INTRODUCTION

NEW STUDIES SUPPORT AMD LINK TO BLUE LIGHT EXPOSURE

Over the past two years there have been a number of studies investigating the mechanisms involved in the development of Age-related Macular Degeneration (AMD). The results of these studies have consistently supported the premise that blue light exposure raises the level of oxidative stress in retinal cells and promotes the development of AMD. The European Eye Study (EUREYE) has now confirmed that blue light exposure combined with low levels of serum antioxidants is associated with the formation of AMD.(1a)

The results of this new research on the pathogenesis of AMD provides compelling evidence that increased exposure to blue light over a lifetime will advance the onset of AMD, and increase the likelihood of blindness later in life. This has lead some prominent researchers on the mechanisms involved in the development of AMD to now assert that "It is photo-oxidative stress, or the cumulative exposure to free radicals from blue light over a lifetime that causes AMD".(1b)

BACKGROUND:

AMD is a condition of advanced degeneration of the macular portion of the retina. The macula is a small portion of the retina that includes the region of the retina that is primarily responsible for detailed color vision. Over 8 million Americans suffer from visual problems as a result of AMD. AMD currently leads to progressive blindness in over 35% of persons over the age of 75. Because genetics factors play a part in AMD susceptibility, more than half the people with a family history of AMD will develop severe vision loss in their lifetime. With increasing life span the incidence of blindness from AMD is increasing rapidly, and it has been said that AMD is reaching epidemic levels in the U.S. and other developed countries.(1c)

The largest American epidemiological study on visual function found that an daily increase in exposure to sunlight of only 3 hours for young adults (in their teens through thirties) will advance the onset of AMD later in their lives by 10 years. A 10 year advance in onset of AMD effectively doubles the likelihood of becoming blind during a lifetime. After age forty, the protective mechanisms of the retina deteriorate progressively with age. Thus, older people are at an even higher risk of retinal damage from light exposure.(1d)

Visible blue light is the only component of sunlight implicated in the development of AMD. Accordingly, many retinal specialists are now expressing concern regarding lifetime exposure to blue light and recommending that people limit their exposure to blue light wavelengths throughout their lives, from both sunlight and artificial light sources.(1e)

While a number of factors have been linked to the development of AMD, over the past decade studies have indicated that cumulative oxidative stress over a lifetime is implicated in the pathogenesis of AMD. The following is an annotated referenced overview of very recent studies on the primary mechanisms by which blue light contributes to increased oxidative stress and the development of AMD. For brevity and clarity of the overview, much of the detailed explanation of these mechanisms are included in the annotations that accompany the references.

THE DEVELOPMENT OF AMD AND OXIDATIVE STRESS IN RPE AND PC CELLS

Numerous studies have implicated high levels of oxidative stress in macular photoreceptor cells (rods and cones) and the adjacent retinal pigment epithelium (RPE) cells as causative factors in the development of AMD. Oxidative stress occurs when the level of pro-oxidants exceeds the level of antioxidants in a cell and results in the oxidation of cellular components and consequent loss of cellular function. In this discussion, molecules and cellular components that are no longer functional because of oxidative activity will be included in the term "oxidative debris".(2)

The retinal pigment epithelium (RPE) is a monolayer of flat, polygonal, pigmented cells lying between the photoreceptor cells and the choroid, and is an integral part of the blood retinal barrier. The basal side of the RPE is in contact with Bruch's membrane whereas the apical side faces the photoreceptor cell outer segments. Bruch's membrane is an extra-cellular structure that acts like a sieve through which nutrients and waste products exchange between the RPE epithelium and the blood bearing choroid. (3)

A major function of RPE cells is to maintain the survival and normal functioning of photoreceptor cells (PCs). RPE cells control nutrient/waste products exchange of the PCs. Every day each PC sheds about 10% of its outer segment. The outer segment of a PC is the outermost portion of the PC, the region where light is absorbed and where photo-oxidative damage occurs. The tips of the outer segment of a PC are surrounded by the apical processes of an adjacent RPE cell. When a PC sheds the tip of its outer segment, this material is engulfed by the adjacent RPE cell. In this manner the RPE cells ingest much of the oxidative debris created as a byproduct of light absorption by PCs. Additionally the RPE cell reconfigures and recycles the *trans* configuration of Retinal molecules released from bleached visual chromophores back to the outer segments of photoreceptor cells in a *cis* form that can be used to re-synthesize active visual pigments. This process is termed "the visual cycle". Failure of any one of these functions can result in degeneration of the retina, loss of vision, and blindness.(3)

PC's and RPE cells are particularly vulnerable to oxidative damage because they are exposed to high levels of oxygen and light energy, and contain high levels of lipids and lipoproteins that are easily damaged by the radical oxygen species (ROS) induced in this environment. The consequences of oxidative damage to RPE and photoreceptor cells is severe because they are non-replicating (post-mitotic), and are not replaced after birth. These cells must therefore survive a lifetime of oxidative insult. Additionally, starting at about age 40 there is an age-related deterioration of the mechanisms that protect these cells from oxidative damage.(4)

BLUE LIGHT AND OXIDATIVE STRESS IN THE RETINA.

It is the short wavelength portion of the visible spectrum, the blue light region, that is associated with an increase in the level of oxidative stress in retinal cells. A strong indication that blue light wavelengths contribute to the development of AMD is found in the relationship between the density of the blue-light-absorbing yellow macular pigment and the development of AMD. Macular pigment is made up of the carotenoids lutein and zeaxanthin and covers the macula, a small region of the retina containing the fovea. The fovea is the region of the retina responsible for detailed color vision. The density of macular pigment, which determines its ability to absorb blue light, is greatest over the fovea. Macular pigment density has been found to be inversely related to AMD susceptibility.(5)

The carotenoids lutein and zeaxanthin which make up the macular pigment are derived entirely from diet. While these carotenoids may have a protective antioxidant role in RPE cells, their primary protective role appears to be in the formation of macular pigment. Macular pigment is mostly located near the inner retina, which is too distant from the outer retina to act as an effective source of antioxidant protection of the outer segments of PCs or the adjacent RPE cells. Therefore, much of the protective capability of macular pigment with regard to AMD likely resides in the ability to limit the amount of blue light reaching the PCs and RPE cells in the macular region of the retina. The inhibitory effect that macular density has on the development of AMD can be considered as a strong indication that reducing exposure of the outer retina to blue light exposure delays or prevents the onset of AMD. (6)

BLUE LIGHT AND OXIDATIVE DAMAGE IN PHOTORECEPTOR CELLS.

Absorption of visible light by photosensitive pigments in photoreceptor cells is a major contributor to oxidative stress in the retina and results in the formation of oxidative molecular damage in PCs. The amount of oxidative damage produced in a PC appears to be proportional to the photon catch; i.e. the amount of light absorbed by the photoreceptor pigment in the cell.7

Blue light in particular appears to increase the amount of photo-oxidative damage generated in PCs. Blue light absorption can short-circuit the lengthy metabolic visual cycle through a process termed photoreversal, and greatly increase the photon catch in the PC. Higher levels of light absorbed by PCs would cause increased formation of oxidative damage.7

> A Brief explanation of Photoreversal
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> Normally, when a photo-pigment in a photoreceptor cell absorbs light, it bleaches and the photo-pigment disassociates into an opsin and the all-*trans* configuration of the Retinal molecule. However, only Retinal in the *cis* configuration can recombine with an opsin to form a photo-pigment. Because the all-*trans* configuration cannot bind with an opsin, it becomes unavailable for light absorption until the *trans*- Retinal is converted to the 11-*cis* configuration of Retinal. This occurs in a lengthy metabolic process called "the visual cycle".(8)
>
> However, if the intermediary formed when the photo-pigment molecule absorbed light should then absorb blue light (< 480 nm), photoreversal causes a photoreceptor pigment to rapidly become unbleached, and again available for light absorption, short-circuiting the visual cycle. This appears to increase the amount of light absorbed by PCs by several orders of magnitude. Since the degree of photic damage in the PC is related to the amount of light absorbed, i.e. the photon catch, this greatly increases the amount of oxidative damage produced in the PC.(9)

BLUE LIGHT, LIPOFUSCIN, AND OXIDATIVE STRESS IN RPE CELLS.

Every day the spent tips of the outer segments of PCs are phagocytosed (engulfed) by an adjacent RPE cell, where it is ingested and degraded by the RPE cell's lysosomes. In post-mitotic, non-replicating cells, including RPE cells, indigestible materials collect in the lysosomes as lipofuscin, also known as the aging pigment. Retinal lipofuscin in RPE cells is an aggregate of highly oxidized cross-linked proteins and lipids that is primarily composed of material derived from the phagocytosed PC outer segments.(10)

A major component of retinal lipofuscin is A2E, a phototoxic indigestible molecule formed in the lysosomes of RPE cells from precursors derived from all-*trans*-retinal. When A2E absorbs blue light it generates highly reactive radical oxygen species (ROS). A2E and its derivatives appear to be the primary source of blue light induced ROS production by RPE lipofuscin. Blue light absorption by A2E also results in the formation of epoxides that can damage DNA and trigger cell death.(11)

Retinal lipofuscin begins to accumulate in RPE cells at birth and increases with age. When exposed to blue light retinal lipofuscin generates ROS, raising the level of oxidative stress in RPE cells. Blue light exposure of lipofuscin inhibits proteosome activity, thus reducing the ability of these RPE cells to degrade waste material. Blue light absorption by lipofuscin can also inactivate lysosomal hydrolases and impair lysosomal stability. Extensive accumulation of retinal lipofuscin in RPE cells is associated with increased oxidative stress that can cause the malfunctioning or death of the RPE cells. Given the multiple functions of the RPE in the maintenance of photoreceptor cells, it is not surprising that RPE dysfunction has been implicated in AMD.(12)

BLUE LIGHT, MITOCHONDRIA, AND OXIDATIVE STRESS IN RPE CELLS.

RPE cells are among the most metabolically active cells in the body. They contain large numbers of mitochondria, the "power sources" of cells. Damaged mitochondria produce high levels of ROS which can significantly contribute to the oxidative stress in aging cells, particularly post-mitotic cells like RPE cells. Mitochondria are also sensitive to damage from ROS. Thus ROS formation contributes to further damage of mitochondria, which in turn contributes to further ROS formation. This is a self escalating cycle.(13)

Mitochondria contain chromophores that generate ROS when they absorb blue light. Blue light absorption and ROS also damage mitochondria DNA (mtDNA) causing additional ROS production and raising the level of oxidative stress in the RPE cell.(14)

A2E and damaged mitochondria act synergistically to increase ROS production in RPE cells exposed to blue light. Extensive levels of retinal lipofuscin containing A2E can raise the oxidative stress generated by damaged mitochondria in RPE cells from sub-lethal to lethal levels, and contribute to the development of AMD.(15)

BLUE LIGHT, MELANIN, AND OXIDATIVE STRESS IN RPE CELLS

A protective element that was thought to reduce levels of oxidative stress in RPE cells is the black pigment, retinal melanin. In humans, retinal melanin production in RPE cells appears to only occur pre-birth and in very young children. Melanin granules collect in structures called melanosomes, where they absorb stray light, and act as powerful anti-oxidants. However, there is an age-related reduction of melanin granules within RPE cells.(16)

Melanosomes are degraded by blue visible light that reaches RPE cells. Degraded melanosomes become pro-oxidant and further contribute to the level of oxidative stress.(17)

Aged melanosomes in RPE cells can become coated with lipofuscin and are referred to as melanolipofuscin. The absorption of blue light by chromophores in lipofuscin and melanolipofuscin can generate high levels of ROS in the RPE membrane, contributing to increased levels of oxidative damage in the RPE and adjacent tissues and promoting self-induced cell death (apoptosis).(18)

BLUE LIGHT, OXIDATIVE STRESS IN RPE CELLS, and AMD

Exposure to blue light contributes to the escalating cycle of actions that increase the level of oxidative stress in the RPE cell with age. There is now strong evidence that chronic oxidative stress in RPE cells over decades has a major role in the development of AMD.

Oxidative stress in RPE cells induces an inflammatory response in the retina, which can exacerbate the generation of ROS. Low level chronic inflamation in the retina has been proposed as an important component of AMD. An immune system response to inflammation is activation of the complement system. RPE cells produce many of the 30 or more proteins that make up the complement system. Genetic studies have confirmed that activation of the complement system is involved in the development of AMD.(19)

The characteristics of AMD include the malfunctioning and degeneration of PC's and RPE cells, excessive formation of lipofuscin within RPE cells, a build up of drusen and basal deposits between the RPE and Bruch's membrane as well as the thickening of Bruch's membrane, and increased immune system activity including activation of the complement system.(20)

With age there is increased levels of lipofuscin accumulation within the lysosomes of RPE cells. A2E, its adducts and other blue light absorbing chromophores contained in lipofuscin generate increasing amounts of ROS. These ROS and reactive lipid and protein fragments leak out of the lysosome into the intracellular cytoplasm and attack other organelles within the RPE cell generating increasing levels of ROS, causing the dysfunction of the RPE cell and limiting its ability to service adjacent PCs. Lipofuscin accumulation and the resulting increase in photo-oxidative stress has been found to be is associated with extracellular deposition on the basal side of the RPE.(20)

The formation of soft drusen between the RPE and Bruch's membrane in the macular region of the retina is considered the hallmark of AMD. Many recent studies have demonstrated links between oxidative damage, inflammation, the complement system, RPE lipofuscin, basal deposits, and drusen formation. (21)

The build up of soft drusen in the macular area creates a barrier that limits the ability of RPE cells to absorb oxygen and nutrients from the choriocapillaris and to expel waste material into the blood stream through the Bruch's membrane/chroroid complex. This contributes to the malfunctioning and death of RPE cells, and results in the death of overlying PCs and a corresponding deterioration of vision. This progressive degeneration of photoreceptor cells in the foveal region of the macula leads to the form of blindness known as geographic atrophy, or the "dry" form of AMD.(22)

Extensive accumulation of lipofuscin and increased oxidative stress within RPE cells in the macular region and the resulting basal deposits and drusen formation are also associated with the promotion of abnormal blood vessels growing from the choriocapillaris through the RPE, a condition known as choroidal neovascularization (CNV). This invasion of the retinal space by these abnormal immature blood vessels typically results in hemorrhage, exudation, scarring, and serous retinal detachment. Leakage from these small, weak abnormal blood vessels into the space between the RPE and the PCs results in a rapid death of PCs in the region and the rapid development of blindness, and is known as the "wet" form of AMD.(23)

SUMMARY

Substantial progress has recently been made in the understanding of mechanisms by which blue light absorption contributes to increased levels of oxidative stress in the retina over a lifetime. It is now apparent that a lifetime of oxidative stress contributes to the formation and accumulation of material between the RPE cells, the cells that provide essential support to photoreceptor cells, and the Bruch's membrane/choroid complex through which the RPE cells access oxygen and nutrients and expel waste products. As chronic, long-term oxidative stress can interfere with the proper functioning of the retina and vision, there is a strong rationale for limiting exposure to blue light wavelengths throughout life.

