin may prevent free-radical damage to DNA by sequestering iron. Millholland et al demonstrated in chicken corneal epithelial cells that a unique nuclear transport molecule, ferritoid, is necessary to transport ferritin into the nucleus [38]. Because the transparent cornea is exposed to UV light—a source of oxidative damage--Cai et al propose that the corneal epithelial cells have evolved a unique means to limit oxidative damage to their DNA.

Excess corneal iron may be toxic. Intracorneal foreign bodies frequently leave rust rings which are traditionally surgically removed. Iron chelators such as desferrioxamine have been shown to decrease iron-containing rust rings that exist after a foreign body injury to the cornea. McGuinness and Knight-Jones showed that desferrioxamine, a compound that binds ferric ions to form ferrioxamine, successfully eliminated the smaller rust rings, but was unsuccessful in eliminating the larger rings[39]. Also, in 1965 Galin et al studied the effect of desferrioxamine

drops in removing experimentally-produced corneal rust stains in rabbits. Interestingly, the desferrioxamine treated eyes healed more quickly than the control group[40]. This raises the possibility of expanding the use of iron-chelators to prevent morbidity from iron-containing foreign bodies by decreasing iron induced corneal oxidative damage.

3.2 Etiology of Iron lines

Excess deposition of iron--corneal iron lines--has been observed in a number of corneal diseases as well as following several surgical procedures [41]. Iron lines exist in the basal epithelial cells of the cornea and are visible upon slit lamp examination. Electron microscopy revealed that ferritin is abundant in iron lines [42]. Although the etiology and clinical impact of iron lines is still incompletely understood, the existence of iron lines in the corneal epithelium may help elucidate a relationship between iron deposition and changes in the structure of the corneal surface[43].

There are four types of known iron lines: Hudson-Stahli Line, Fleischer's Ring, Stocker's Line, and Ferry's Line[44]. Additionally, in the last two decades there have been reports of iron lines following radial keratotomy[45], refractive keratoplasty[46], intrastromal corneal ring insertion[47], as well as another iron line, Coat's Ring, that develops in Bowman's layer after metallic foreign body in the cornea[48].

There are different hypotheses offering an explanation for the etiology of the iron lines:

(1) Tear Pooling Hypothesis (Gass 1964): Gass published his observations on the Hudson-Stahli line as well as Fleischer's Ring and Stocker's line in 1964 reporting that iron is deposited in the basal epithelial cells of the cornea[49]. He observed histopathological similarities between Fleischer's Ring and Stocker's line and considered them variations of the Hudson-Stahli line in the presence of corneal pathology. This work has become the basis for the tear-pooling hypothesis for the development of iron lines. Because iron exists in tears and is deposited in the corneal epithelium along the line of lid closure in Hudson-Stahli lines, it is hypothesized that the tear pooling along the line of lid closure results in increased deposition. However, later work in tear physiology demonstrated that there is more mucus in tears than was previously thought and some argue that it would be less likely for tears to pool at this viscosity[50]. Additionally, a Hudson-Stahli line was reported in a patient with neuroparalytic keratitis and absent tear production even following noxious endonasal stimuli[51].

(2) Basal-Cell-Migration theory (Rose and Lavin 1987): Basal epithelial cells migrate from the periphery towards the center of the eye and proliferate to become the superficial epithelial cells. Rose and Lavin proposed that the migration of basal epithelial cells would slow at the interpalpebral fissure when the cells originating cranially and caudally meet. As a result cells along this line would be the oldest cells with the most extracellular accumulated pigment[52]. Rose and Lavin propose that although intracellular pigment would decrease with age and repeated mitotic divisions, the extracellular pigment would increase as neighboring cells divide and pigment would be released as cellular debris.

(3) Tear Desiccation hypothesis (Assil 1993): Assil hypothesized that iron may deposit in areas with the greatest tear film instability. He proposes that instead of iron lines forming under tear pools, iron deposits in areas of tear dessication. The evaporation of tears would increase the relative concentration of iron. Given that iron lines exist in association with changes in the corneal landscape, those changes may increase local tear film instability [47]. It seems reasonable that if tear desiccation were responsible for increasing iron deposits, other types of deposits may also occur in the region of dessication.

(4) Senescent basal cell hypothesis (Assil in 1993): In this theory, Assil proposes that iron accumulates in cells with the least turnover.

These hypotheses for the etiology of iron lines suggest an association between iron deposition and changes in the corneal surface epithelium. In reviewing the iron lines within the cornea, we propose another hypothesis for the potential etiology of iron lines: the lactoferrin/transferrin receptor hypothesis. In agreement with previous work, we believe that changes on the corneal surface may damage the corneal epithelial cells. We propose that basal epithelial cell stress leads to an increase in transferrin or lactoferrin receptor expression causing increased iron binding and uptake. Other studies have demonstrated an increase in transferrin-receptor expression in response to cellular stress[53]. This hypothesis could be tested by immunolabeling for transferrin or lactoferrin receptor in post mortem or surgically excised corneal tissue containing iron lines.

In the next sections, we will examine the major iron lines of the cornea. Hopefully understanding more about the various iron lines within the eye will help reveal iron's role in corneal pathophysiology.

3.3 The aging normal retina and the Hudson-Stahli line

The Hudson-Stähli line is a horizontal line formed at the lower third of the eye corresponding approximately to where closed lids meet.

The Hudson-Stahli line is typically seen in apparently healthy corneas developing in the fifth and sixth decade, although there have been reports of Hudson-Stahli lines occurring throughout the lifetime. Rose and Lavin reported that both the length and density of the iron line increase with age[55]. Histologically, the pigmented Hudson-Stahli line represents iron deposition in the cytoplasm of epithelial cells.

Additionally, Hudson-Stahli lines may be associated with keratoconjunctivitis sicca or dry eye. Dry eye is a perturbation of the tear film that leads to various disturbances of ocular surface cells, including the cornea[56,57]. Tears smooth the irregularities on the surface of the eye allowing for proper refraction [58]. Since a healthy cornea is predominately avascular, the tear film also serves to bathe the cornea in a mucinous lubricating layer rich in antioxidant defenses —protecting the cornea from cumulative oxidative damage from ultraviolet light [59]. Dry eye has been shown to lead to increases in reactive oxygen species within the corneal epithelium [59]. Additionally, Seal et al compared tear proteins in patients with keratoconjunctivitis sicca and demonstrated a significant decrease in lactoferrin and a simultaneous increase in ceruloplasmin concentration. The decrease in lactoferrin seen in dry eye could lead to an increase in potentially toxic free iron. Upregulation of ceruloplasmin may occur to convert ferrous iron to its less toxic ferric form and protect the eye from oxidative damage. The decrease in lactoferrin concentration associated with dry eye is the basis for the lactoferrin tear test used as a measure of dry eye in diseases like Sjogrens and keratoconjunctivitis sicca[60]. Interestingly, it was shown in a rabbit dry eye model that lactoferrin eye drops attenuated damage to corneal epithelial cells[61] and, recently, Dogru et al demonstrated that oral lactoferrin has potential as a treatment to improve tear stability and ocular surface epithelium in Sjogren's patients[62]. These data suggest a relationship between accumulation of oxidative stress and the development of corneal epithelial changes in dry eye. Further research into oxidative stress and corneal changes is necessary in order to understand how and if oxidative damage contributes to the formation of iron lines. Ultimately, if iron induced oxidative stress contributes to cellular damage leading to dry eye, then there is an opportunity for iron chelators or antioxidant drops to potentially have a therapeutic role in the treatment of corneal diseases.

3.4 Keratoconus and Fleischer's ring

Keratoconus is bilateral disease of unknown etiology that usually develops in adolescence in which the cornea protrudes away from the eye forming a cone-shape.