We believe this review of recent studies provides compelling evidence that cumulative blue light exposure significantly contributes to the development of AMD. The evidence provided indicates that this occurs at a level that would advance the onset of AMD by several years for many of the users of light therapy. While there are some who claim that this causality is yet to be proven, a growing number of experts in AMD pathology are now recommending that it would be beneficial to limit exposure to blue light wavelengths over a lifetime.(24)

With the state of knowledge that exists today, there appears to be an unwarranted risk of damage to the vision of light therapy users from increased exposure to blue light wavelengths, when a safe and effective alternative is available.

1a.. **The European Eye Study (EUREYE) has now confirmed that blue light exposure combined with low levels of serum antioxidants is associated with the formation of AMD.**

"We found that the combination of blue light exposure and low plasma concentrations of antioxidants was also associated with the early stages of AMD, which are common in the population, and that blue light exposure in middle age might be more damaging than at younger ages." *Sunlight Exposure, Antioxidants, and Age-Related Macular Degeneration*. AE Fletcher et al. Arch Ophthalmol. Oct 22, 2008; 126:1396-1403.

1b. **This has lead some prominent researchers on the mechanisms involved in the development of AMD to now assert that "It is photo-oxidative stress, or the cumulative exposure to free radicals from blue light over a lifetime that causes AMD"**.

"According to Dr Stephen Beatty, Director of the Macular Pigment Research Group (MPRG) and consultant ophthalmologist at Waterford Regional Hospital ... 'It is photo-oxidative stress, or the cumulative exposure to free radicals from blue light over a lifetime that causes AMD." *Age-Related Macular Degeneration*. Danielle Barron. Irish Medical News July 9, 2007.

1c.) **AMD is a condition of advanced degeneration of the macular portion of the retina. The macula is a small portion of the retina that includes the region of the retina that is primarily responsible for detailed color vision. Over 8 million Americans suffer from visual problems as a result of AMD. AMD currently leads to progressive blindness in over 35% of persons over the age of 75. Because genetics factors play a part in AMD susceptibility, more than half the people with a family history of AMD will develop severe vision loss in their lifetime. With increasing life span the incidence of blindness from AMD is increasing rapidly, and it has been said that AMD is reaching epidemic levels in the U.S. and other developed countries.**

"AMD affects a region of the human retina called the macula, which lies in the central axis of vision. The macula is a region 6 mm in diameter... Because the life span of humans continues to increase as a function of improved nutrition and increased awareness of environmental factors, AMD is expected to nearly double in the next 25 years. To place this in perspective, 35% of the human population of 75 years or older has some degree of AMD. Projections by the National Institute of Aging suggest that one in five people in the USA will be 65 or older by 2030. Individuals 85 and older could exceed 10 million at that time." *New insights and new approaches toward the study of age-related macular degeneration*. Bok. Proc Natl Acad Sci USA. 2002 Nov 12; 99(23):14619-21.

"With increasing age, a gradually larger proportion of participants had advanced AMD; 54% (469 of 863) of all those 75 years and older had advanced AMD, 64% (258 of 406) of all those 85 years and older, 74% (37 of 50) of all those 95 years and older, and all, [100%] (eight of eight) 100 years and older had advanced AMD." *Age-related Macular Degeneration in Very Old Individuals with Family History.* Asbjorg et al American Journal of Ophthalmology 2007; 143(5): 889-890.

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"Age-related macular degeneration is the leading cause of irreversible blindness in people 50 years of age or older in the developed world. More than 8 million Americans have age-related macular degeneration, and the overall prevalence of advanced age-related macular degeneration is projected to increase by more than 50% by the year 2020." *Age-related macular degeneration.* N Engl J Med. 2008 Jun 12;358(24):2606-17. Jager RD, Mieler WF, Miller JW.

"An extraordinary fraction of the population is at risk for the development of AMD. For example, the Beaver Dam Eye Study found that nearly 20% of the population between 65 and 75 years of age is affected with either early or late age-related maculopathy and also that >35% of the population >75 years of age is similarly affected. These numbers are especially alarming given that the US Census Bureau has predicted that the number of people in these two age groups will increase by 80% in the next 25 years." Stone EM, Sheffield VC, Hageman GS. Molecular genetics of age-related macular degeneration. Hum Mol Genet. 2001 Oct 1;10(20):2285-92.

"Age-related macular degeneration (AMD) is accompanied by considerable consequences regarding the psychosocial quality of life. A considerable body of research literature now indicates, for instance, an increased rate of depression and substantial loss of everyday capabilities in AMD patients." *Quality of life by limited vision in old age: the example of age-related macula degeneration*. Der Ophthalmologe Online first July 16, 2008

"Age-related macular degeneration (ARMD) is the most common cause of blindness in older patients and is a major health care epidemic in developed countries." *Evaluation of the ABCR and glutathione peroxidase-3 genes in familial and sporadic cases of exudative age-related macular degeneration*. Shastry BS. Int J Mol Med. 2004 Oct;14(4):753-7.

1d. **The largest American epidemiological study on visual function found that an daily increase in exposure to sunlight of only 3 hours for young adults (in their teens through thirties) will advance the onset of AMD later in their lives by 10 years. A 10 year advance in onset of AMD effectively doubles the likelihood of becoming blind during a lifetime. After age forty, the protective mechanisms of the retina deteriorate progressively with age. Thus, older people are at an even higher risk of retinal damage from light exposure.**

"While controlling for age and sex, we found that participants exposed to the summer sun for more than 5 hours a day during their teens, in their 30s, and at the baseline examination were at a higher risk of developing increased retinal pigment and early ARM by 10 years than those exposed less than 2 hours per day during the same periods."*Sunlight and the 10-Year Incidence of Age-Related Maculopathy: The Beaver Dam Eye Study, Correction.* SC Tomany et al. Arch Ophthalmol. Mar 2005; 123(3):362

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“This paper is, to our knowledge, the first to objectively quantify lifetime exposure to sun and to evaluate the relationship between this and ARM....Our study shows that facial wrinkle length is positively related to late ARM prevalence, so lifetime exposure to sunlight and late ARM are considered to be positively related.” *Age-related maculopathy and sunlight exposure evaluated by objective measurement.* M Hirakawa, et al. British J. of Ophthalmology 2008;92:630-634.

"In our study, the associations of blue light exposure in those with low antioxidant levels appeared stronger at older ages, reaching a peak at ages 50 to 59 years. Penetration of shorter wavelengths of blue light into the retina decreases with age, principally as a result of a yellowing of the lens and a decreasing pupil diameter. The aging eye also accumulates chromophores, indicating increased susceptibility to oxidative damage from blue light." *Sunlight Exposure, Antioxidants, and Age-Related Macular Degeneration*. AE Fletcher et al. Arch Ophthalmol. Oct 22,2008; 126:1396-1403.

1e. **Visible blue light is the only component of sunlight implicated in the development of AMD. Accordingly, many retinal specialists are now expressing concern regarding lifetime exposure to blue light and recommending that people limit their exposure to blue light wavelengths throughout their lives, from both sunlight and artificial light sources.**

"The retina is vulnerable to the damaging effects of light. While wavelengths in the UV radiation range are largely absorbed by the cornea and lens, the retina is exposed to visible light, including blue light. Animal and laboratory studies have shown that blue light damages the retinal pigment epithelium and choriocapillaris through generation of reactive oxygen species and may be a factor in the pathogenesis of age related macular degeneration (AMD)."..."Lowering retinal exposure to blue light and ensuring that intake of key antioxidant nutrients is sufficient are the main recommendations from our study." *Sunlight Exposure, Antioxidants, and Age-Related Macular Degeneration*. AE Fletcher et al. Arch Ophthalmol. Oct 22, 2008; 126:1396-1403.

"As a result of the transmission properties of the cornea and lens, only visible light reaches the RPE in the adult human eye." *Role of Ocular Melanin in Ophthalmic Physiology and Pathology* D-N Hu et al. Photochem Photobiol. 2008 May-Jun;84(3):639-44.

“Unlike most previous studies of IOLs using only solar radiation, it examines retinal protection from 2 widely used artificial lamps in indoor and outdoor lighting. This was essential because lamps have different radiation spectra than solar radiation and because they are widely used for extended durations in the developed world. Even if a source has relatively low intensity, the retinal phototoxicity dose is determined by the product of intensity and duration.”
“For computing age-related comparative retinal phototoxicity ....3 relevant light sources were identified and selected as follows: solar radiation; Arcstream high-intensity lamp,...and Cool White Deluxe fluorescent lamps”

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In conclusion, our data show that accumulation of lipofuscin results in a significant increase in retinal phototoxicity with age. Interaction between the filtering curve of an IOL and the radiation spectrum of the light source determines the retinal protection provided by the IOL against this phototoxicity, independent of age. This protection is most noticeable in the discriminating 420 to 480 nm range of wavelengths, where significant absorbance of the A2E component of the accumulated lipofuscin occurs." *New approach to evaluate retinal protection by intraocular lenses against age-related lipofuscin accumulation –mediated retinal phototoxicity.* D Carson, TH Margrain, A Patel. J Cataract Refract Surg 2008; 34:1785–1792

2) **Numerous studies have implicated oxidative stress in macular photoreceptor cells (rods and cones) and the adjacent retinal pigment epithelium (RPE) as a causative factor in the development of AMD. Oxidative stress occurs when the level of pro-oxidants exceeds the level of antioxidants in a cell and results in the oxidation of cellular components and consequent loss of cellular function. In this discussion, molecules and cellular components that are no longer functional because of oxidative activity will be included in the term "oxidative debris"**

"There is good evidence that oxidative stress is involved in the pathogenesis of age-related macular degeneration (AMD)... Our findings confirm and expand the results of previous studies reporting that lipoproteins and phospholipids are contained in drusen, and that oxidation of these materials is strongly related to AMD... These findings indicate that oxidative stress is probably involved in the pathogenesis of AMD." *Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration* M. Suzuki, M. Kamei, H. Itabe, K. Yoneda, H. Bando, N. Kume, Y. Tano Molecular Vision 2007; 13:772-778

"Oxidative stress causes retinal pigment epithelium (RPE) cell dysfunction and is a major risk factor leading to the development of dry-type age-related macular degeneration.... Growing evidence indicates that oxidative stress injury to the retinal pigment epithelium (RPE) plays an important role in the etiology of AMD. The RPE is at high risk for oxidative injury due to its location in a highly oxygenated environment, its high levels of light exposure, and generation of reactive oxygen species (ROS) during POS [photorecptor outer segment] phagocytosis." *Alpha-2 But not Alpha-1 AMP-activated protein kinase mediates oxidative stress-induced inhibition of retinal pigment epithelium cell phagocytosis of photoreceptor outer segments.* Qin S, De Vries GW J Biol Chem. 2008 Mar 14; 283(11):6744-51

"Mounting evidence suggests that oxidative stress caused by reactive oxygen intermediates is a significant mechanism in the pathogenesis of age-related macular degeneration (AMD). ... These findings suggest that oxidative stress compromises the viability of RPE cells and CECs (choroidal endothelial cells)." *Growth-related effects of oxidant-induced stress on cultured RPE and choroidal endothelial cells* W. Eichler, A. Reichea, Y. Yafaia, J. Langea and P.Wiedemanna. Experimental Eye Research Oct 2008; 87(4): 342-348.

"Retinal oxidative stress caused by light exposure has been implicated in the pathogenesis of age-related macular degeneration (AMD) and other retinal degenerations. Photooxidative stress is exacerbated by an imbalance between light-induced reactive oxygen species (ROS) and antioxidants. Photooxidative stress has been implicated as a mechanism of retinal light damage." *Isoprostane F2A-VI, A New Marker of Oxidative Stress, Increases Following Light Damage to the Mouse Retina*. Dentchev T, Yao Y, Pratico D, Dunaief J. Molecular Vision Feb 2007. 13;Vol 13; 190-5

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"Oxidative stress occurs when the level of oxidants (ROIs) in a system exceeds the detoxifying capacity of its antioxidants. ROIs, which include free radicals, hydrogen peroxide and singlet oxygen, are unstable by-products of oxygen metabolism and interact with macromolecules causing damage to cells and tissues." *The rationale and evidence base for a protective role of macular pigment in age-related maculopathy.* E Loane, C Keliher, S Beatty, J M Nolan. Br J Ophthalmol. 2008 Sep;92(9):1163-8.

"Oxidative stress is thought to be an important contributing factor to the initiation and progression of AMD. The retina is vulnerable to oxidative stress because of its exposure to light and oxygen... In particular, damage to the retinal pigment epithelium by oxidative stress is an early indication of AMD." *Activation of Caspase-8 and Caspase-12 Pathways by 7-Ketocholesterol in Human Retinal Pigment Epithelial Cells.* Luthra S et al, Investigative Ophthalmology and Visual Science. Dec 2006;47:5569-5575

"Several studies indicate that oxidative stress is one of the causes of age-related macular degeneration (AMD), the leading cause of irreversible vision loss among people age 60 and older in the United States."*Increased expression of ceruloplasmin in the retina following photic injury.* Chen L, Dentchev T, Wong R, Hahn P, Wen R, Bennett J, Dunaief JL. Mol Vis 2003 Apr; 9:151-8.

“Evidence that Oxidative Stress is Responsible for ARM:
ROIs [Reactive Oxygen Intermediates] are produced in all cells as a by-product of metabolism, and additionally, in the retina, by photochemical reactions between light and oxygen. ROIs are likely to be particularly abundant in the retina because of its high metabolic rate, and because of the abundance of photosensitisers which increase photochemical production of ROIs in response to incident light energy .... Finally, several studies have demonstrated a link between the pro oxidant effects of light exposure, dietary fatty acids and retinal lipofuscin, and an increased risk for ARM." *Macular Carotenoids and Age-related Maculopathy.* O'Connell, et al. Ann Acad Med Singapore 2006;35:821-30

"These data support the hypothesis that oxidized lipoproteins are one trigger for initiating early events in the pathogenesis of AMD.” *Oxidized low density lipoproteins induce a pathologic response by retinal pigmented epithelial cells.*" Yamada Y, Tian J, Yang Y, Cutler RG, Wu T, Telljohann RS, Mattson MP, Handa JT. J Neurochem. 2008 May;105(4):1187-97

"AMD is an advanced stage of a deteriorative process that takes place in all eyes ... Beginning early in life, and continuing throughout the life span, cells of the RPE gradually accumulate sacs of molecular debris. These residual bodies (lipofuscin) are remnants of the incomplete degradation of abnormal molecules which have been damaged within the RPE cells or derived from phagocytized rod and cone membranes. Progressive engorgement of RPE cells with these functionless residues is associated with the extrusion of aberrant materials which accumulate in Bruch's membrane and aggregate in the form of drusen and basal laminar deposits. These excretions contribute to the further deterioration of the RPE. Loss of vision results from death of visual cells due to degeneration of RPE cells, or the effects of leakage from neovascular membranes that invade the region of abnormal extracellular deposits." *Pathophysiology of age-related macular degeneration*. Young RW. Surv Ophthalmol.1987; 31(5):291-306.