This protrusion results in thinning of the central base of the conical cornea. One of the characteristics of keratoconus is the formation of a Fleischer's ring, a pigmented ring highlighting the circumference of the base of the cornea. Electron microscopy by Iwamoto and Devoe showed that Fleischer's ring consists of ferritin deposits in the cytoplasm of corneal epithelial cells as well as in widened epithelial intercellular space[42]. Fleischer initially thought that the ruptures in Bowman's membrane that can occur in keratoconus caused the iron accumulation. However, Gass noted variable rupturing of Bowman's membrane and concluded that Fleischer's ring is not necessarily related to breaks in Bowman's membrane[49].

Recently, Hiratsuka et al reported a corneal epithelial ring similar to Fleischer's ring in patients with thinning and protrusion of their cornea from corneal diseases other than keratoconus [43]. For example, one reported patient sustained trauma to his cornea as a child that resulted in thinning of the central cornea with Fleischer's ring-like iron deposition.

Hiratsuka et al offers two alternate explanations relating the thinning of the corneal stroma and iron deposition. The first explanation is based on the theory that disturbance of corneal epithelium leads to iron accumulation; the downstream effect is that the disruption of the interaction between epithelium and stroma changes the metabolism of collagen fibers and thins the stroma. Their alternate theory suggests that changes in iron metabolism may occur in the epithelium which directly causes thinning of the stroma. Evidence for this link is seen in iron-requiring proteins in collagen synthesis. Iron is a required co-factor in the formation of hydroxylysine that affects the diameter of collagen fibers in the cornea[64]. Hydroxylysine levels have been reported to be lower in patients with keratoconus than in controls[65], implying an abnormality in iron metabolism could affect the development of iron lines and potentially cause stromal thinning.

3.5 Pterygium and Stocker's Line

Stocker's line is a vertical iron line formed at the edge of an advancing pterygium. Pterygium is a fibrovascular proliferative disorder in which conjunctival tissue grows medially to cover the clear cornea[66].

Although pterygia do not commonly disrupt vision, they frequently cause irritation and inflammation and can be disturbing to patients because of the abnormal appearance of the eye. Pterygia are frequently observed in patients who work outdoors[68]. This suggests a relationship between sun exposure and the formation of pterygium likely mediated by ultraviolet light's oxidative damage to epithelial cells[69-72]. The role, if any, of iron in the development of pterygia is still unknown, but its participation in oxidative damage is a potential contributing factor.

3.6 Post-Glaucoma surgery and Ferry's Line

In 1968, Ferry reported another iron line in the cornea following surgery for glaucoma[73]. Glaucoma is a group of diseases historically characterized by high intraocular pressures. More recently, it is thought that glaucoma is an optic neuropathy where cumulative damage to the retinal ganglion cell axons of the optic nerve cause visual field loss and, potentially, blindness [74]. However, the mainstay of treatment for glaucoma remains medical, laser and surgical interventions to lower intraocular pressure. The surgical treatment creates a filtration bleb of the conjunctiva that increases the amount of aqueous fluid that can exit and decreases the pressure within the eye. In 1968, Ferry first reported cases of a golden-brown line that appears

anterior to the filtering bleb. Ferry stained a section of the iron line from a patient undergoing surgery and reported iron in the cytoplasm of the basal epithelial cells. He also described an association between the development of Ferry's line and increased height of the bleb as well as increased level of tension of the bleb. Without an elevated bleb, the Ferry's line does not form. Ferry proposed that repeated trauma of lid closure on the elevated, unyielding bleb initiates a cascade of events that causes iron deposition. Ultimately, the exact cause of the iron line formation is unknown as well as whether it is necessarily harmful to the eye; however, the existence of the Ferry line is another example of how perturbations to the corneal epithelium result in iron deposition.

3.7 Miscellaneous iron deposits

In addition to the four major iron lines, there are other instances of abnormal iron depositions within the cornea that are clinically apparent. Most notable are Coat's white ring of the cornea and Salzmann's nodular degeneration. The white rings of the cornea were first described by Coats in 1912—the opacity is typically a small ring .5-1.0 mm in diameter below the epithelium or within Bowman's membrane. Although Coats initially believed the rings to be congenital and composed of calcium or lead[75], in 1969, Nevins and Elliot excised a Coat's white ring from a human eye and demonstrated that the ring contained iron deposits[76] and no calcium. Their sections of the white ring stained with Gomori's iron stain and were confirmed by electron probe x-ray microanalysis. Additionally, it is now widely believed that the ring develops following corneal trauma[76-78]. Lastly, in 1981, Reinach and Baum reported cases of a pigmented line at the base of the corneal [79]

Salzmann's nodular degeneration is a rare corneal degeneration characterized by the appearance of blueish-white nodules in the cornea. The pigmented lines under the nodules are in Bowman's layer and deep epithelium and are presumed to be iron-containing[81].

4. Diseases of the Lens

4.1 Cataract

The most common disease affecting the lens is the formation of opacities called cataracts. The term cataracts is derived from Latin “catarractes” meaning “waterfall” because the opacity of the lens sometimes appears as a white waterfall-like haze[82]. The formation of cataracts is a painless and yet progressive process that can significantly impair vision. Cataracts are the leading cause of morbidity and decreased functional ability of the elderly[83].

Although the pathogenesis of cataracts is incompletely understood, there is increasing evidence linking oxidative stress to cataractogenesis. Firstly, some of the risk factors for the development of cataracts include exposure to sunlight and cigarette smoking which cause oxidative damage and impair antioxidants respectively. Increasing exposure to cigarette smoking [84] and ultraviolet exposure from sunlight[85] results in increasing the odds of developing cataracts. Secondly, oxidation of proteins in the lens increases with age, and occurs before cataract formation[16], raising the possibility that oxidation could initiate cataractogenesis. Bhuyan and Bhuyan in 1986 compared tissue from cataract and normal lens and demonstrated oxidation of membrane lipids in the cataractous lens and none in the normal tissue[86]. Also, normally the embryonic nucleus of the lens has a gel-like consistency, but hardens in cataracts. The hardened gel in the cataractous lens nucleus may represent fibers with novel cross-linking caused by oxidative damage[17]. Spector posits that the loss of ability to repair and replace damaged molecules in the older lens fiber cells may increase the lens' susceptibility to oxidative damage[16].

Iron is thought to be potentially involved in the etiology of cataracts primarily through its participation in the generation of oxygen free radicals. Zigler et al demonstrated that iron-

catalyzed reactions produce lens oxidative damage similar to that implicated in the development of cataracts[87]. However, the authors note that a better understanding of the metal ions in the lens—the quantity and reactivity in the lens-- is necessary before adequate evaluation of their role in the oxidizing processes. More evidence relating iron to cataractogenesis includes reports of cataract formation following ocular siderosis from an iron foreign body in the lens[14,18-20,88]. Ocular siderosis describes the clinical condition caused by retained iron-containing foreign body in the eye following trauma. Hope-Ross et al[88] as well as Sneed and Weingeist[89] reported cataract formation in the majority of their patients with siderosis.

The discovery of a genetic syndrome with premature development of cataracts offers new insights into the link between iron and cataractogenesis . The recently recognized autosomal dominant disease hereditary hyperferritinaemia cataract syndrome (HHCS) is characterized by 5-20 fold increase in serum L-ferritin concentrations, low to normal serum iron and transferrin saturation, and the development of bilateral nuclear cataract[90]. Ferritin is composed of H-subunits (21kD) and L-units (19kD) in varying ratios depending on the tissue. Prior to the discovery of HHCS, hereditary hemochromatosis – abnormal iron absorption causing iron overload—was considered the only genetic disease with increased ferritin levels[91]. HHCS differs from hereditary hemochromatosis because ferritin levels remain elevated--unrelated to total body iron levels--and HHCS lacks iron overload in parenchymal organs. The only significant clinical manifestation of HHCS is caratact formation. Girelli et al localized the mutation in HHCS to the ferritin L-subunit gene in the region of the iron-responsive element (IRE)[90]. In the posttranscriptional regulation of ferritin, binding of IRE to iron-regulatory proteins is necessary to inhibit ferritin translation. In 2000, Mumford et al examined the lens tissue from an individual with HHCS and demonstrated abundant crystalline deposits of L-ferritin within the cataract [92]

In 2002, Brooks et al studied five members of one family with HHCS and discovered the mutation in the L-ferritin gene (C33T) as well as confirmed the presence of light-diffracting crystalline deposits[93].

However, the mechanism of cataract formation in HHCS is still unknown. Mumford et al assert that the crystalline deposits within the HHCS cataract may represent a separate mechanism of cataract formation or, less likely, that the increased production of L-ferritin potentiates oxidative damage by limiting ferritin's ability to store iron. The histologic data demonstrating L-ferritin crystalline deposits within the HHCS cataract that are not seen in age-related cataract suggest a mechanism beyond oxidative damage. However, recent studies by Goralska et al showed the formation of inclusion bodies of L-ferritin in older lenticular epithelial cells which raises the possibility of these ferritin aggregates participating in not only genetic cataract formation but also age-related cataractogensis[19]. In conclusion, cataract formation is a devastating and pervasive disease and hopefully improving our understanding of iron and iron-related proteins' role in the development of cataracts will lead to novel therapeutics.

5. Disease of the Retina

In the retina, iron is necessary for phototransduction, but the build-up of excess iron can be toxic. The generation of new photoreceptor disc membranes requires the iron-containing enzyme fatty acid desaturase [94]. The RPE enzyme RPE65 that converts 11-*cis*-retinal to all-*trans*-retinyl as part of the retinoid cycle necessary for phototransduction requires iron[95]. However, excess iron in the RPE can be destructive by forming the reactive oxygen species through the Fenton reaction. Excess iron in the RPE and photoreceptors is a proposed participant in the development of age-related macular degeneration[23].

5.1. Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a degenerative disease of the central portion of the retina resulting in central vision loss. AMD is the leading cause of blindness in developed countries among people 65 years and older[96]. Patients with AMD initially present with drusen, which are subretinal deposits of extracellular matrix, proteins and lipids (sometimes oxidized) as well as immune mediators, in the macula. Although drusen do not appear to cause visual disturbances, AMD can progress to vision loss due to either geographic atrophy also known as “dry” AMD or with chorodial neovascularization known as “wet” AMD[22]. Geographic atrophy is seen in the center of the macula as depigmentation. Choroidal neovascularization (CNV) is extension of the choroidal vessels growing anteriorly into the retina. CNV leaks and bleeds which creates a blister of fluid under the retina that distorts vision. The fluid can be appreciated with slit lamp and hand-held lens[2,3].

The pathogenesis of AMD--while incompletely understood--involves perturbation of the retinal pigment epithelium (RPE). The most likely mechanisms for pathogenesis of AMD include cumulative oxidative damage and inflammation. There is increasing evidence correlating oxidative damage and the development of age-related macular degeneration[22, 97]. In 2001, the Age-Related Eye Disease Study demonstrated that therapy with antioxidants and zinc significantly reduced the progression of AMD, indirectly suggesting that oxidative damage may be involved in the pathogenesis of AMD[98]. A recent study by Shen et al showed direct evidence of increased oxidative damage in AMD retinas; immunoflorescent staining of biomarkers of oxidative damage were demonstrated in AMD retinas compared to controls [99]. In addition to oxidative damage, inflammation is implicated in AMD pathogenesis because of the genetic linkage between AMD and polymorphisms in components of the complement cascade[100-102]. Wong et al's review[2] summarizes how iron regulation is linked to inflammation through the iron regulatory hormone hepcidin. Hepcidin is synthesized in the liver and inhibits iron efflux from cells, ultimately increasing iron storage. During inflammation, hepcidin is upregulated and as a result iron storage in tissues increases[103].

There is increasing evidence that excess iron may play a role in the pathogenesis of AMD. Firstly, in 2003, we examined post-mortem eyes with AMD and found that the AMD retinas had significantly increased iron levels compared to age-matched control retinas[104] Some of the iron in the RPE and Bruch's membrane in these tissue sections was chelatable. Secondly, patients with the rare autosomal recessive disease aceruloplasminemia, who lack the ferroxidase ceruloplasmin (Cp), have increased RPE iron and develop early onset maculopathy that resembles AMD[105] Ceruloplasmin (Cp) is a ferroxidase necessary for iron export from the cell. Cp converts ferrous iron ($Fe^{2+)}$ to ferric iron (Fe^{3+}) facilitating iron export from the cell. Additionally, abnormal iron accumulations have been detected in the brains of patients with neurodegenerative diseases including very common diseases like Alzheimer's and Parkinson's disease. However, no evidence directly links iron accumulations to pathogenesis. Also, in mice with knockout of ceruloplasmin (Cp) and its homolog hephaestin (Heph), there are increased iron levels in the RPE and retinal degeneration with similarities to AMD[106] Ultimately, these initial data suggest that disruption of iron homeostasis can lead to retinal changes similar to AMD.

Lastly, iron foreign bodies in the eye cause retinal degeneration[88]. In 1972, Masciulli et al injected elemental iron, ferrous and ferric iron into the vitreous of squirrel monkey eyes to study the effect on the retina[107]. The iron powder and ferrous iron proved more damaging than the ferric form which appears to reinforce the principle that ferrous iron which enters the Fenton reaction is more toxic. Masciulli et al also demonstrated that the severity of the retinal damage—pyknosis (nuclear condensation), degeneration of photoreceptor cells, and inner retinal edema—is correlated to the dose of iron. Additionally, Declerecq et al implanted an iron wire in rabbit eyes and compared them to rabbit eyes having undergone a sham operation

[108]. Degeneration of RPE was seen as well as iron deposits in the lenticular, ciliary, and iris epithelia. However, the authors note that the cellular damage appreciated was not proportional to the presence of stainable iron in the tissues. Together, these data suggest that iron-induced oxidative stress and perhaps iron-mediated changes during inflammation may contribute to age-related retinal damage and AMD.

5.2 Hereditary iron overload states

There are four hereditary iron overload states associated with retinal pathology: aceruloplasminemia, hereditary hemochromatosis (HH), pantothenate kinase associated neurodegeneration (PKAN), and Friedreich's ataxia (FRDA). Firstly, aceruloplasminemia is a rare autosomal recessive disease where patients lack the ferroxidase ceruloplasmin (Cp) and, as discussed earlier, have increased iron accumulation in their liver, pancreas, brain and retina [109]. Patients with aceruloplasminemia develop early onset maculopathy that resembles AMD [105].

Secondly, hereditary hemochromatosis is the most common hereditary iron overload condition. HH presents primarily with parenchymal iron accumulations, but iron has been demonstrated in the RPE, sclera, and ciliary epithelium as well[112,2]. The most common mutation causing hereditary hemochromatosis is a mutation of a class I histocompatibility leukocyte antigen (HLA-1) gene, HFE[110]. The mutation in HFE impairs hepcidin upregulation [111]. It seems that without hepcidin to limit iron import from the intestine, iron accumulates in parenchymal tissues. Additionally, HFE is expressed in the RPE of the mouse retina [97,115] and HFE knockout mice were shown to have retinal degeneration suggesting a possible link between HFE associated iron homeostasis and retinal degeneration. This hypothesis could be tested by studying of the incidence of AMD in patients with HH.