3. **The retinal pigment epithelium (RPE) is a monolayer of flat, polygonal, pigmented cells lying between the photoreceptor cells and the choroid, and is an integral part of the blood retinal barrier. The basal side of the RPE is in contact with Bruch's membrane whereas the apical side faces the photoreceptor cell outer segments. Bruch's membrane is an extra-cellular structure that acts like a sieve through which nutrients and waste products exchange between the retinal pigment epithelium and the blood bearing choroid.**

A major function of RPE cells is to maintain the survival and normal functioning of photoreceptor cells (PCs). RPE cells control nutrient/waste products exchange of the PCs. Every day each PC sheds about 10% of its outer segment. The outer segment of a PC is the region where light is absorbed and where photo-oxidative damage occurs. The tips of the outer segment of a PC are surrounded by the apical processes of an adjacent RPE cell. When a PC sheds the tip of its outer segment, this material is engulfed by the adjacent RPE cell. In this manner the RPE cells ingest much of the oxidative debris created as a byproduct of light absorption by PCs. Additionally the RPE cell reconfigures and recycles the *trans* configuration of Retinal molecules released from bleached visual chromophores back to the outer segments of photoreceptor cells in a *cis* form that can be used to re-synthesize active visual pigments. This process is termed "the visual cycle". Failure of any one of these functions can result in degeneration of the retina, loss of vision, and blindness.

"The retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood-retina barrier. Basal membrane of the RPE is in contact with Bruch's membrane whereas the apical membrane faces the photoreceptor outer segments. The close structural interactions of RPE cells with the outer retina indicate that the major functions of the RPE layer are to maintain the survival and normal functioning of photoreceptors by controlling nutrients/ waste products exchange, phagocytizing shed outer segments, shuttling retinoids to synthesize visual pigments, and producing trophic factors necessary for photoreceptor survival. Failure of any one of these functions can result in degeneration of the retina, loss of visual function, and blindness." *Alpha-2 But not Alpha-1 AMP-activated protein kinase mediates oxidative stress-induced inhibition of retinal pigment epithelium cell phagocytosis of photoreceptor outer segments.* Qin S, De Vries GW. J Biol Chem. 2008 Mar 14;283(11):6744-51

"The RPE plays an essential role in maintaining the health of the retina. The RPE is also the site of pathologic processes in a wide variety of retinal disorders including monogenic retinal dystrophies, age-related macular degeneration, and retinal detachment."
"The RPE consists of a monolayer of flat polygonal cells sandwiched between the outer segments of retinal photoreceptors and the choroidal vasculature. The RPE performs a variety of essential functions to support the retina: it forms part of the blood-retina barrier and mediates exchange of metabolites between the retina and the choroid; it stores and recycles the visual pigment chromophore (the visual cycle); it engulfs and digests the tips of photoreceptor outer segments; and it maintains the ionic composition of the subretinal space." *The Genomic Response of the Retinal Pigment Epithelium to Light Damage and Retinal Detachment*. A Rattner et al. The Journal of Neuroscience, Sep 24, 2008; 28(39):9880-9889
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"RPE cells form a polarized monolayer epithelium between the photoreceptors of the neurosensory retina and the choroidal capillary bed. Daily phagocytosis of outer segment tips (OS) shed by adjacent photoreceptors is a vital task of the RPE. RPE cells are postmitotic and face each ~30 photoreceptor outer segments in the human eye, all of which shed their distal tip containing stacked membrane disks once a day. Diurnal phagocytosis and digestion of thousands of OS disks for life renders RPE cells the most active phagocytes in the body."
"Photoreceptor function strictly depends on efficient RPE phagocytosis of spent OS. Complete failure of RPE cells to engulf OS causes rapid photoreceptor degeneration...The continuous nature of outer segment renewal implies that any delay in OS removal by aged or damaged RPE will gradually cause OS components to accumulate. RPE cells are at risk for oxidative damage due to their location in the highly oxygenated environment of the outer retina and their exposure to light." *The age-lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells.* C. Vives-Bauza, M.Anand, A.K. Shirazi, J. Magrane, J. Gao, H.R. Vollmer-Snarr, G. Manfredi, S.C. Finnemann J Biol Chem. Sep 2008; 283(36):24770-80.

"An intact retinal pigment epithelium (RPE) represents an essential condition for the visual process. This post-mitotic RPE monolayer combines different functions such as degradation of photoreceptor outer segments, vitamin A cycle, support of retinal metabolism and maintenance of the outer blood-retina barrier. As a consequence of excessive metabolism, high oxygen levels, exposition to light of short wave length and ensuing radical formation, the RPE is highly dependent on protective systems. In spite of differentiated defence mechanisms, aging processes cause cumulative RPE damage, representing a major component of age-related macular degeneration (AMD), the leading cause of irreversible severe vision loss in people over 50 years old." [*Pathomechanisms for aging of retinal pigment epithelium (RPE) and prophylactic therapy options in regard to AMD*] Schütt F, Kopitz J, Yu A, Welge-Lüssen U Klin Monatsbl Augenheilkd. 2008 Jun; 225(6):548-54.

"In the normal human eye, the retina pigment epithelium (RPE) forms a hexagonal cell monolayer that lines Bruch's membrane internally and separates the neural retina from the choriocapillaris. The RPE is responsible for maintaining the integrity of the neural retina, choriocapillaris, and Bruch's membrane. The apical RPE processes surround the distal tip of the outer segments. The RPE performs several crucial functions important for maintaining the outer retina, including phagocytosis of the distal tips of outer segments, recycling of visual pigment, and transferring nutrients from the choriocapillaris to the neural retina. In addition, the RPE is responsible for maintaining the integrity of the choriocapillaris, as surgical RPE removal or pharmacological RPE damage by intravitreal injection of ornithine or iodate leads to secondary choriocapillaris atrophy."
"Age-related macular degeneration (AMD) is characterized by cellular changes in the RPE, choriocapillaris, and outer retina and by structural changes within Bruch's membrane that include diffuse thickening, accumulation of drusen, basal laminar, and basal linear deposits, collagen cross-linking in the inner and outer collagen layer, calcification, and fragmentation of the elastin layer and lipidization." *Bruch's membrane aging decreases phagocytosis of outer segments by retinal pigment epithelium*. Sun K, Cai H, Tezel TH, Paik D, Gaillard ER, Del Priore LV. Mol Vis. 2007 Dec 21;13:2310-9.
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"Bruch's membrane is an extra cellular structure that acts like a sieve through which nutrients and waste products exchange between the retinal pigment epithelium and the blood bearing choroid. With age and age-related macular degeneration (AMD), debris accumulates in this membrane, resulting in increased thickness and decreased permeability." *Characterization of Lipid Oxidation Products and Proteins in 's Membrane from Normal and AMD Donor Eyes.* X. Gu, K. Shadrach, M. Sun, K. A. West, L. Shan, S. L. Hazen, R. G. Salomon, J. G. Hollyfield, J. W. Crabb. Association of Bimolecular Research Facilities Annual Meeting 2003 Poster P137-T.

4. **PC's and RPE cells are particularly vulnerable to oxidative damage because they are exposed to high levels of oxygen and light energy, and contain high levels of lipids and lipoproteins that are easily damaged by the radical oxygen species (ROS) induced in this environment. The consequences of oxidative damage to RPE and photoreceptor cells is severe because they are non-replicating (post-mitotic), and are not replaced after birth. Theses cells must therefore survive a lifetime of oxidative insult. Additionally, starting at about age 40 there is an age-related deterioration of the mechanisms that protect these cells from oxidative damage.**

"Oxidative stress has been suggested to be a major contributing factor for retinal degeneration in AMD. The retinas constant exposed to light and a relatively high oxygen pressure, which is close to that found in arterial blood, contributes to light-induced oxidative stress in the retina which may result in oxidative damage to biomolecules in these cells. RPE cells are post mitotic and therefore must respond to a life time of oxidative insult. While there are numerous mechanisms for preventing and combating oxidative injuries, by middle-age many of these anti-oxidative mechanisms have begun to break down, which can increase the susceptibility of RPE cells to accumulated damage. LF [lipofuscin] and MLF [melanolipofuscin] granules are thought to result from the accumulation of undegradable material in RPE cells. Modifications, including oxidation, may render the molecules in these granules undegradable by the cell, contributing to their accumulation." *Proteomic and Phototoxic Characterization of Melanolipofuscin: Correlation to Disease and Model for its Origin.* Warburton S, et al. Molecular Vision March 1, 2007; 13:318-329

"Several reports have shown that oxidative mechanisms constitute the initial stimulus that triggers apoptosis, thereby contributing to the progression of AMD. The retina is highly susceptible to photo-oxidative damage due to its high oxygen demand, life-long exposure to light and the presence of polyunsaturated fatty acids highly enriched in the photoreceptor outer segments This scenario is aggravated with age, for there is a reduction in the local antioxidative enzymes in the RPE ...The age-related increase in oxidative stress leads to cellular events which in turn induce the histopathological changes associated with AMD, as described above." *Age and disease-related structural changes in the retinal pigment epithelium*. Vera L Bonilha. Clinical Ophthalmology Feb 2008 2(2):413-424

"The RPE-choroid complex is known to be exposed to the highest oxidative stress in the eye. RPE cells are postmitotic; they will not divide after they have matured. Thus the exposure of the cells to high oxidative stress and light will last throughout the life of the individual, as may any modifications induced by oxidative stress and the resulting cellular dysfunction." *Age-dependent Photoionization Thresholds of Melanosomes and Lipofuscin Isolated from Human Retinal Pigment Epithelium Cells.* Hong L. et al Photochemistry and Photobiology 2006; 82(6):1475-81.

"However, when photoreceptors are stressed, the renewal of outer segments alone is not capable of overcoming the higher rates of oxidizing and detrimental chemical reactions, and the health of the entire photoreceptor cell is at risk *" An Hypothesis to Account for the Renewal of Outer Segments in Rod and Cone Photoreceptor Cells: Renewal as a Surrogate Antioxidant.* Barry S. Winkler Invest Ophthalmol Vis Sci. 2008 Aug;49(8):3259-61.

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"The retinal pigment epithelium (RPE) is a single layer of post-mitotic cells which functions both as a selective barrier to and a vegetative regulator of the overlying photoreceptor layer, thereby playing a key role in its maintenance.". *The role of the retinal pigment epithelium: topographical variation and ageing changes.* Boulton M, Dayhaw-Barker P. Eye 2001 Jun;15 (Pt 3):384-9.

“One of the highlights of postmitotic aging is the intracellular accumulation of highly oxidized and cross-linked proteins, known as lipofuscin. Lipofuscin is insoluble and not degradable by lysosomal enzymes or the proteasomal system, which is responsible for the recognition and degradation of misfolded and oxidatively damaged proteins. These aggregates have been found in various cell types, including heart, liver, kidney, neuronal tissue, and dermal tissue, and are associated with the life span of a single postmitotic cell and, consequently, of the whole organism.” *Lipofuscin: formation, distribution, and metabolic consequences*. Tobias Jung, Nicolle Bader, and Tilman Grune. Ann N Y Acad Sci. 2007 Nov;1119:97-111

5. **It is the short wavelength portion of the visible spectrum, the blue light region, that is associated with an increase in the level of oxidative stress in retinal cells. A strong indication that blue light wavelengths contribute to the development of AMD is found in the relationship between the density of the blue-light-absorbing yellow macular pigment and the development of AMD. Macular pigment is made up of the carotenoids lutein and zeaxanthin and covers the macula, a small region of the retina containing the fovea. The fovea is the portion of the retina responsible for detailed color vision. The density of macular pigment, which determines its ability to absorb blue light, is greatest over the fovea. Macular pigment density has been found to be inversely related to AMD susceptibility.**

"Due to its function of light perception, the eye is exposed to high levels of radiation of the optical spectrum. Most of the ultraviolet and infrared radiation is absorbed in the cornea and lens, and mostly only radiation of the visible spectrum can reach the retina. Visible light can cause retinal damage by photomechanical, photothermal, and photochemical mechanisms. The most important mechanism of light damage to the retina under daily conditions or when using ophthalmologic light sources is the photochemical light toxicity caused by light-induced chemical reactions. The extent of damage depends on several factors, such as wavelength, exposure time, and irradiance. Particularly the shorter portion of the visible light spectrum (blue light) is responsible for photochemical damage to the retina." [*Light exposition in vitreoretinal surgery : I. Basics*.] A E Hoh, T Ach, R Amberger, and S Dithmar. Ophthalmologe, Oct 2008; (10):905-910

"There is a growing body of evidence implicating oxidative stress and/or cumulative blue light damage in the process. The carotenoids lutein (L) and zeaxanthin (Z), to the exclusion of all other carotenoids, are concentrated in the macula, where they are collectively referred to as macular pigment (MP)." ... "The concentration of MP peaks at the center of the fovea and is optically undetectable at an eccentricity of 5° ..Because of its yellow color and position anterior to the photoreceptor outer segments, MP acts as a short-wavelength light filter for the foveal photoreceptors" *Spatial profile of macular pigment and its relationship to foveal architecture.* Nolan JM, Stringham JM, Beatty S, Snodderly DM. IOVS. May 2008; 49(5):2134-42

"There is an increasing body of evidence supporting the hypothesis that the macular pigment carotenoids, lutein and zeaxanthin, play an important role in protection against AMD, by filtering out blue light at a pre-receptoral level, or by quenching free radicals."
"The central retina, known as the macula, is responsible for central and color vision because of its high concentration of cone photoreceptors. The macula is characterized by a yellow color, attributable to the presence of macular pigment (MP). MP is composed of lutein and zeaxanthin, two hydroxycarotenoids, which are entirely of dietary origin..The concentration of MP peaks at the center of the macula, known as the fovea, where zeaxanthin is the dominant carotenoid.
AMD is the most common cause of irreversible blindness in people over 50 years of age in the developed world. ...At the macula, MP filters out blue light at a prereceptoral level and quenches free radicals. These actions are consistent with the hypothesis that MP protects against AMD. Several studies in humans have demonstrated the beneficial effects of xanthophylls in preventing the onset and progression of AMD." *Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration*. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S. Surv Ophthalmol. 2008; 3(1):68-81.

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"Conclusion Higher dietary intake of lutein/zeaxanthin was independently associated with decreased likelihood of having neovascular AMD, geographic atrophy, and large or extensive intermediate drusen." *The Relationship of Dietary Carotenoid and Vitamin A, E, and C Intake With Age-Related Macular Degeneration in a Case-Control Study*. AREDS* Report No. 22 Arch Ophthalmol. 2007; 125:1225-1232. * Age-Related Eye Disease Study Research Group

6. **The carotenoids lutein and zeaxanthin which make up the macular pigment are derived entirely from diet. While these carotenoids may have a protective antioxidant role in RPE cells, their primary protective role appears to be in the formation of macular pigment. Macular pigment is mostly located near the inner retina, which is too distant from the outer retina to act as an effective source of antioxidant protection of the outer segments of PCs or the adjacent RPE cells. Therefore, much of the protective capability of macular pigment with regard to AMD likely resides in the ability to limit the amount of blue light reaching the PCs and RPE cells in the macular region of the retina. The inhibitory effect that macular density has on the development of AMD can be considered as a strong indication that reducing exposure of the outer retina to blue light exposure delays or prevents the onset of AMD.**

"The Putative Protective Effect of Macular Pigment Against AMD.
MP peaks at the center of the fovea, with optically negligible levels outside the macula. Within the layer structure of the retina, the highest concentration of MP is seen in the receptor axon layer and the inner plexiform layer. The peak absorbance spectrum of MP is at 460 nm, and MP therefore filters out damaging blue light at a pre-receptoral level. It has been estimated that MP absorbs approximately 40% of blue light before it is incident on the photoreceptors. This is a particularly important function, as exposure to high-energy wavelengths can result in photochemical retinal injury."
"The peak concentration of MP at the center of the fovea is also consistent with its role as an optical filter. For example, it has been demonstrated that short wavelength cones ('s' cones) suffer a loss in sensitivity with increasing age. However, it has also been observed that this loss in sensitivity is less severe at the fovea, where MP peaks, suggesting a protective effect of the pigment."
"The presence of these carotenoids in the iris suggests a role in filtering out damaging short-wavelength visible light, whereas these carotenoids in the ciliary body are likely to have an antioxidant function only, and both mechanisms (light screening and antioxidation) may be operative in the RPE/choroid." *Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration*. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty Surv Ophthalmol. 2008 Jan-Feb; 53(1):68-81.