Lastly, two other hereditary iron overload diseases associated with retinopathy exist: pantothenate kinase associated neurodegeneration (PKAN) and Friedreich's ataxia. Recently, discovery of mutations affecting iron metabolism in these two rare genetic neurodegenerative diseases suggest that iron overload may play a causal role[112]. Although the consequences of the iron accumulation from these hereditary iron overload states in the retina is still being investigated, they represent important examples of how disruptions in iron homeostasis may lead to retinal disease.

6. Disease of the Optic Nerve

6.1 Introduction

Diseases of the optic nerve (optic neuropathies) affect millions of people and are a significant cause of morbidity and vision loss. Optic neuropathies include a range of pathologies including optic neuritis, ischemic optic neuropathy, traumatic optic neuropathy and glaucoma. The common mechanistic pathway uniting these diverse diseases is the eventual retinal ganglion cell death[113]. It seems that damage to retinal ganglion cell axons initiates cell death through apoptosis, an orderly cell suicide cascade[114]. The process by which axonal damage results in retinal ganglion cell death is still unknown. However, recently, it has been suggested by Lieven et al and Nguyen et al and others that reactive oxygen species (ROS) are crucial players in the signaling pathway in apoptosis following axonal injury[115,116].

Although currently there is no direct link between excess iron and development of optic neuropathies, ferrous iron's ability to form highly reactive oxygen species may play a role in optic neuropathy pathogenesis. In this section, the evidence for oxidative stress in the development of optic neuropathies is reviewed as well as, in the case of traumatic optic neuropathy, the role of the ferroxidase ceruloplasmin.

6.2 Optic Neuritis

Optic neuritis is inflammation of the optic nerve seen in many autoimmune diseases, but is most commonly associated with multiple sclerosis (MS)[117]. MS is a progressive demyelinating disease of the central nervous system. The pathogenesis of the neurodegeneration of multiple sclerosis and optic neuritis is not well understood. Previously demyelination was viewed as the predominant contributor to disease activity from MS; however there is increasing interest in the role of axonal and neuronal cell death in disability from MS and optic neuritis. Currently, there is evidence that release of reactive oxygen species (ROS) may play a role in optic nerve injury[118,119]. The generator of the majority of ROS appears to be cellular mitochondria[120]. Following retinal ganglion cell injury, there is a release of intracellular superoxide anions from the mitochondria. Recently, Qi et al demonstrated that the neurodegeneration seen in experimental autoimmune encephalomyelitis (EAE) --the mouse model for MS—can be suppressed by increasing the expression of a mitochondrial antioxidant gene, superoxide dismutase-2 (SOD2)[25]. Although further research is necessary, advances in understanding the role of oxidative damage in optic neuritis may lead to novel uses for antioxidant therapy and potentially for discovering a link between iron-related production of ROS and optic neuritis.

6.3 Ischemic optic neuropathy

Ischemia and reperfusion is known to increase oxidative stress [121]. It is possible that ischemic ocular diseases may also involve this mechanism. These diseases include anterior ischemic optic neuropathy (AION), diabetic retinopathy, retinal vein occlusions, and retinopathy of prematurity. For example, Szabo et al demonstrated a decrease in oxidative stress induced by ischemia and reperfusion of a diabetic rat retina by administration of an oral antioxidant [122]. The exact role of oxidative stress in ischemic optic neuropathy is being investigated and raises the possibility of iron's participation in the generation of oxidative stress.

6.4 Traumatic optic neuropathy

There is increasing evidence that oxidative stress is involved in the destruction of retinal ganglion cells following traumatic optic neuropathy. Traumatic optic neuropathy refers to posttraumatic loss of vision with associated relative afferent papillary defect. The pathophysiology of traumatic optic neuropathy is not well understood, but may involve either avulsion or direct damage to the optic nerve as well as damage that indirectly occurs following closed head trauma. A well-studied model of traumatic optic neuropathy involves crushing the axons of rodent optic nerves with forceps[27,28,123]. Retinal ganglion cell axotomy causes apoptosis[124]—a likely common pathway in the damage due to many optic neuropathies. Hence, studies of rodents following optic nerve crush may reveal new insights into the biology of optic neuropathy. Swanson et al demonstrated that decreasing oxidative stress by adding a sulfhydryl reductant is protective against retinal ganglion cell death after optic nerve axotomy [28]. Additionally, Levin and Geszvain demonstrated an increase in expression of ceruloplasmin in the retina after crush injury. They posit that this upregulation may help protect the retina from iron-induced oxidative stress[27].

The link between oxidative stress and the development of retinal ganglion cell death may extend to hereditary optic neuropathy. Patients with mitochondrial mutations in different subunits in the oxidative phosphorylation pathway develop Leber's hereditary optic neuropathy (LHON). LHON causes degeneration of retinal ganglion cells and their axons which eventually leads to central vision loss. Although the mechanisms are not entirely evident, like in the crush model, retinal ganglion cell death appears to be caused by oxidative stress leading to apoptosis[124]. Due to the LHON mutations, electrons in the oxidative phosphorylation pathway may react with oxygen and generate oxidative stress. Qi et al demonstrated that increasing the expression

of mitochondrial antioxidant defenses like SOD2 could protect cells from oxidative damage [123].

Although currently there is no direct link between excess iron and development of optic neuropathies, iron may play a role in optic neuropathy pathogenesis through generation of oxidative stress. It is our hope that increased understanding into the role of oxidative damage in the development of optic neuropathies will lead to new potential treatments including antioxidants or iron chelators.

6.5 Glaucoma

Glaucoma is a potentially blinding disease caused by progressive death of retinal ganglion cells and classically associated with increased intraocular pressure (IOP). Because the eye is an enclosed space, maintaining fluid homeostasis is crucial in the regulation of eye pressure. Aqueous humor flows from the ciliary body, fills the posterior chamber, and enters the anterior chamber through the pupil to exit the eye at the angle between the iris and the arch of the cornea through the trabecular meshwork. Although increased IOP is strongly associated with development of glaucoma, mechanisms of the ensuing ganglion cell death are incompletely understood. Oxidative stress [125,126] and glutamate neurotoxicity [127,128] may be important. It is hypothesized that oxidative damage contributes to the apoptotic process of retinal ganglion cells. Moreno et al demonstrated in an experimental rat model of glaucoma that there is a decrease in the antioxidant defenses of the retina--including superoxide dismutase (SOD), catalase and glutathione--as compared to control retinas[129]. This evidence supports a theory in which increased intraocular pressure disturbs the protective antioxidant machinery and contributes to retinal damage from oxygen free radicals. Liu et al demonstrated evidence of products of oxidative damage throughout the retina after acute pressure elevation in the eye similar to that seen in glaucoma[130]. Although increased products of oxidative damage in the retina suggest a role for oxidative damage in glaucoma pathogenesis, the authors raise the question why the neuronal glaucoma is restricted to retinal ganglion cells if there is oxidative damage in all layers. Liu et al postulate that either the RGCs may be more vulnerable to damage because of longer axons with more lipids or perhaps there are additional unique stresses to the RGCs.

Although iron's relationship to glaucoma is still not well understood, there is some evidence that iron-related proteins may be linked to glaucoma. Increased levels of transferrin have been reported in the aqueous humor in human glaucoma[131]. However, it is unclear whether this represents simply a more permeable blood-aqueous barrier or increased endogenous production. Farkas et al found increased levels of the iron related proteins--transferrin, ceruloplasmin and ferritin--in experimental monkey glaucoma as well as glaucomatous human eyes[29]. They also documented increased retinal mRNA levels of transferrin, ceruloplasmin and ferritin in glaucomatous monkey eyes. These changes in iron-related gene expression suggest that iron may be involved in glaucoma pathogenesis. Further, glaucoma pathogenesis may be linked to glutamate excitotoxicty, and recent evidence provides a potential link between glutamate toxicity and increased iron uptake by neurons[127,128]. Ultimately more research is needed to understand iron's role in the development of glaucoma, but these data support the potential for an oxidative damage mechanism as well as offer some hope that antioxidant therapy might be helpful in the future.