"Macular pigment is entirely of dietary origin, is ideally located, and exhibits important optical and antioxidant properties, which suggest that this pigment protects against ARM....Zeaxanthin is a powerful antioxidant that is concentrated, together with its constitutional isomer lutein, at the macula. Together, these antioxidants are believed to play a key role in protecting against oxidative stress and, by extension, against ARM. Several studies have reported an inverse association between risk of ARM and serum concentrations and dietary levels of lutein and zeaxanthin."
"The protection that retinal antioxidants confer against ARM must be exercised over many years, and decades before the onset of disease, because the oxidative basis of ARM pathogenesis is a chronic and cumulative process. In other words, the appropriate age profile of subjects to be investigated for any protective effect that diet confers against ARM is the 20–60-y age group and to relate findings to established and putative risk factors for this condition....Nevertheless, our finding that the most important and universal risk factor for ARM, age, is associated with a relative lack of dietary zeaxanthin is an important and novel finding" *Diet and risk factors for age-related maculopathy*. ED O'Connell et al, American Journal of Clinical Nutrition 2008; 87(3); 712-722.

“Taken in sum, these human cadaver eye data are consistent with the hypothesis that retinal carotenoids inhibit formation and oxidation of A2E in the underlying RPE it is more likely that inhibition of A2E formation by ingested carotenoids is mediated by a light filtering effect rather than a direct antioxidant mechanism because although there was a significant increase in RPE carotenoids in response to supplementation, levels still remained about ten times lower than the overlying retina.”
“Our findings provide evidence for a new mechanism for the potential protective effect of lutein and zeaxanthin in degenerative eye disorders, inhibition of A2E formation and oxidation.”
Retinal carotenoids can attenuate formation of A2E in the retinal pigment epithelium. P. Bhosale, B Serban, and PS Bernstein. Arch Biochem Biophys 15 March 2009; 483(2): 175-181

“Macular pigment is a natural barrier protecting the central retina against oxidative damage. It is formed by two dihydroxycarotenoids, lutein and zeaxanthin. The prereceptoral location of the macular pigment permits it to act as an optical filter that absorbs short-wavelength visible light. Carotenoids also demonstrate antioxidant activity. Eyes with a predisposition to develop AMD or which already have developed the disease have considerably less macular pigment and a greater risk of oxidative damage compared with healthy eyes.” [*The potential role of oxidative stress in the pathogenesis of the age-related macular degeneration (AMD)*] M Drobek-Slowik, D Karczewicz, and K Safranow Postepy Hig Med Dosw, Jan 1, 2007; 61: 28-37.

"In the retina, short-wavelength blue light initiates photosensitisation with the consequential generation of ROIs (Reactive Oxygen Intermediates). L[utein] and Z[eaxanthin], by virtue of absorbing blue light en route to the photoreceptors, may prevent this short-wavelength light from producing ROIs. From this perspective, the blue light filtering property of the macular carotenoids can be considered as a passive, or indirect, antioxidant function. Macular carotenoids are well-suited to act as an optical filter to the potentially damaging blue light for numerous reasons. First, the absorbance spectrum of macular carotenoids peaks at 460 nm, which corresponds to the wavelength of blue light..." *Macular Carotenoids and Age-related Maculopathy*. O'Connell, et al. Ann Acad Med Singapore 2006;35:821-30.

"By absorbing blue light, the macular pigment protects the underlying photoreceptor cell layer from light damage, possibly initiated by the formation of reactive oxygen species during a photosensitized reaction. There is ample epidemiological evidence that the amount of macular pigment is inversely associated with the incidence of age-related macular degeneration, an irreversible process that is the major cause of blindness in the elderly." *Biologic Mechanisms of the Protective Role of Lutein and Zeaxanthin in the Eye.* N.I. Krinsky et al., Annu Rev Nutr 2003; 23:171-201.

7. **Absorption of visible light by photosensitive pigments in photoreceptor cells is a major contributor to oxidative stress in the retina and results in the formation of oxidative molecular damage in PCs. The amount of oxidative damage produced in a PC appears to be proportional to the photon catch; i.e. the amount of light absorbed by the photoreceptor pigment in the cell.**

Blue light in particular appears to increase the amount of photo-oxidative damage generated in PCs. Blue light absorption can short-circuit the lengthy metabolic visual cycle through a process termed photoreversal, and greatly increase the photon catch in the PC. Higher levels of light absorbed by PCs would cause increased formation of oxidative damage.

"We also observed that blue light alone can inflict apoptotic death of visual cells, indicating to us, not only that rhodopsin readily absorbs blue light and is bleached, but also that this same blue light is absorbed by a photochemically active bleaching product that inflicts cellular damage." "Concerning blue light-induced apoptosis, we have shown that, similar to white light, the photon absorption by rhodopsin plays a critical role. A special feature of blue light, however, comes into play by the fact that rhodopsin bleaching product(s) strongly absorb in the blue (and near UV) range and possibly induce detrimental photochemical lesions in photoreceptors." *The Dark Side of Light: Rhodopsin and the Silent Death of Vision. The Proctor Lecture.* C.E. Reme Investigative Ophthalmology and Visual Science. 2005;46:2672-2682

"The pathogenesis of age-related maculopathy (ARM), the most common cause of visual loss after the age of 60 years, is indeed a complicated scenario that involves a variety of hereditary and environmental factors. The pathological cellular and molecular events underlying retinal photochemical light damage, including photoreceptor apoptosis, have been analysed in experimental animal models. Studies of age-related alterations of the retina and photoreceptors, the accumulation of lipofuscin in retinal pigment epithelium (RPE) cells, and the formation of drusen have greatly contributed to our knowledge. A new concept of an inflammatory response to drusen has emerged, suggesting immunogenic and systemic reactions in Bruch's membrane and the subretinal space. Oxidative stress and free radical damage also impact on the photoreceptors and RPE cells in the ageing eye. Based on the photoelectric effect, a fundamental concept in quantum physics, the consequences of high-energy irradiation have been analysed in animal models and cell culture. Short-wavelength radiation (rhodopsin spectrum), and the blue light hazard (excitation peak 440 nm), have been shown to have a major impact on photoreceptor and RPE function, inducing photochemical damage and apoptotic cell death." Algvere PV, Marshall J, and Seregard S. Review Article: *Age-Related Maculopathy and the Impact of Blue Light Hazard*. Acta Ophthalmologica Scandinavica 2006; 84(1):4 -15

“However, when photoreceptors are stressed, the renewal of outer segments alone is not capable of overcoming the higher rates of oxidizing and detrimental chemical reactions, and the health of the entire photoreceptor cell is at risk.” *An Hypothesis to Account for the Renewal of Outer Segments in Rod and Cone Photoreceptor Cells: Renewal as a Surrogate Antioxidant.* Barry S. Winkler Invest Ophthalmol Vis Sci. 2008 Aug;49(8):3259-61.

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"The photoreceptors in the retina, designed to initiate the cascade of events which link the incoming light to the sensation of 'vision', are susceptible to damage by light, particularly blue light. The damage can lead to cell death and diseases." *A2E and blue light in the retina: the paradigm of age-related macular degeneration.* Shaban H, Richter C. Biol Chem. 2002; 383(3-4):537-45.

"In the retina this damage may lead to the development of age-related macular disease. The retina is particularly susceptible to oxidative stress for several reasons: the retina is subject to high levels of radiation, particularly blue light; oxygen consumption by the retina is greater than that of other tissues; Photoreceptor outer segments contain a high proportion of polyunsaturated fatty acids, whose double bonds are a rich source of electrons; the retina contains photosensitisers, which make cells and tissues sensitive to the influence of irradiation; phagocytosis by the RPE generates ROI.." Bartlett H, Eperjesi, F. *A randomised controlled trial investigating the effect of nutritional supplementation on visual function in normal, and age-related macular disease affected eyes: design and methodology.* Nutrition Journal 2003, 2:12.

"Light is essential for vision but the trade off is the generation of potentially damaging reactive oxygen species within the eye." Boulton M, Rozanowska M, Rozanowski B. *Retinal Photodamage.* J Photochem Photobiol. B 2001 Nov 15;64(2-3):144-61.

"The photoreceptors of the retina present a puzzling phenomenon: they can be injured or even destroyed by light, the very element they are designed to detect." Reme CE et al. *Apoptosis in the Retina: The Silent Death of Vision.* News Physiol Sci 2000; 15: 120-124

8. **Normally, when a photo-pigment in a photoreceptor cell absorbs light, it bleaches and the photo-pigment disassociates into an opsin and the all-*trans* configuration of the Retinal molecule. However, only Retinal in the *cis* configuration can recombine with an opsin to form a photo-pigment. Because the all-*trans* configuration cannot bind with an opsin, it becomes unavailable for light absorption until the *trans*- Retinal is converted to the 11-*cis* configuration. This occurs in a lengthy metabolic process called "the visual cycle".**

"The visual cycle is a chain of biochemical reactions that regenerate visual pigment following exposure to light. Initial steps, the liberation of all-trans retinal and its reduction to all-trans retinol by retinol dehydrogenase (RDH), take place in photoreceptors."
"Photon absorption by a visual pigment within rod and cone photoreceptors produces a *cis*-to-*trans* isomerization of its retinal chromophore, resulting in an activation of the visual pigment (R* or metarhodopsin II). This initial photochemical event triggers the activation of the visual transduction cascade that eventually leads to transmission of a visual signal from the photoreceptor to other cells within the retina and thence to the brain. Once the chromophore has undergone photoisomerization, the visual pigment is said to be "bleached," i.e., it is no longer able to absorb photons in the "visual" region of the spectrum. The regeneration of the visual pigment to its previous dark state occurs by a series of biochemical reactions referred to as the visual cycle."
"The initial steps in the visual cycle occur immediately following photon absorption and appearance of the active form of the visual pigment. These initial steps are slow thermal reactions that result in separation of the all-*trans* retinal chromophore from opsin, the apoprotein portion of the visual pigment, followed by the reduction of all-*trans* retinal to all-*trans* retinol. All of these steps occur within the photoreceptor outer segment. Beyond this point, the visual cycle for rod and cone photoreceptors appears to diverge. In rods, retinol translocates from the outer segment via the intercellular matrix to the retinal pigment epithelium, where it is subjected to a multistep enzymatic conversion to 11-cis retinal. After this regenerative isomerization, the 11-*cis* retinal chromophore is translocated back to rod outer segments, where it condenses with the apoprotein to regenerate the dark visual pigment. In cones, recent work has proposed that all-*trans* retinol is likely to translocate from outer segments to Müller cells, where enzymatic isomerization to 11-*cis* retinol is suggested to occur. It is proposed that after transfer of this chromophore back to cones, oxidation of the retinol to retinal is followed by recombination of retinal with opsin to form the dark visual pigment." *Visual Cycle: Dependence of Retinol Production and Removal on Photoproduct Decay and Cell Morphology* P. Ala-Laurila, A.V. Kolesnikov, R.K. Crouch, E. Tsina, S.A. Shukolyukov, V.I. Govardovskii, Y. Koutalos, B. Wiggert, M.E. Estevez, and MC Cornwall. J. Gen. Physiol. 2006; 128(2): 153-169

"In vertebrate retinal photoreceptors, the absorption of light by rhodopsin leads to photoisomerization of 11-*cis*-retinal to its all-*trans* isomer. To sustain vision, a metabolic system evolved that recycles all-*trans*-retinal back to 11-*cis*-retinal. The importance of this visual (retinoid) cycle is underscored by the fact that mutations in genes encoding visual cycle components induce a wide spectrum of diseases characterized by abnormal levels of specific retinoid cycle intermediates. In addition, intense illumination can produce retinoid cycle by-products that are toxic to the retina. Thus, inhibition of the retinoid cycle has therapeutic potential in physiological and pathological states."*Metabolic basis of visual cycle inhibition by retinoid and nonretinoid compounds in the vertebrate retina.* Golczak M, Maeda A, Bereta G, Maeda T, Kiser PD, Hunzelmann S, von Lintig J, Blaner WS, Palczewski K.. J Biol Chem. Apr 11, 2008 ; 83(15): 9543-54.

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“Visual (retinoid) cycle is a fundamental metabolic process in the vertebrate retina responsible for production of 11-*cis*-retinal, the chromophore of rhodopsin and cone pigments. 11-*cis*-retinal is bound to opsins forming visual pigments, and when the resulting visual chromophore 11-*cis*-retinylidene is photoisomerized to all-trans-retinylidene, all-trans-retinal is released from these receptors. Toxic byproducts of the visual cycle formed from all-trans-retinal often are associated with lipofuscin deposits in the retinal pigmented epithelium (RPE)....
Evidence presented here indicates ...delayed all-*trans*-retinal clearance and A2E accumulation successfully reproduce...all the major features of AMD, providing genetic proof that abnormal reactions of the visual cycle can cause progressive changes similar to AMD” *Retinopathy in mice induced by disrupted all-trans-retinal clearance* A. Maeda, T. Maeda, M. Golczak, and K. Palczewski. J Biol Chem. 2008 Sep 26;283(39):26684-93.

“Visual perception begins with the absorption of a photon by an opsin pigment, inducing isomerization of its 11-*cis*-retinaldehyde chromophore. After a brief period of activation, the resulting all-trans-retinaldehyde dissociates from the opsin apoprotein rendering it insensitive to light. Restoring light sensitivity to apo-opsin requires thermal re-isomerization of all-trans-retinaldehyde to 11-*cis*-retinaldehyde via an enzyme pathway called the visual cycle in retinal pigment epithelial (RPE) cells. Vertebrates can see over a 10(8)-fold range of background illumination. This implies that the visual cycle can regenerate a visual chromophore over a similarly broad range....We show...in human RPE cells that this mobilization is mediated by a protein called "RPE-retinal G protein receptor" (RGR) opsin. These data establish that RPE cells are intrinsically sensitive to light.” *Retinal Pigment Epithelium-Retinal G Protein Receptor-Opsin Mediates Light-dependent Translocation of All-trans-retinyl Esters for Synthesis of Visual Chromophore in Retinal Pigment Epithelial Cells.* Radu RA, Hu J, Peng J, Bok D, Mata NL, Travis GH. J Biol Chem. 2008 Jul 11;283(28):19730-8.