7. Potential Therapies

7.1 Antioxidants

Given the growing evidence that oxidative damage contributes to many diseases within the eye, there is hope that medical therapies targeting oxygen free radicals will prove beneficial.

The Age-Related Eye Disease Study demonstrated a protective effect of an antioxidant cocktail of zinc, vitamin C, vitamin E, and beta-carotene in macular degeneration progression[98]. Additionally, in a 2007 AREDS case-control study, populations with higher dietary intake of macular carotenoids (lutein and zeaxanthin) were independently associated with decreased likelihood of having neovascular AMD, geographic atrophy, and large drusen[132]. Macular carotenoids may impact AMD development through their ability to filter blue light as well as reduce the impact of oxygen free radicals[133]. Additionally, melatonin, a highly lipophilic molecule with local activity in the retina, is thought to have potential benefit through its action as an antioxidant[129,134]. It has been demonstrated in guinea pig retina that melatonin attenuated oxidative damage in reperfusion injury[135].

The potential of antioxidant therapy in preventing cataractogenesis remains unclear. In 2001, an AREDS study also demonstrated no effect of antioxidants on the progression of age-related cataracts despite the oxidative damage component of cataractogenesis [98] . However, more recently it was shown that long-term use of dietary antioxidants like vitamin E may reduce the advancement of lens opacification [136]. Antioxidants remain an exciting horizon for potential improvement in the treatment of eye disease.

7.2 Chelators

Iron chelators are currently being investigated for their benefit in limiting iron-induced oxidative damage. Iron chelation is the mainstay of treatment for blood-transfusion related iron overload, but is also being investigated for the treatment of some neurological degenerative diseases including Alzheimer's and Parkinson's disease[137]. In order for a systemic iron chelator to be effective for neurodegenerative disease, it needs to selectively bind iron[138] as well as be lipid soluble enough to cross the blood-brain or blood-retina barrier[139]. The best known iron chelator in clinical use for transfusion-related iron-overload is desferrioxamine (DFO, Desferal). DFO is an effective hexadentate iron chelator and has been in clinical use since the 1970s[140]. However, administration requires continuous intravenous infusion or daily intramuscular injections due to its rapid renal excretion and poor absorption in the gut [141]. As a result, DFO is limited by expense and difficulty of use. A new form of DFO, a starch-conjugated form, allowing once-weekly administration is currently in clinical trials [142]. Additionally, there have been reports of retinal toxicity with high dose DFO use in iron overload diseases[143]. While the mechanism is likely to be RPE iron deficiency, this is uncertain and further research is needed to develop a chelator without retinal toxicity with potential to mediate iron-induced oxidative damage in ophthalmic diseases. One recent advance in this regard is the development of iron prochelators; chelators that bind iron only when activated by oxidative stress[144] .

Two other systemic iron chelators that have recently gained approval for clinical use are deferiprone (Ferriprox) and deferasirox (Exjade). The benefits of deferiprone and deferasirox include effective oral administration and longer half life than DFO. Additionally, deferiprone and deferasirox offer hope for increased efficacy in specific tissues. Glickstein et al demonstrated more rapid chelation with deferiprone and deferasirox than DFO in several cultured cell types[145]. To our knowledge, the ocular toxicity associated with DFO has not been reported with deferiprone and deferasirox. While more studies are necessary to determine the long term safety and efficacy of the oral iron chelators, they remain promising novel therapeutics for potentially treating ocular diseases.

Lastly, another chelator, salicylaldehyde isonicotinoyl hydrazone (SIH), is being studied as a potential therapy for iron-induced oxidative damage. SIH is a highly lipophilic chelator and as a result has increased cell permeability. A number of studies have demonstrated that SIH may have a role in mitigating oxidative-stress induced damage to cardiomyocytes[146-148]. In our studies, SIH protects cultured RPE cells against hydrogen peroxide toxicity [149].

Additionally, we are testing the efficacy of SIH in the cp/heph retinal iron overload mouse model.

7.3 Dietary iron limitation

Given iron's ability to generate oxygen free radicals through the Fenton reaction and the documented increase in iron stores within tissues like the retina over time, it seems plausible that limiting dietary iron may decrease oxidative damage. Tuomainen et al demonstrated that men with increased iron stores are at an increased risk for an acute myocardial infarction [150]. Additionally, a decrease in risk of MI in blood donors compared to those who did not donate blood has been reported[151]. Our lab is currently investigating the role of a low-iron diet in the development of retinal degeneration in mice. Although a minimum of iron is necessary for life, decreasing lifetime iron stores through dietary iron limitation remains an intriguing therapeutic possibility.

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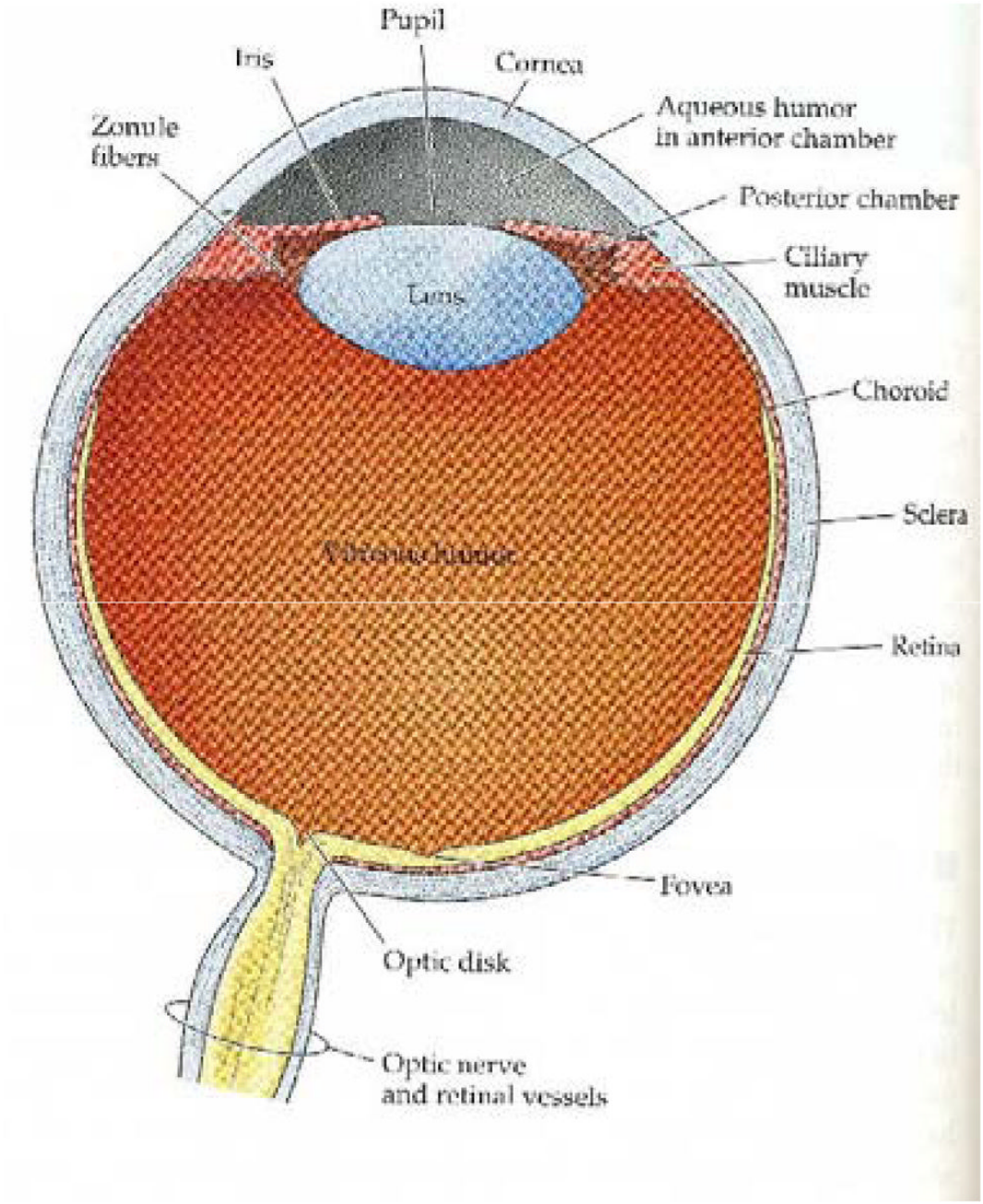