"During visual excitation, rhodopsin undergoes photoactivation and bleaches to opsin and all-*trans*-retinal. To regenerate rhodopsin and maintain normal visual sensitivity, the all-*trans* isomer must be metabolized and re-isomerized to produce the chromophore 11-*cis*-retinal in biochemical steps that constitute the visual cycle and involve the retinal pigment epithelium. A key step in the visual cycle is isomerization of an all-*trans* retinoid to 11-*cis*-retinol in the RPE." *A photic visual cycle of rhodopsin regeneration is dependent on Rgr*. Chen P, Hao W, Rife L, Wang XP, Shen D, Chen J, Ogden T, Van Boemel GB, Wu L, Yang M, Fong HK. Nature Genet. 2001 Jul;28(3):256-60.

9. However, if the intermediary formed when the photo-pigment molecule absorbed light should then absorb blue light (< 480 nm), photoreversal causes a photoreceptor cell to rapidly become unbleached, and again available for light absorption . This appears to increase the amount of light absorbed by PCs by several orders of magnitude. Since the degree of photic damage in the PC is related to the amount of light absorbed, i.e. the photon catch, this greatly increases the amount of oxidative damage produced in the PC.

"Light induced retinal injury manifests endogenously programmed apoptotic cell death in the photoreceptors and the RPE in addition to toxic bio-oxidation. To elucidate the underlying pathomechanims, effort has been directed to search for the molecular initiator of such apoptotic pathway and accordingly the intrinsic photon receptor of the retina, rhodopsin, has become an obvious target of interest. As the availability of rhodopsin to capture photons depends critically on the rate of visual pigment regeneration, the expression of Rpe65 gene in the RPE, crucial to the re-isomerizaton of all-trans retinal to 11-cis retinal, has also been implicated."
"Rhodopsin recycling can occur in vitro via rapid photoreversal of bleaching by short-wavelength visible light (blue light) distinctive from the slower metabolic pathway via the RPE. With this process rhodopsin is regenerated from retinoid intermediates by rapid photochemical reactions many times faster than the metabolic pathway...these results have provided important clues to the possible mechanisms underlying phototoxicity and highlighted the rationale for avoiding excessive exposure of the retina to high frequency radiation such as ultraviolet and blue light." *Toxicology of the Retina: Advances in Understanding the Defence Mechanisms and Pathogenesis of Drug- and Light-Induced Retinopathy.* Siu et al. Clinical and Experimental Ophthalmology 2008; 36:176-185

"Acute white-light damage to rods depends on the amount of rhodopsin available for bleaching during light exposure. ... Because photoreversal is faster than metabolic regeneration of rhodopsin by several orders of magnitude, the photon catch capacity of the retina is significantly augmented during blue-light illumination, which may explain the greater susceptibility of the retina to blue light than to green light. ... Blue light can also affect function of several blue-light-absorbing enzymes that may lead to the induction of retinal damage... When a visual pigment molecule is excited by photon absorption, rhodopsin rapidly decays (1 ms) through bleaching intermediates to metarhodopsin I and II (MI and MII). High-energy blue light is absorbed by MII, which is photoreversed back to original chromophore 11-cis retinal attached to its apoprotein opsin. In high-photon fluxes, 1 molecule of rhodopsin can be reversed many times (>50 times within 30 min). CONCLUSIONS: Short time exposure to blue light has deleterious effects on retinal morphology..... Photoreversal of bleaching, which occurs only in blue but not in green light, increases the photon-catch capacity of the retina and may thus account for the difference in the damage potential between blue and green light." Grimm C, et al. *Rhodopsin-Mediated Blue-Light Damage to the Rat Retina: Effect of Photoreversal of Bleaching.* Invest Ophthalmol Vis Sci 2001 Feb; 42(2):497-50.

"Blue light can efficiently restore functional rhodopsin from bleaching intermediates through a process termed photoreversal of bleaching. This process does not depend on the visual cycle via the pigment epithelium but nevertheless enables rhodopsin molecules to absorb the critical number of photons required to induce retinal degeneration." *Protective effect of halothane anesthesia on retinal light damage: inhibition of metabolic rhodopsin regeneration*. Keller C, Grimm C, Wenzel A, Hafezi F, Reme C. Invest Ophthalmol Vis Sci. 2001 Feb;42(2):476-80.

10. **Every day the spent tips of the outer segments of PCs are phagocytosed (engulfed) by an adjacent RPE cell, where it is ingested and degraded by the RPE cell's lysosomes. In post-mitotic, non-replicating cells, including RPE cells, indigestible materials collect in the lysosomes as lipofuscin, also known as the aging pigment. Retinal lipofuscin in RPE cells is an aggregate of highly oxidized cross-linked proteins and lipids that is primarily composed of material derived from the phagocytosed PC outer segments.**

"One of the highlights of postmitotic aging is the intracellular accumulation of highly oxidized and cross-linked proteins, known as lipofuscin. Lipofuscin is insoluble and not degradable by lysosomal enzymes or the proteasomal system, which is responsible for the recognition and degradation of misfolded and oxidatively damaged proteins. These aggregates have been found in various cell types, including heart, liver, kidney, neuronal tissue, and dermal tissue, and are associated with the life span of a single postmitotic cell and, consequently, of the whole organism. Lipofuscin formation appears to depend on the rate of oxidative damage to proteins, the functionality of mitochondrial repair systems, the proteasomal system, and the functionality and effectiveness of the lysosomes." *Lipofuscin: formation, distribution, and metabolic consequences*. T. Jung, N. Bader, and T. Grune. Ann N Y Acad Sci. Nov 2007;1119:97-111

"Exposure to intense light is thought to acutely induce retinal damage by generating the production of high doses of lipid peroxidation– derived DNA-reactive aldehydes that trigger photoreceptor cell apoptosis. Besides forming DNA lesions, the reactive aldehydes resulting from lipid peroxidation are also capable of easily forming protein adducts. However, if such protein damage occurs in the photoreceptor outer region, the permanent renewal of the outer segments will clear away and replace the damaged proteins. Thus, persistent damage of the retinal outer region by such protein modifications appears unlikely. The retinal pigment epithelium, which has to phagocytose and degrade all material shed from the photoreceptor outer region, may be affected by damaged POS[Photoreceptor Outer Segment] proteins." *Effects of lipid peroxidation-related protein modifications on RPE lysosomal functions and POS phagocytosis* Kaemmerer E, Schutt F, Krohne TU, Holz FG, Kopitz J. Invest Ophthalmol Vis Sci. 2007 Mar;48(3):1342-7.

"A characteristic of aging of postmitotic cells such as cardiac muscle, neurons, and retinal pigment epithelium (RPE) is the accumulation of lipofuscin. .. In most cell types, lipofuscin is a product of cellular autophagy. In RPE cells, however, a major component of the lipofuscin is derived from the phagocytosis of the rod outer segment (ROS) disc membranes. *Retinal Pigment Epithelium Cell Damage by A2-E and its Photo-Derivatives.* Hammer M., et al. Molecular Vision. Nov 1, 2006 12:1348-54

"In the RPE, lipofuscin is derived primarily from phagocytosis of shed photoreceptor outer segments and is associated with a functioning retinoid visual cycle. ....Our observations...are consistent with early theories suggesting a role for lipoxidation in lipofuscin formation." *Retinal Pigment Epithelium Lipofuscin Proteomics.* K-P Ng, B Gugiu, K Renganathan, MW Davies, X Gu, JS. Crabb, SR Kim, MB Rózanowska, VL Bonilha, ME Rayborn, RG Salomon, JR Sparrow, ME Boulton, JG. Hollyfield, and JW Crabb. Mol Cell Proteomics. Jul, 2008; 7(7):1397-405.

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"Indeed, storage bodies containing autofluorescent age lipids termed "lipofuscin" build up in human RPE over time as a direct consequence of incomplete OS [Outer Segment] digestion. While some other post-mitotic cells build up age lipid granules with age, excess accumulation of RPE lipofuscin containing specific retinoids is associated with several hereditary retinal degenerations and may contribute to AMD." *The age-lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells.* C. Vives-Bauza, M.Anand, A.K. Shirazi, J. Magrane, J. Gao, H.R. Vollmer-Snarr, G. Manfredi, S.C. Finnemann J Biol Chem. Sep 2008; 283(36):24770-80.

11. **A major component of retinal lipofuscin is A2E, a phototoxic indigestible molecule formed in the lysosomes of RPE cells from precursors derived from photo-excited all-*trans*-Retinal produced in PCs. When A2E absorbs blue light it generates highly reactive radical oxygen species (ROS). A2E and its derivatives appear to be the primary source of blue light induced ROS production by RPE lipofuscin. Blue light absorption by A2E also results in the formation of epoxides that can damage DNA and trigger cell death.**

"The nondegradable pigments that accumulate in retinal pigment epithelial (RPE) cells as lipofuscin constituents ... The best characterized of these fluorophores is A2E,... Evidence indicates that photochemical mechanisms initiated by excitation from the blue region of the spectrum may contribute to the adverse effects of A2E accumulation, with the A2E photooxidation products being damaging intermediates" *Characterization of peroxy-A2E and furan-A2E photooxidation products and detection in human and mouse retinal pigment epithelial cell lipofuscin.* Jang YP et al. J. Biol Chem. 2005 Dec 2;280(48):39732-9.

"Overproduction of fluorescent lipofuscin pigment, i.e. dihydro-N-retinylidene- N retinylphosphatidyl- ethanolamine (A2PE-H2), in photoreceptor cells and its subsequent conversion and accumulation in the form of A2E in RPE as indicated by fundus autofluorescence, precedes macular degeneration and visual loss in AMD.... A2E accumulation causes retinal and macular degeneration" *Metabolic basis of visual cycle inhibition by retinoid and nonretinoid compounds in the vertebrate retina.* Golczak M, Maeda A, Bereta G, Maeda T, Kiser PD, Hunzelmann S, von Lintig J, Blaner WS, Palczewski K. J Biol Chem. Apr 11, 2008; 283(15): 9543-54.

"When A2E is exposed to blue light, singlet oxygen molecules are generated, which add to the carbon–carbon double bonds of A2E along the side arms. The highly reactive photooxidized A2E species that are generated likely account for the cellular damage ensuing from A2E irradiation." *Immunochemical recognition of A2E, a pigment in the lipofuscin of retinal pigment epithelial cells.* Abeywickrama C, Matsuda H, Jockusch S, Zhou J, Jang YP, Chen BX, Itagaki Y, Erlanger BF, Nakanishi K, Turro NJ, Sparrow JR. Proc Natl Acad Sci U S A. 2007 Sep 11;104(37):14610-5.

"A2E is the major fluorophore of lipofuscin and acts as a photosensitizer to generate reactive oxygen species inside the cells upon exposure to blue light. An increasing body of literature indicates that oxidative stress and dysfunction of RPE are associated with the pathogenesis of AMD, the leading cause of blindness in industrialized countries." *Oxidative inactivation of the proteasome in RPE: A potential link between oxidative stress and upregulation of IL-8.* Fernandes AF, Zhou J, Zhang X, Bian Q, Sparrow JR, Taylor A, Pereira P, Shang F J Biol Chem. Jul 25, 2008; 283(30):20745-53

"Thus, blue-light irradiation of A2-E may induce photooxidation, and the resulting epoxides may trigger the apoptosis cascade yielding DNA fragmentation... In conclusion, our data support the hypothesis of direct DNA damage by oxidized A2-E ... Certainly, oxidative processes are responsible for the detrimental effect of A2-E to RPE cells under irradiation." *Retinal Pigment Epithelium Cell Damage by A2-E and its Photo-Derivatives.* Hammer M., et al. Molecular Vision. Nov 1, 2006 12:1348-54.

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“Apparently, once formed, the RPE cell has no means to either degrade or transport LF (lipofuscin) material and granules into the extracellular space via exocytosis. Subsequently, these granules are trapped in the cytoplasmic space. Previous studies have shown that various LF compounds such as A2-E (N-retinylidene-N-retinylethanol-amine), a dominant fluorophore, possess toxic properties which may interfere with normal cell function via various molecular mechanisms. There are many other compounds in LF in addition to A2-E. These include precursors of A2-E, molecules formed by the mixture of oxygen-containing moieties within photooxidized A2-E, reactions between retinoids and other constituents other than ethanolamine, and peroxidation products of proteins and lipids.” *Fundus Autofluorescence and Progression of Age-related Macular Degeneration.* Steffen Schmitz-Valckenberg, Monika Fleckenstein, Hendrik P.N. Scholl, and Frank G. Holz. Survey of Ophthalmology. Jan-Feb 2009. 54(1): 96-117

CONCLUSIONS: A2E is essential to blue [450 nm] light-induced hRPE[human RPE] cell damage. Only blue light exposure and without A2E lead to little cell injury. hRPE cells in old people which contain much lipofuscin are sensitive to blue light injury. [*Blue light-induced damage to human retinal pigmented epithelial cells mediated by A2E*]. Zhonghua Yan Ke Za Zhi, November 1, 2007; 43(11): 1017-21 H Sheng, Y Lu, and FL Qing

"When the pyridinium bisretinoid A2E, an age-related fluorophore in the retinal pigment epithelium (RPE), is irradiated with blue light, photochemical events are initiated that can ultimately provoke cell death." *DNA is a target of the photodynamic effects elicited in A2E-laden RPE by blue-light illumination.* Sparrow JR, Zhou J, Cai B. Invest Ophthalmol Vis Sci. 2003 May; 44(5): 2245-51

12. **Retinal lipofuscin begins to accumulate in RPE cells at birth and increases with age. When exposed to blue light retinal lipofuscin generates ROS, raising the level of oxidative stress in RPE cells. Blue light exposure of lipofuscin inhibits proteosome activity, thus reducing the ability of these RPE cells to degrade waste material. Blue light absorption by lipofuscin can also inactivate lysosomal hydrolases and impair lysosomal stability. Extensive accumulation of retinal lipofuscin in RPE cells is associated with increased oxidative stress that can cause the malfunctioning or death of the RPE cells. Given the multiple functions of the RPE in the maintenance of photoreceptor cells, it is not surprising that RPE dysfunction has been implicated in AMD.**

"Remarkable accumulation of lipofuscin, the remnant of lysosomes in the RPE cells, is an early-onset senescent change in human RPE cells-as observed in other cell types. Lipofuscin begins to accumulate after birth, and its accumulation becomes prominent by the 4th decade of life. In addition, insoluble components in lipofuscin increase with age ."
"In conclusion, the accumulation of lipofuscin, especially involving insoluble components increasing with age, may be implicated in the pathogenesis of AMD." *Glycoxidized particles mimic lipofuscin accumulation in aging eyes- a new age-related macular degeneration model in rabbits* T. Yasukawa. Graefe's Arch Clin Exp Ophthalmol Oct, 2007; 245(10):1475-85

"We have demonstrated unequivocally that the toxic components of lipofuscin are associated with the granules, which contain phototoxic bisretinoids and many different lipids...Oxidative stress has been associated with a host of age-related pathologies and long been suspected of contributing to lipofuscinogenesis. Previous studies have demonstrated that RPE lipofuscin is a potent generator of reactive oxygen species including superoxide anion, singlet oxygen and lipid hydroperoxides that have the capacity to escape the lysosome and modify other cellular compartments such as nuclear DNA and the plasma membrane, as demonstrated in cell culture. Given the highly conjugated structures of lipids and retinoids, and their susceptibility to attack by free radicals and singlet oxygen, lipofuscin granules must be considered a rich source of highly reactive oxidative fragmentation products...A2E has been shown to fragment upon irradiation [430 nm] and complement to be activated in serum overlying irradiated[430 nm] A2E-containing RPE cells, which raises the possibility that reactive fragments may escape from RPE cells"
"This finding is consistent with previous studies demonstrating RPE lipofuscin to be a potent generator of reactive oxygen species and supports the hypothesis that such species, including reactive fragments from lipids and retinoids, contribute to the mechanisms of RPE lipofuscin pathogenesis."
"Toward a pathogenic mechanism for RPE lipofuscin, we hypothesize that oxidative cleavage of lipid and retinoid precursors within the granule generates a plethora of reactive fragments, some of which diffuse through membranes and modify intracellular and extracellular components with physiological consequences." *Retinal Pigment Epithelium Lipofuscin Proteomics.* K-P Ng, B Gugiu, K Renganathan, MW Davies, X Gu, JS. Crabb, SR Kim, MB Rózanowska, VL Bonilha, ME Rayborn, RG Salomon, JR Sparrow, ME Boulton, JG. Hollyfield, and JW Crabb. Molecular & Cellular Proteomics 2008; 7:1397-1405.

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"Besides being a product of lipid peroxidation processes, lipofuscin itself may also represent a source of lipid peroxidation damage. It has been shown that exposure of lipofuscin-fed RPE cells to short-wavelength-visible light causes photo-oxidative processes generating several oxygen-reactive species, which also produce increased levels of MDA and HNE. Thus, the RPE not only has to deal with lipid peroxidation–modified substrates resulting from phagocytosis but also with substrate modifications generated directly in the lysosomal compartment....Photoreactivity of lipofuscin is also likely to inactivate lysosomal hydrolases and impair lysosomal stability." *Effects of lipid peroxidation-related protein modifications on RPE lysosomal functions and POS phagocytosis.* Kaemmerer E, Schutt F, Krohne TU, Holz FG, Kopitz. Invest Ophthalmol Vis Sci. 2007 Mar;48(3):1342-7.

"Dysfunction of the ubiquitin-proteasome pathway (UPP) is associated with several age-related degenerative diseases. The objective of this study was to investigate the effect of oxidative stress on the UPP in cultured human retina pigment epithelial cells. ... exposure of A2E-loaded ARPE-19 cells to blue light resulted in a 40% to 60% reduction in proteasome activity. Loading of A2E or exposure to blue light alone had little effect on proteasome activity.
CONCLUSIONS. These data show that the proteasome in ARPE-19 is susceptible to oxidative inactivation, whereas activities of the ubiquitin-conjugating enzymes are more resistant to oxidative stress. Oxidative inactivation of the proteasome appears to be one of the mechanisms underlying stress-induced accumulation of ubiquitin conjugates in the cells." *The Proteasome: A Target of Oxidative Damage in Cultured Human Retina Pigment Epithelial Cells.* Xinyu Zhang, Jilin Zhou, Alexandre F. Fernandes, Janet R. Sparrow, Paulo Pereira, Allen Taylor, and Fu Shang.. Investigative Ophthalmology and Visual Science. 2008; 49(8):3622-3630.

"The RPE is essential for maintenance of retinal health because it is necessary for the phagocytic uptake and degradation of the constantly shed photoreceptor outer segments (POS). Since RPE lysosomes are the major source for degradation of POS every day, impaired lysosomal function due to aging is the major cause of accumulation of POS, a precursor of lipofucsin granules and extracellular drusen. The increased lipofucsin granules and drusen in retina are the hallmarks of an early-stage of AMD. To explain the complex etiology of AMD, other pathogenic mechanisms such as RPE cell death, oxidative damage of cellular components, mitochondrial dysfunction, inflammation and activation of the innate immune system and the accumulation of toxic compounds such as advanced glycation end products have been proposed." *Carotenoid derived aldehydes-induced oxidative stress causes apoptotic cell death in human retinal pigment epithelial cells.* Kalariya NM, Ramana KV, Srivastava SK, van Kuijk FJ. Exp Eye Res. Jan 2008; 86(1):70-80.

"The retina has the highest metabolic rate and oxygen consumption in the body. The high metabolic rate and oxygen consumption is usually accompanied by generation of reactive oxygen species. Chronic exposure to light could also increase the production of reactive oxygen species. Therefore, retinal pigment epithelium (RPE) is a primary target of oxidative stress."
"Oxidative stress, which refers to cellular damage caused by reactive oxygen species, has been implicated in the onset and progression of many age-related diseases, including AMD...Age-related accumulation of lipofuscin in RPE is another source of oxidative stress. Lipofuscin is a mixture of non-degradable protein-lipid aggregates derived from the ingestion of photoreceptor outer segments." *Oxidative inactivation of the proteasome in RPE: A potential link between oxidative stress and upregulation of IL-8.* Fernandes AF, Zhou J, Zhang X, Bian Q, Sparrow JR, Taylor A, Pereira P, Shang F. J Biol Chem. Jul 25, 2008; 283(30):20745-53.

“Lipofuscin accumulation in the RPE is a common downstream pathogenic pathway in various monogenic and complex retinal diseases including age-related macular degeneration (AMD). Lipid peroxidation-induced modification of proteins is thought to play a role in lipofuscinogenesis and may contribute to RPE dysfunction....The initiation of lipid peroxidation also requires the generation of oxygen-derived free radicals and the presence of polyunsaturated fatty acids. The outer retina is exposed to light in an oxygen-rich environment, and unsaturated fatty acids are present in high concentrations in the photoreceptor membranes of the retina. Accordingly, evidence for light-induced lipid peroxidation reactions in the retina has been reported in several studies.” *Effects of lipid peroxidation-related protein modifications on RPE lysosomal functions and POS phagocytosis* Kaemmerer E, Schutt F, Krohne TU, Holz FG, Kopitz J. Invest Ophthalmol Vis Sci. 2007 Mar;48(3):1342-7.

"Blue visible light damage to retinal pigment epithelial cells occurs through a photooxidative mechanism and the resultant damage is hypothesized to induce or exacerbate age-related macular degeneration... Therefore the inhibition of production of HGF {Hepatocyte Growth Factor] by visible light, especially by blue light, may enhance the phototoxic effects of visible light on the RPE and retinal neurons and plays an important role in the development of damage to RPE and retinal neuron after irradiation with blue light" *Blue Light Irradiation Inhibits the Production of HGF by Human Retinal Pigment Epithelium Cells In Vitro*. Chu R et al. Photochemistry and Photobiology 2006: 82(5): 1247-51

"Because the RPE is vital to the integrity of the photoreceptor cells, the demise of RPE cells brings about the loss of photoreceptors... Studies performed over the past several years have pointed to the fluorophores that constitute the lipofuscin of RPE cells as being crucial factors in the degeneration of these cells in macular degeneration. Of added importance is the fact that lipofuscin accumulates with age in the RPE cells of all eyes... Much of this indigestible pigment originates in the photoreceptor cell, with deposition in the RPE occurring because it is the responsibility of the RPE to internalize membranous debris discarded daily by the photoreceptor cell." *Therapy for macular degeneration: Insights from acne.* Sparrow JR. PNAS April 15, 2003; 100(8):4353-4354.

“In conclusion, our data show that accumulation of lipofuscin results in a significant increase in retinal phototoxicity with age. Interaction between the filtering curve of an IOL and the radiation spectrum of the light source determines the retinal protection provided by the IOL against this phototoxicity, independent of age. This protection is most noticeable in the discriminating 420 to 480 nm range of wavelengths, where significant absorbance of the A2E component of the accumulated lipofuscin occurs.” *New approach to evaluate retinal protection by intraocular lenses against age-related lipofuscin accumulation –mediated retinal phototoxicity.* Dennis Carson, PhD, DABT, Tom H. Margrain, PhD, Anil Patel, PhD J Cataract Refract Surg 2008; 34:1785-1792

“Here, we show that A2E, a quaternary amine and retinoid by-product of the visual cycle, causes the accumulation of free and esterified cholesterol in RPE cells. ...Our results provide direct evidence that A2E causes aberrant cholesterol metabolism in RPE cells which could likely contribute to AMD progression.” *The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells.* Aparna Lakkaraju, Silvia C. Finnemann, and Enrique Rodriguez-Boulan. Proc. Natl. Acad. Sci. USA, Jun 26, 2007; 104(26):11026-31.

13. **RPE cells are among the most metabolically active cells in the body. They contain large numbers of mitochondria, the "power sources" of cells. Damaged mitochondria produce high levels of ROS which can significantly contribute to the oxidative stress in aging cells, particularly post-mitotic cells such as RPE cells. Mitochondria are also sensitive to damage from ROS. Blue light absorption and oxidative stress can damage mtDNA, increasing ROS production and the level of oxidative stress in the RPE cell. Thus ROS formation contributes to further damage of mitochondria, which in turn contributes to further ROS formation. This is a self escalating cycle.**

"Oxidative stress is implicated in wide range of age-related disorders including...macular degeneration ....Superoxide production precedes subsequent reactions that form potentially more dangerous reactive oxygen species (ROS) species such as the hydroxyl radical (OH), hydrogen peroxide (H(2)O(2)) and peroxynitrite (OONO(-)). The major source of ROS in the mitochondria, and in the cell overall, is leakage of electrons from complexes I and III of the electron transport chain. It is estimated that 0.2-2% of oxygen taken up by cells is converted to ROS, through mitochondrial superoxide generation, by the mitochondria. ...mitochondria are perhaps the most significant contribution to ROS production affecting the aging process. In addition to producing ROS, mitochondria are also a target for ROS which in turn reduces mitochondrial efficiency and leads to the generation of more ROS in a vicious self-destructive cycle.*" Mitochondrial function and redox control in the aging eye: Role of MsrA and other repair systems in cataract and macular degenerations.* Brennan LA, Kantorow M. Exp Eye Res. Feb 2009;88(2):195-203

"AMD, diabetic retinopathy and glaucoma are the leading cause of severe visual loss in developed countries. Although the pathogenesis of these diseases has yet to be fully elucidated, oxidative stress is considered to be a significant contributor and mitochondria represent the major source of endogenous reactive oxygen species (ROS) in most cell types.
ROS are highly reactive molecules which can cause oxidative damage to proteins, nucleic acids and lipids. ROS can be free radicals (i.e. species capable of independent existence that contain one or more unpaired electrons), oxygen species which have been elevated to a higher energy level (e.g. singlet oxygen) or strong oxidizing agents (e.g. hydrogen peroxide).
There is strong evidence for mitochondrial dysfunction being involved in AMD...The last decade has seen direct evidence of mitochondrial dysfunction in macular degeneration....Mitochondria are the major source of intracellular ROS in most cell types. Consistent with the notion that the mitochondria may contribute to cellular degeneration is the fact that this organelle is uniquely vulnerable to oxidative damage.
The mitochondrial genome is inherently unstable as it is repeatedly exposed to spontaneous chemical reactions arising from a wide variety of insults, e.g. ROS, cigarette smoke, blue light and alkylating agents. Perhaps the most important target of ROS in the mitochondria is the mtDNA.
Conclusions: The findings discussed in this article highlight the importance of mtDNA damage and strongly suggests a central role for genomic instability in the etiology of AMD and possibly other retinal diseases involving oxidative stress. *Mitochondrial DNA damage and its potential role in retinal degeneration.* Jarrett SG, Lin H, Godley BF, Boulton ME. Prog Retin Eye Res. Nov, 2008;27(6):596-607

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"Our findings suggest that RPE cells rely on mitochondria to generate ATP for [photoreceptor cell] OS [outer segment] uptake and that A2E interferes with this process...Because post-mitotic RPE cells must support overlying photoreceptor neurons for life, decrease in any of their support functions will eventually impair photoreceptor function and vision."
"Mitochondria play a central role in aging and in the pathogenesis of age-related neurodegenerative diseases, mainly for two reasons: they are the main source of cellular ATP and the major cellular source of reactive oxygen species. Oxidative damage to mitochondria can lead to a spiral of noxious effects, whereby damaged mitochondria in turn release more reactive oxygen species, increasing oxidative damage and leading eventually to dysfunctional or defective mitochondria. Post-mitotic tissues like the RPE are most susceptible to this damage, particularly because they are more likely to accumulate somatic mutations in their mitochondrial DNA (mtDNA). Mutations of mtDNA and decreases in RPE cell number during aging and AMD have been described." *The age-lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells.* Cristofol Vives-Bauza, Monika Anand, Arash K. Shirazi, Jordi Magrane, Junping Gao, Heidi R. Vollmer-Snarr, Giovanni Manfredi, and Silvia C. Finnemann J. Biol. Chem Sep 5, 2008;283(36):24770-80.

CONCLUSIONS. mtDNA oxidative damage seems to be the "trigger" for cell dysfunction in high glucose *B* treated HRECs by setting in motion the vicious circle of mtDNA damage leading to ROS overproduction and further mtDNA damage, which may explain in part early vascular damage in diabetic retinopathy. *Mitochondrial DNA Oxidative Damage Triggering Mitochondrial Dysfunction and Apoptosis in High Glucose B Induced HRECs* Lin Xie, Xiaobo Zhu, Yiqun Hu, Tao Li, Yi Gao, Yu Shi, and Shibo Tang Invest Ophthalmol Vis Sci. 2008;49:4203-4209

14. **Mitochondria contain chromophores that generate ROS when they absorb blue light. Blue light absorption and ROS also damage mitochondria DNA (mtDNA) causing additional ROS production and raising the level of oxidative stress in the RPE cell.**

"Light can be absorbed by mitochondrial enzymes such as cytochrome and flavin oxidases causing the generation of reactive oxygen species, and we have suggested this may pose a risk to ganglion cell survival if their energy state is compromised,....Our findings support the proposal that the interaction of light, particularly the blue component, with intra-axonal ganglion cell mitochondria may be deleterious under certain circumstances, and suggest that reducing the light energy impinging upon the retina might benefit patients with certain optic neuropathies." *Light affects mitochondria to cause apoptosis to cultured cells: possible relevance to ganglion cell death in certain optic neuropathies*. Neville N. Osborne, Guang-Yu Li, Dan Ji, Heather J. Mortiboys, Sandra Jackson. Journal of Neurochemistry 2008; 105(5): 2013 - 2028

"Mitochondria control numerous metabolic reactions within a cell including oxidative processes that generate reactive oxygen species (ROS) as a byproduct. Under physiologic conditions, cells possess several antioxidant defense mechanisms, which prevent such reactive intermediates from becoming toxic. However, it is now well established that sustained or intense cellular insults, such as aging or ischemia, can cause mitochondria to produce excess and uncontrolled levels of ROS and that this can cause cell death via the intrinsic apoptotic pathway."
"studies have shown that components of the visible spectrum can be absorbed by biologic chromophores in cells, such as astrocytes and epithelial cells, to cause cellular dysfunction and even death. It is believed that the blue region of the spectrum (approximately 400–500 nm) is particularly likely to induce these reactions, since it has relatively high energy and can penetrate through tissues to cells and their organelles. It can be theorized, therefore, that mitochondria, which are laden with prominent chromophores (e.g., cytochrome C oxidase, flavins, and flavoproteins), can be affected in a detrimental way by components of visible light." "This is supported by observations that isolated, light-exposed mitochondria release several ROS, including singlet oxygen, superoxide, and hydroxyl radicals."
"The idea that light can eventually cause apoptosis is a logical one, since the stimulation of ROS production in cells is known to play an intricate role in the activation of intracellular death pathways. It is now believed that ROS are central to the intrinsic system of apoptosis."
The influence of visible light exposure on cultured RGC-5 cells . J.P.M. Wood, G. Lascaratos, A.J. Bron, N.N. Osborne. Mol Vis. 2007 Feb 11; 14:334-44.