Figure 1.
A schematic drawing of the cross-section of the eye. Reprinted with permission from Purves' Neuroscience published by Sinauer Associates in 1997. See legend for figure captions[15]).

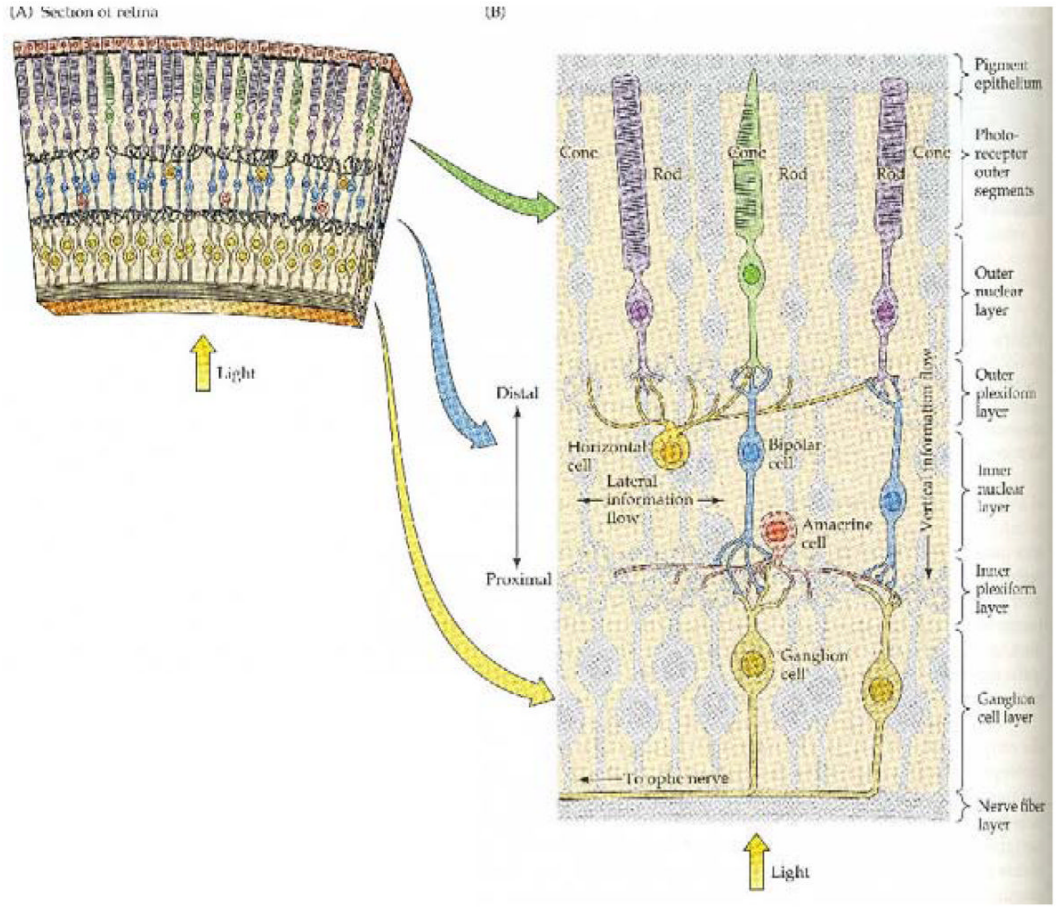

Figure 2.

A schematic drawing of the cell layers within the retina. (A) Section of the retina showing the cell layers of the retina. (B) A diagram of the arrangement of the retinal cells. Reprinted with permission from Purves' Neuroscience published by Sinauer Associates in 1997 [15])

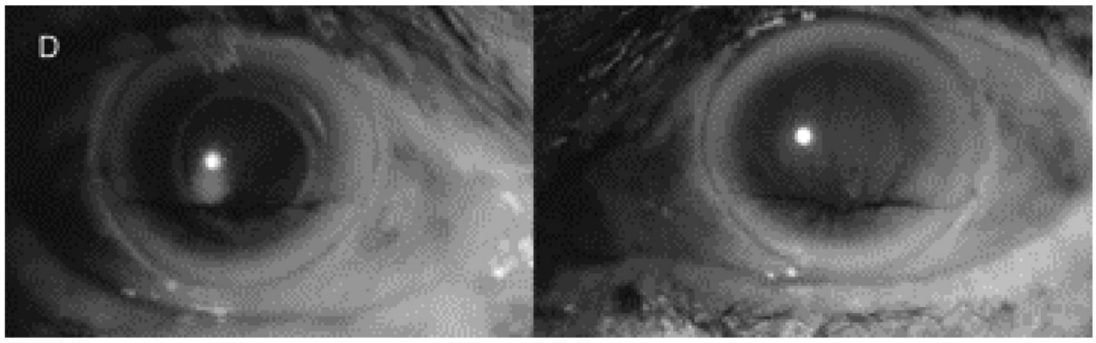

Figure 3.
UV photographs of bilateral corneas with Hudson-Stahli lines in a healthy elderly man. Reprinted with permission from Every et al. “Ultraviolet Photography of the In Vivo Human Cornea Unmasks the Hudson-Stähli Line and Physiologic Vortex Patterns” published in IOVS in 2005 [54])