"Several lines of evidence indicate a role for mitochondria in the pathogenesis of AMD. First, mitochondria are the major source of superoxide anion in the cell. The superoxide anion can generate highly toxic hydroxyl radicals and hydrogen peroxide that damage the cell by reacting with proteins, DNA, and lipids. Oxidative stress appears to play an important role in AMD, since human donor eyes affected by AMD contain increased levels of protein adducts resulting from the oxidative modification of carbohydrates and lipids and higher levels of antioxidant enzymes. Second, mitochondrial (mt)DNA is more susceptible than nuclear DNA to damage from oxidation and blue light, and mtDNA damage in the retina and RPE accumulates with age." *Mitochondrial Proteomics of the Retinal Pigment Epithelium at Progressive Stages of Age-Related Macular Degeneration.* CL Nordgaard, PP Karunadharma, X Feng, T Olsen, and DA. Ferrington Invest. Ophthalmol. Vis. Sci. 2008 49(7): 2848-2855.

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“Mitochondria are the major source of superoxide anion in the cell. The superoxide anion generates highly toxic hydroxyl radicals and hydrogen peroxide that damage the cell by reacting with proteins, DNA, and lipids and ultimately, by inducing cell death. Several lines of evidence indicate that mitochondria play a central role in aging and in the pathogenesis of AMD....Blue light irradiation and treatment with hydrogen peroxide cause long-lasting mtDNA but not nuclear DNA mutations in cultured RPE cells. Moreover, mtDNA damage accumulates in the retina and RPE with age.

“Recently, several studies offered direct evidence of mitochondrial alterations in AMD...we have also found a drastic decrease in normal mitochondria in the photoreceptors and RPE cells of the AMD eyes as compared to that of the normal age-control eyes. ...The susceptibility of RPE mtDNA to oxidative damage, together with the failure of mtDNA repair provides an intriguing and plausible candidate mechanism for a mitochondria-based model of AMD and retinal degeneration”.

Molecular Pathology of Age-Related Macular Degeneration. Ding, X., Patel, M., Chan, C-C. Progress in Retinal and Eye Research 2009 (1):1-18

15. **A2E and damaged mitochondria act synergistically to increase ROS production in RPE cells exposed to blue light. Extensive levels of retinal lipofuscin containing A2E can raise the oxidative stress generated by damaged mitochondria in RPE cells from sub-lethal to lethal levels, and contribute to the development of AMD.**

"Exposure of A2E-loaded RPE cells to short wavelength (blue) visible light causes oxidative damage and may result in loss of lysosomal integrity and RPE apoptotic cell death that is regulated by the mitochondrial protein Bcl-2. Lipofuscin/A2E accumulation and mitochondrial damage are both prominent features of RPE aging....Taken together, these data provide exciting new insight into the complexity of RPE defects associated with aging: minor lysosomal A2E load and moderate mitochondrial inefficiency each by themselves may cause no detectable harm. However, our results demonstrate that they mutually enhance each other's impact to significantly impair vital RPE activities." *The age-lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells.* Cristofol Vives-Bauza, Monika Anand, Arash K. Shirazi, Jordi Magrane, Junping Gao, Heidi R. Vollmer-Snarr, Giovanni Manfredi, and Silvia C. Finnemann J. Biol. Chem, Papers In Press July 10, 2008

BACKGROUND: Lipofuscin occurs in association with various blinding diseases, including ARMD. Formation of lipofuscin is considered to be initiated by the inability of the RPE lysosome to degrade constituents of phagocytosed material resulting in its intralysosomal accumulation. Thus, the deposition of abnormal retinoid adducts causing the autofluorescent properties of RPE lipofuscin originates from abnormal products of the retinoid cycle contained in phagocytosed photoreceptor outer segments. The major lipofuscin retinoid conjugate A2-E was previously shown to exert toxic effects on RPE cells by directly damaging lysosomal function and structure. However, A2-E was also proposed to severely harm extralysosomal RPE cell structures during the pathogenesis of ARMD. This would require release or leakage of A2-E from the lysosomal compartment with subsequent targeting of other cellular compartments.
CONCLUSIONS: Since A2-E was suggested to act as a proapoptotic molecule via a mitochondrial pathway, we postulate that upon reaching a critical intralysosomal concentration, A2-E is released from the lysosome and then specifically targets the outer mitochondrial membrane thereby initiating apoptosis of the RPE cell. This may also apply correspondingly to other lipofuscin-associated molecules that cause leakage of the lysosomal membrane. *Accumulation of A2-E in mitochondrial membranes of cultured RPE cells.* Schutt F, Bergmann M, Holz FG, Dithmar S, Volcker HE, Kopitz J. Graefes Arch Clin Exp Ophthalmol. 2007; 245(3):391-8.

"Melanin in the long-lived melanosomes of the retinal pigment epithelium (RPE) may undergo photobleaching with aging, which appears to diminish the antioxidant function of melanin and could make photobleached melanosomes less efficient in protecting biomolecules from oxidative modification. ...The results support the growing body of evidence that photobleaching of RPE melanosomes, which is believed to occur with aging, changes the physicochemical properties of the organelle and reduces the likelihood that the granules perform an antioxidant function."*Photobleaching of melanosomes from retinal pigment epithelium: I. Effects on protein oxidation.* Janice M. Burke Photochem Photobiol. 2007 Jul-Aug;83(4):920-4.

16. **A protective element that was thought to reduce levels of oxidative stress in RPE cells is the black pigment, retinal melanin. In humans, retinal melanin production in RPE cells appears to only occur pre-birth and in very young children. Melanin granules collect in structures called melanosomes, where they absorb stray light, and act as powerful anti-oxidants. However, there is an age-related reduction of melanin granules within RPE cells.**

"Formation of melanosomes occurs in the RPE early in fetal development, then ceases within a few weeks (14). Polymerization of melanin within these melanosomes continues until, at approximately 2 years of age in humans, the RPE contains only mature melanosomes.
The melanin content of the RPE decreases significantly in aged human eyes. Therefore, melanin biosynthesis either is absent in adult human RPE cells or occurs only at a very slow rate
Biochemical protective effects in the RPE may also play a role in the occurrence of AMD. Melanin in the RPE can act against ROS and protect the neural retina. With age, the constant exposure of the RPE to high levels of oxygen and light might diminish the antioxidant properties of melanin. Under these conditions melanin may become pro-oxidant, adding to the accumulation of the singlet-oxygen-producing pigment lipofuscin in the cytoplasm of aged RPE cells and ultimately leading to AMD." *Role of Ocular Melanin in Ophthalmic Physiology and Pathology* Dan-Ning Hu, John D. Simon and Tadeusz Sarna. Photochem Photobiol. 2008 May-Jun;84(3):639-44

"As the retinal pigment epithelium (RPE) ages, a number of structural changes occur, including loss of melanin granules..." *Age and disease-related structural changes in the retinal pigment epithelium*. Vera L Bonilha Clinical Ophthalmology Feb 2008 2(2):413-424

"Melanin in the human retinal pigment epithelium (RPE) is believed to play an important photoprotective role. However, unlike in skin, melanosomes in the RPE are rather long-lived organelles, which increases their risk of modifications resulting from significant fluxes of light and high oxygen tension..... We have previously shown that a similar loss in the content of the RPE melanin occurs during human lifetime, which may suggest that the normal antioxidant properties of human RPE melanin become compromised with aging." *Photobleaching of retinal pigment epithelium melanosomes reduces their ability to inhibit iron-induced peroxidation of lipids.* Zadlo A et al. Pigment Cell Research Feb 2007; 20(1): 52-60

17. **Melanosomes are degraded by blue visible light that reaches RPE cells. Degraded melanosomes become pro-oxidant and further contribute to the level of oxidative stress.**

"Melanosomes, melanolipofuscin, and lipofuscin granules were isolated from human RPE donors older than 60. Melanosomes were photodegraded by exposure to blue light."
"Human melanosomes act as effective antioxidants by preventing iron ion-induced oxidation. Photodegradation of melanosomes results in the loss of these antioxidant properties while it preserves their ability to deactivate cationic photosensitizers. ...Photodegradation of melanosomes with acute blue light decreases their ability to protect from iron-mediated oxidation and, on top of that, the degraded melanosomes become a susceptible target for iron-mediated oxidation themselves. It appears that iron ions bound to the surface of partially degraded melanin are more susceptible to reduction by ascorbate and their redox cycling will elicit oxidation. Such a scenario where iron binds to degraded melanin but can be easily reduced by ascorbate and the subsequently formed free radicals are scavenged within melanosomes contributing to oxygen depletion explains the observed effects where photodegraded melanosomes appear to be more susceptible than native melanosomes to metal ion catalysed oxidation mediated by ascorbate." *Human RPE melanosomes protect from photosensitized and iron-mediated oxidation but become pro-oxidant in the presence of iron upon photodegradation*. Rózanowski B, Burke JM, Boulton ME, Sarna T, Rózanowska M. Invest Ophthalmol Vis Sci. 2008 Jul;49(7):2838-47

"Melanosomes of the retinal pigment epithelium (RPE) are long lived organelles that may undergo photobleaching with aging, which can diminish the antioxidant efficiency of melanin. Photobleaching of RPE melanosomes therefore makes cells containing them more sensitive to light-induced cytotoxicity. This observation raises the possibility that aged melanosomes increase RPE cell photic stress in situ, perhaps contributing to reduced tissue function and to degeneration of the adjacent retina that the RPE supports. *Photobleaching of Melanosomes from Retinal Pigment Epithelium: II. Effects on the Response of Living Cells to Photic Stress*. Janice M. Burke " Photochem Photobiol. 2007 Jul-Aug;83(4):925-30.

"Exposures to blue light in the presence of OHMs [old human melanosomes] resulted in abnormal cell morphology, up to approximately 75% decrease in mitochondrial activity, loss of lysosomal pH and cell death. OHMs contained significantly less melanin than YHMs [young human melanosomes] , supporting the hypothesis that melanin undergoes degradation during RPE aging. Our results demonstrate that aged MS [melanosomes] can be phototoxic to human RPE cells and support a contributing role of MS in RPE aging and in the pathogenesis of age-related macular degeneration".
In particular, the ability of MS to photogenerate superoxide and their susceptibility to undergo photooxidation increases strongly with aging. Our results clearly demonstrate that aged human MS are phototoxic to RPE cells. ... The increased phototoxicity of aged human RPE MS is likely to be related to their increased photoreactivity in comparison with that of young MS. ....There may be several different factors contributing to the age-related increase in photoreactivity and phototoxicity of RPE MS. Several recent studies demonstrate the loss of antioxidant properties and enhancement of pro-oxidant properties of photodegraded melanin. In particular, photo-oxidative degradation of RPE MS leads to a substantial increase in their photoreactivity. *The phototoxicity of aged human retinal melanosomes.* Rózanowska B, Cuenco J, Davies S, Shamsi FA, Zad?o A, Dayhaw-Barker P, Rózanowska M, Sarna T, Boulton ME. Photochem Photobiol. 2008 May-Jun;84(3):650-7.

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"CONCLUSIONS: Oxidative stress to the RPE is believed to contribute to sight threatening diseases such as age-related macular degeneration, ... it is increasingly likely that RPE melanosomes are sensitive to aging changes and that aging changes could be due at least partly to the effects of light exposure. As shown here, experimental treatment of isolated melanosomes with visible light produces changes similar to those that occur with aging of human RPE melanosomes within eyes, including reduced melanin content and an increased capacity to photogenerate superoxide. The consequence of chronic light exposure may therefore be to render RPE melanosomes less competent to perform an antioxidant function and to help protect aged RPE cells from oxidative injury." *Effects of Photodegradation on the Physical and Antioxidant Properties of Melanosomes Isolated from Retinal Pigment Epithelium*. Zareba M. et al. Photochem Photobiol: 2006; Jul-Aug 82,(4):1024-1029

18. **Aged melanosomes can become coated with lipofuscin and are referred to as melanolipofuscin. The absorption of blue light by chromophores in lipofuscin and melanolipofuscin can generate high levels of ROS in the RPE membrane, contributing to increasing levels of oxidative damage in the RPE and adjacent tissues and promoting self-induced cell death (apoptosis).**

"These data suggest that thin deposits of lipofuscin on the surface of RPE melanosomes are common in the aged eye. The presence of lipofuscin on the surface could account for some or all of the age-dependent absorption, fluorescence and reactivity properties of RPE melanosomes with age. Lipofuscin has been shown to be one of the major factors contributing to the increased aerobic photoreactivity of the RPE, and on a quantitative basis is more reactive under blue light exposure than the melanosome. Because of its increased aerobic photoreactivity, adhesion of lipofuscin to the surface of a melanosome would compromise the ability of melanin to act as an antioxidant and render the organelle more reactive. Complex granules composed of melanosomes coated by a thick lipofuscin layer, called melanolipofuscin, have been observed and exhibit reactivity between 'pure' melanosomes and lipofuscin". *Surface Elastic Properties of Human Retinal Pigment Epithelium Melanosomes.* S Guo et al. Photochemistry and Photobiology, 2008, 84: 671-678

"There is a close association between the level of oxidative stress in the RPE and its two major pigments, melanin and lipofuscin. Melanin in the form of melanosomes in the RPE is generally considered to be an antioxidant....The accumulation of lipofuscin in the eye is hypothesized to contribute to the age-related increase in aerobic photoreactivity of the RPE and is phototoxic to RPE cells in culture. With age, human RPE cells loses melanosomes and gains lipofuscin."..." These data suggest that thin deposits of lipofuscin on the surface of retinal pigment epithelium melanosomes are common in the aged eye and that this renders the melanosomes more pro-oxidant." *Age-dependent Photoionization Thresholds of Melanosomes and Lipofuscin Isolated from Human Retinal Pigment Epithelium Cells.* Hong L. Et al. Photochemistry and Photobiology: Dec 2006; 82(6):1475-1481.