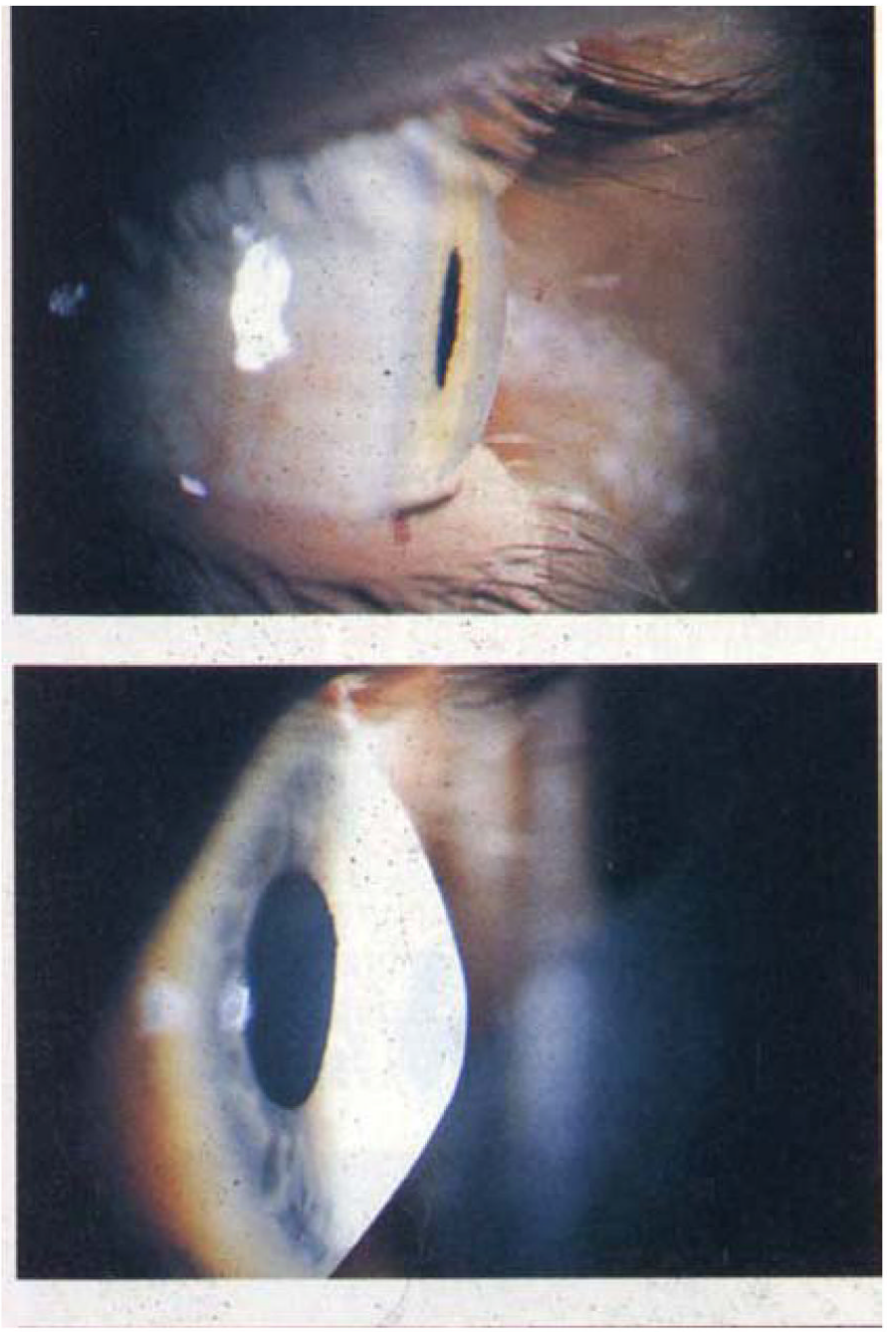

Figure 4.
Slit lamp photograph of a normal and keratoconus eye in profile. Reprinted with permission from Lawless et al "*Keratoconus: diagnosis and management*" in Australian and New Zealand Journal of Ophthalmology by Blackwell Synergy in 1989[63]).

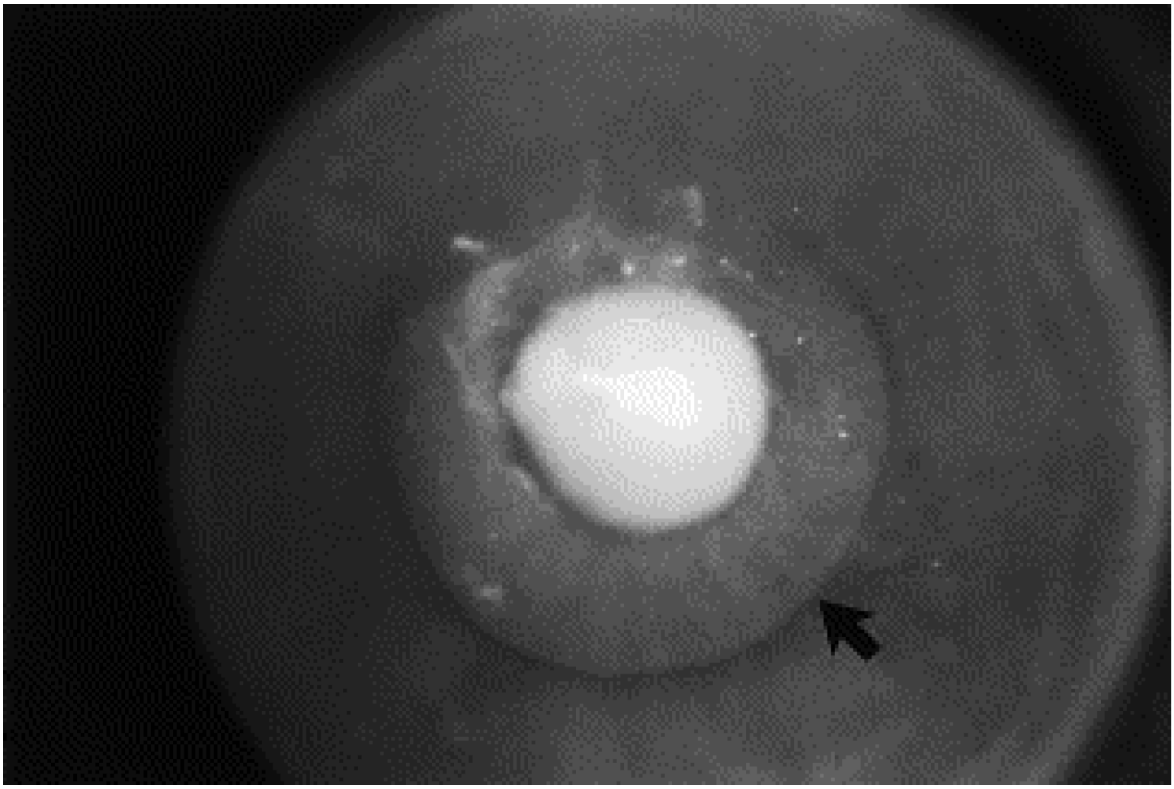

Figure 5.
Blue filter photography of the cornea of trauma patient with secondary keratoconus and a Fleischer's ring. Round Fleischer's ring-like pigment is observed (arrow). Reprinted with permission from Hiratsuka et al. “Secondary Keratoconus with Corneal Epithelial Iron Ring Similar to Fleischer's Ring” in Japanese Journal of Ophthalmology by SpringerLink in 2000 [43].

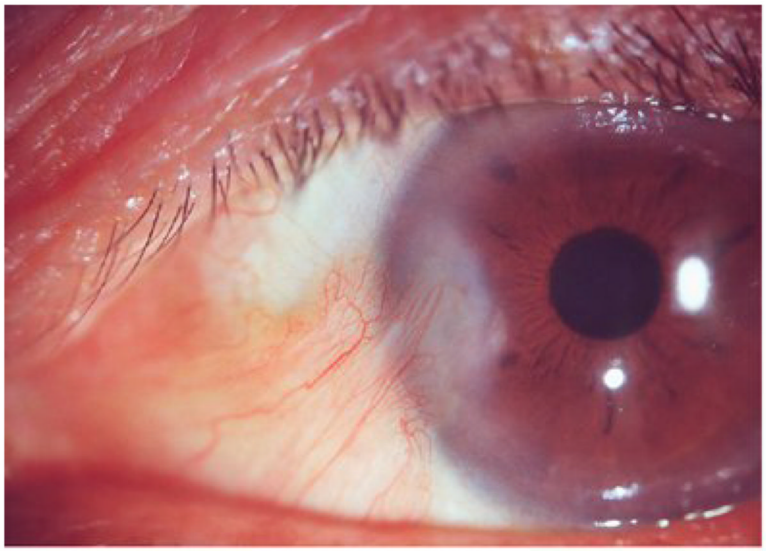

Figure 6.
Photograph of a pterygium in the right eye. Reprinted by permission from Macmillan Publishers Lts in Memarzadeh et al “Comparison of de-epithelialized amniotic membrane transplantation and conjunctival autograft after primary pterygium excision” in Eye 2006 [67]).

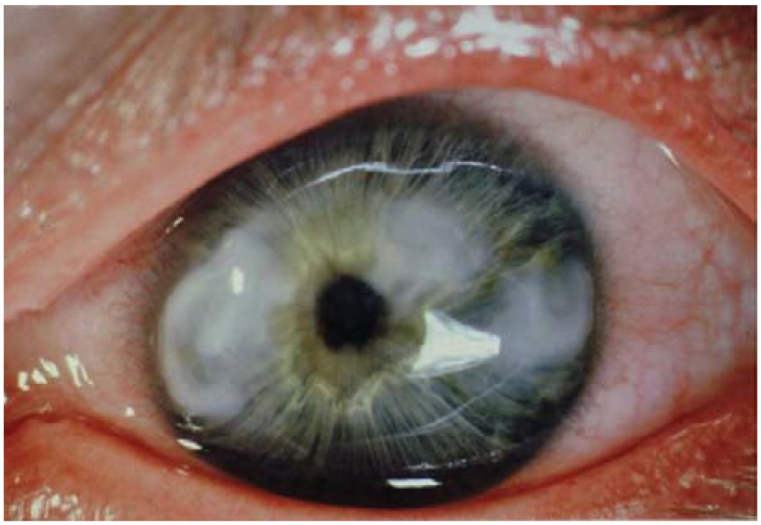

Figure 7.

A photograph of a patient with Salzmann's nodular degeneration. The left eye of a patient with Salzmann's nodular degeneration shows three blueish-white nodules located within the cornea. Reprinted with permission from Germundsson et al. “Phototherapeutic keratectomy in Salzmann's nodular degeneration” in Acta Ophthalmology Scandinavia by Blackwell Synergy in 2004 [80].

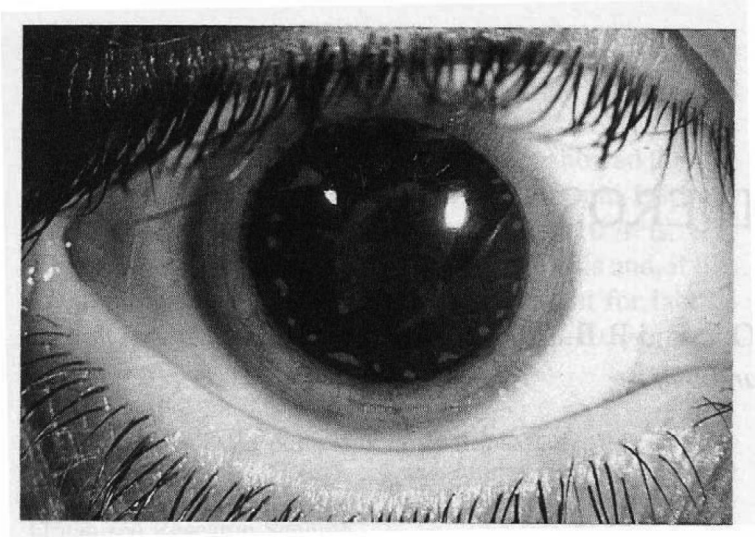

Figure 8.
A slit lamp photograph showing iron deposits beneath the anterior lens capsule and the anterior lens opacity in ocular siderosis. Reprinted with permission from Hope-Ross et al. “Ocular Siderosis” in Eye by Nature Publishing Group[88].

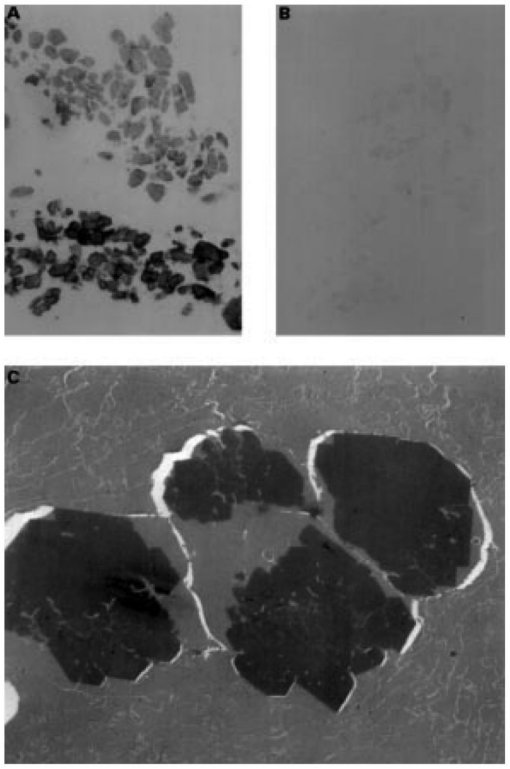

Figure 9.
Light microscopy of hyperferritinaemia cataract syndrome lenses. (A) Light microscopic appearance of crystalline inclusions within the lens stroma showing dense staining with monoclonal anti-L-ferritin (×400). (B) The deposits were not stained with anti-H-ferritin (×400). (C) Appearance of inclusions at ×50 000 magnification showing square-shaped crystal morphology. The crystals were not associated with any cellular elements, but appeared to lie free within the stroma. Reprinted with permission from Mumford et al. “The lens in hereditary hyperferritiniaemia cataract syndrome contains crystallin deposits of L-ferritin” in Br. J. Ophthal. by BMJ Publishing Group Ltd. [92]

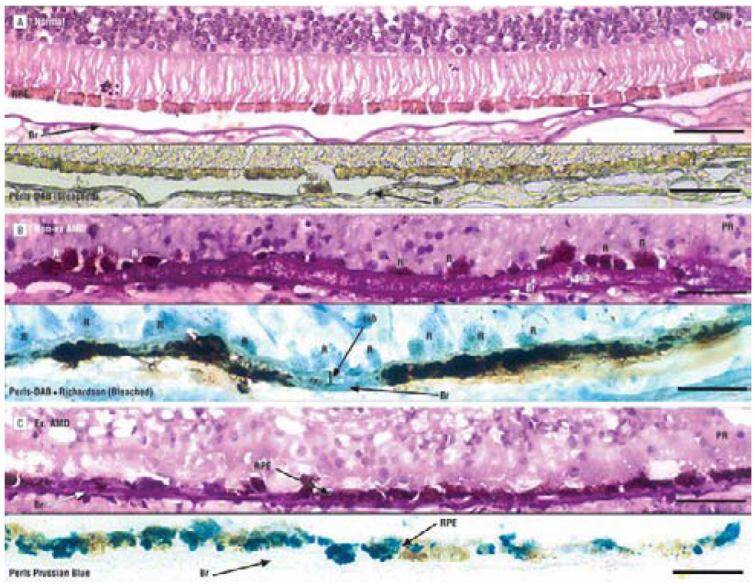

Figure 10.

Photomicrographs of increased Perls'-positive iron in age-related macular degeneration (AMD)–affected retinas. (A) Healthy macula has no Perls-3,3′-diaminobenzidine (DAB) stain after bleaching (inset) (B) Geographic atrophic macula with severe photoreceptor loss, RPE atrophy, and sub-RPE deposits. Bruch's membrane and sub-RPE deposits (sub) are positive for iron (inset). (C) Exudative AMD retina demonstrating significant photoreceptor loss with RPE atrophy and thickened BR. The RPE contains iron detectable by the Perls Prussian blue stain without DAB enhancement (inset). Scale bars indicate 50 μm. Reprinted figure and legend with permission from Hahn et al. “Maculas Affected by Age-Related Macular Degeneration Contain Increased Chelatable Iron in the Retinal Pigment Epithelium and Bruch's Membrane” in Arch Ophthalmol © 2003, American Medical Association. All rights reserved. [104]).