"However, with aging there are an increasing number of RPE complex granules, melanolysosomes and melanolipofuscin; these contain melanin and lipid components that account for considerably more of the complex granules than in melanosomes. Eventually, by the age of 90 melanolipofuscin represents almost all pigment granules in the RPE
CONCLUSIONS: Our results demonstrate that there is an age-dependent shift in the pathways with which ascorbate interacts in the RPE. ...Interestingly, photoexcited melanolipofuscin initiates the pro-oxidant effects with much greater efficiency than to be expected based on its melanin content. The pro-oxidant effects of photoexcited melanin-ascorbate interactions are strongly wavelength dependent, this being the greatest for the shortest wavelength studied (340 nm) and steeply decreasing with increasing wavelength but remaining detectable even at 600 nm*." The Pro-oxidant Effects of Interactions of Ascorbate with Photoexcited Melanin Fade Away with Aging of the Retina.* Bartosz Rozanowski, Janice Burke, Tadeusz Sarna and Magorzata Rozanowska Photochemistry and Photobiology, 2008, 84: 658-670

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"The retinal pigment epithelium (RPE) is a single layer of postmitotic cells containing melanosomes. ... The major component in RPE melanosomes is eumelanin, the black melanin pigment. ... eumelanin exhibits antioxidant properties and can sequester large concentrations of reactive metals ...the function of RPE melanosomes is hypothesized to be protective by reducing light scatter and improving visual acuity through the absorption of light, and through its antioxidant and metal chelation properties....Upon damage, the protective properties of the melanosomes - and the melanin - are compromised. Oxidized melanosomes are less able to function as an antioxidant, and show increased generation of reactive oxygen species. Melanosomes become more efficient in photogenerating superoxide anion as a result of photodegradation."

"Such oxidative damage is essentially unavoidable in the RPE because of the limited melanosome turnover during life, the high oxidative stress associated with the phagocytosis and degradation of rod outer segments, and the constant exposure to light....Specifically blue light exposure of cells containing 60- to 90-year-old human RPE melanosomes shows extensive loss of mitochondrial activity and lysosomal destabilization compared with the corresponding cells containing 20- to 30-year-old human RPE melanosomes."

"These data suggest that thin deposits of lipofuscin on the surface of RPE melanosomes are common in the aged eye. The presence of lipofuscin on the surface could account for some or all of the age-dependent absorption, fluorescence and reactivity properties of RPE melanosomes with age. Lipofuscin has been shown to be one of the major factors contributing to the increased aerobic photoreactivity of the RPE, and on a quantitative basis is more reactive under blue light exposure than the melanosome. Because of its increased aerobic hotoreactivity, adhesion of lipofuscin to the surface of a melanosome would compromise the ability of melanin to act as an antioxidant and render the organelle more reactive. Complex granules composed of melanosomes coated by a thick lipofuscin layer, called melanolipofuscin, have been observed and exhibit reactivity between 'pure' melanosomes and lipofuscin" *Surface Elastic Properties of Human Retinal Pigment Epithelium Melanosomes.*. S Guo et al. Photochemistry and Photobiology, 2008, 84: 671-678

19. **Exposure to blue light contributes to the escalating cycle of actions that increase the level of oxidative stress in the RPE cell with age. There is now strong evidence that chronic oxidative stress in RPE cells over decades has a major role in the development of AMD. Oxidative stress in RPE cells induces an inflammatory response in the retina, which can exacerbate the generation of ROS. Low level chronic inflamation in the retina has been proposed as an important component of AMD. An immune system response to inflammation is activation of the complement system. RPE cells produce many of the 30 or more proteins that make up the complement system. Genetic studies have confirmed that activation of the complement system is involved in the development of AMD.**

"Oxidative stress can trigger inflammation and this can, in turn, exacerbate the generation of reactive oxygen species. ..."Recent studies indicate that inflammation is an important component of AMD and that oxidative stress in RPE can trigger the activation of the complement system. Moreover, complement activation is associated with enhanced expression of IL-8, an important inflammatory cytokine. Increased expression of IL-8 was also reported when RPE were fed oxidized photoreceptor outer segments. The increased expression of IL-8 may account, at least in part, for the inflammatory reactions during development of AMD." *Oxidative inactivation of the proteasome in RPE: A potential link between oxidative stress and upregulation of IL-8.* Fernandes AF, Zhou J, Zhang X, Bian Q, Sparrow JR, Taylor A, Pereira P, Shang F. J Biol Chem. Jul 25, 2008; 283(30):20745-53

"Studies implicate activation of complement amongst the processes involved in the pathogenesis of age-related macular degeneration (AMD)."
" photooxidized forms of A2E can activate complement and we suggested this as a mechanism for inciting low grade inflammation at the RPE-Bruch's membrane interface. In work described here, we have explored complement activation by other bisretinoid pigments of lipofuscin in addition to A2E. ...the source of complement, was placed specifically to the basal side of the cells, a paradigm mimicking the relationship of RPE to Bruch's membrane. Accordingly, when the cells were irradiated at 430 nm to generate A2E photooxidation products, activation of complement was evidenced....AMD has onset in the elder years yet likely develops over multiple decades before diagnosis. Thus contributions to AMD pathogenesis resulting from aberrant complement activation are likely to be chronic. As a stimulus for the complement associated events, we suggest that molecular fragments released by photooxidative processes within RPE lipofuscin, can play a role. *Complement Activation by Bisretinoid Constituents of RPE Lipofuscin*. Jilin Zhou, So Ra Kim, Barbro S. Westlund and Janet R. Sparrow. IOVS 2009; 50(3): 1392-1399.

"Our study showed a strong association between the complement C3 S/F (Arg80Gly) polymorphism and age-related macular degeneration, with similar findings for geographic atrophy and choroidal neovascularization...Their involvement in age-related macular degeneration, together with the finding that drusen contain proteins associated with inflammation and immune-mediated processes, supports the hypothesis that inflammation and complement activation influence the pathogenesis of age-related macular degeneration...."
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"The complement system comprises more than 30 plasma and cell-surface proteins. It mediates the host defense against pathogens and the elimination of immune complexes and apoptotic cells; it also facilitates adaptive immune responses. ...These findings add to our growing understanding of the genetics of age-related macular degeneration and provide conclusive evidence that the complement pathway has a key role in the pathogenesis of this common and debilitating condition." *Complement C3 Variant and the Risk of Age-Related Macular Degeneration* John R.W. Yates, et al for the Genetic Factors in AMD Study Group. New England Journal of Medicine 2007 Aug 9;357(6):553-61

20. **The characteristics of AMD include the malfunctioning and degeneration of PC's and RPE cells, excessive formation of lipofuscin within RPE cells, a build up of drusen and basal deposits between the RPE and Bruch's membrane as well as the thickening of Bruch's membrane, and increased immune system activity including activation of the complement system.**
With age there is increased levels of lipofuscin accumulation within the lysosomes of RPE cells. A2E, its adducts and other blue light absorbing chromophores contained in lipofuscin generate increasing amounts of ROS. These ROS and reactive lipid and protein fragments leak out of the lysosome into the intracellular cytoplasm and attack other organelles within the RPE cell generating increasing levels of ROS, causing the dysfunction of the RPE cell, and limiting its ability to service adjacent PCs. Lipofuscin accumulation and the resulting increase in photo-oxidative stress has been found to be is associated with extracellular deposition on the basal side of the RPE.

"As the retinal pigment epithelium (RPE) ages, a number of structural changes occur, including loss of melanin granules, increase in the density of residual bodies, accumulation of lipofuscin, accumulation of basal deposits on or within Bruch's membrane, formation of drusen (between the basal lamina of the RPE and the inner collagenous layer of Bruch's membrane), thickening of Bruch's membrane, microvilli atrophy and disorganization of the basal infoldings ...These age-related changes progress slowly and vary in severity in different individuals. These changes are also found in age- related macular degeneration (AMD), a late onset disease that severely impacts the RPE, but they are much more pronounced than during normal aging. However, the changes in AMD lead to severe loss of vision." *Age and disease-related structural changes in the retinal pigment epithelium*. Vera L Bonilha Clinical Ophthalmology Feb 2008 2(2):413-424

"Lipofuscin accumulates with age in the retinal pigment epithelium (RPE) in discrete granular organelles and may contribute to age-related macular degeneration."
"In the RPE, lipofuscin is derived primarily from phagocytosis of shed photoreceptor outer segments and is associated with a functioning retinoid visual cycle. The phototoxicity of A2E to RPE cells is well established, however recent *in vitro* studies have also associated complement activation with A2E in RPE cells exposed to light."
Oxidative stress has been associated with a host of age-related pathologies and long been suspected of contributing to lipofuscinogenesis. Previous studies have demonstrated that RPE lipofuscin is a potent generator of reactive oxygen species including superoxide anion, singlet oxygen and lipid hydroperoxides that have the capacity to escape the lysosome and modify other cellular compartments such as nuclear DNA and the plasma membrane, as demonstrated in cell culture."
"This finding is consistent with previous studies demonstrating RPE lipofuscin to be a potent generator of reactive oxygen species and supports the hypothesis that such species, including reactive fragments from lipids and retinoids, contribute to the mechanisms of RPE lipofuscin pathogenesis." *Retinal pigment epithelium lipofuscin proteomics.* Ng KP, Gugiu B, Renganathan K, Davies MW, Gu X, Crabb JS, Kim SR, Rózanowska MB, Bonilha VL, Rayborn ME, Salomon RG, Sparrow JR, Boulton ME, Hollyfield JG, Crabb JW. Mol Cell Proteomics. 2008 Jul;7(7):1397-405.

"Our work supports a novel model of sub-RPE deposit formation in which excessive material first accumulates in the subretinal space, disrupting the physical contact between RPE cells and photoreceptors. To restore the contact, RPE cells migrate toward photoreceptors and form a new layer. The subretinal material is consequently displaced to the sub-RPE location and becomes sub-RPE deposit."
"The present work has revealed a remarkable ability of RPE cells to reestablish direct contact with photoreceptors when the contact is disrupted by excessive extracellular material in the subretinal space. We have also demonstrated that RPE cells circumvent the subretinal material by translocation and reestablishing a new layer between photoreceptors and the subretinal material, and therefore relocating the subretinal material to sub-RPE space. Our work thus revealed a novel mechanism of sub-RPE deposit formation in which extracellular material first accumulates in the subretinal space, separating the RPE from photoreceptors. To reestablish direct contact with photoreceptors, RPE cells migrate toward photoreceptors and form a new RPE layer, which is equivalent to translocation of the RPE layer. Because of the translocation, there is a switch of positions between the original RPE and the deposit. Since the RPE is normally used as a reference to describe the surrounding structures, the location of the deposit after RPE translocation is therefore in the sub-RPE space and it becomes a sub-RPE deposit." *Translocation of the retinal pigment epithelium and formation of sub-retinal pigment epithelium deposit induced by subretinal deposit*. Lian Zhao, Zhenfang Wang, Yun Liu, Ying Song, Yiwen Li, Alan M. Laties, Rong Wen. Molecular Vision 2007; 13:873-80

"Oxidative damage and inflammation are postulated to be involved in age-related macular degeneration (AMD). Here we describe AMD-like lesions in mice after immunization with mouse serum albumin adducted with carboxyethylpyrrole, a unique oxidation fragment of docosahexaenoic acid that has previously been found adducting proteins in drusen from AMD donor eye tissues and in plasma samples from individuals with AMD."
"As a potential initiating signal we evaluated carboxyethylpyrrole (CEP), an adduct that forms from an oxidation fragment of docosahexaenoic acid (DHA)....DHA, the most oxidizable of all longchain polyunsaturated fatty acids, is abundant in the outer retina, where high oxygen tension and light provide a permissive environment for oxidation ...Because DHA is concentrated in RPE and photoreceptor cells, we reasoned that these tissues are a probable source of CEP adducts during aging."
"This oxidation-generated hapten is noteworthy because of the long-recognized association of AMD with oxidative damage....To our knowledge, this is the first study showing that immunization with a hapten generated by oxidative damage to the DHA present in the drusen and plasma from AMD-affected individuals." *Oxidative damage-induced inflammation initiates age-related macular degeneration.* Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Nat Med. 2008 Feb;14(2):194-8

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"CONCLUSIONS: The pattern of accumulation of particles was consistent with a retinal pigment epithelium (RPE) source for the LLPs [lipoprotein-like particles], which explains why once the EL[elastic layer] and ICL [inner collagenous layer] were filled with particles, LLPs continued to accumulate near the RPE, but no further accumulation was found in the OCL [outer collagenous layer]. The quantity of LLP-associated lipids found in BrM [Bruch's Membrane] accounts for a large portion of the accumulated lipids measured in this tissue." [lipoprotein-like particles] (LLPs) inner collagenous layer (ICL), elastic layer (EL),outer collagenous layer (OCL)]. *Morphometric analysis of lipoprotein-like particle accumulation in aging human macular Bruch's membrane.* Huang JD, Curcio CA, Johnson M. Invest Ophthalmol Vis Sci.2008 Jun;49(6):2721-7

"Considering that AMD is a disease affecting the photoreceptors, RPE cells, and choriocapillaris, it is interesting that oxidized phospholipids were detected almost exclusively in the photoreceptors and RPE cells and were not observed in the inner layers of the retina. RPE cells continuously ingest shed photoreceptor discs at their apical border, form phagosomes within the cytoplasm, breakdown the fragments of the outer segment discs in their lysosomes, and exocytose the degraded materials on the basal side. Because an accumulation of oxidized compounds inhibits the function of phagosomes in the RPE, incompletely digested photoreceptors containing oxidized phospholipids are probably exocytosed and observed as debris."
"Our results confirmed that oxidized phospholipids were indeed present in the photoreceptors and RPE cells in the macula region of the normal eyes, and the quantity increased with age. In addition, eyes with AMD showed more intense immunoreactivity to oxidized phospholipids than age-matched normal eyes. These results support our hypothesis that oxidized phospholipids accumulate in the eyes of patients with AMD. These findings would then indicate that oxidative damage of the photoreceptors and RPE cells may be the mechanism for the changes induced in eyes with AMD. We suggest that the oxidation of phospholipids in the photoreceptors may lead to incomplete digestion of the photoreceptor discs phagocytosed by the RPE. This may result in the accumulation of lipofuscin in the RPE and subsequently drusen formation. Finally, the accumulation of lipofuscin could lead to RPE dysfunction, which would then cause the death of photoreceptor cells." *Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration* Mihoko Suzuki, Motohiro Kamei, Hiroyuki Itabe, Kazuhito Yoneda, Hajime Bando, Noriaki Kume, Yasuo Tano. Molecular Vision 2007; 13:772-778

21. **The formation of soft drusen between the RPE and Bruch's membrane in the macular region of the retina is considered the hallmark of AMD. Many recent studies have demonstrated links between oxidative damage, inflammation, the complement system, RPE lipofuscin, basal deposits, and drusen formation.**

"Age-related macular degeneration is the leading cause of visual impairment in the elderly and the most common cause of blindness in Western countries. It affects the macular region of the retina. .... In the early stages of the disease deposits called drusen develop between the retinal pigment epithelium and underlying choroid." *Complement C3 Variant and the Risk of Age-Related Macular Degeneration* John R.W. Yates, et al for the Genetic Factors in AMD Study Group. New England Journal of Medicine 2007 Aug 9;357(6):553-61

"In early AMD, FIAF's [focally increased autofluorescence] colocalization with large, soft drusen and hyperpigmentation is several times greater than chance, suggesting linked disease processes. In advanced atrophic AMD, FIAF is found mostly adjacent to drusen and GA, suggesting that dispersal of drusen-associated lipofuscin is a marker of atrophic disease progression." Autofluorescence Characteristics of Early, Atrophic, and High-Risk Fellow Eyes in Age-Related Macular Degeneration. Smith Rt et al. Invest Ophthalmol Vis Sci. Dec 2006; 47(12):5495-504

"Lipofuscin, which extensively accumulates with age in RPE cells, is hardly soluble, derived in part from oxidation products of the photoreceptor outer segments. ... These results suggest that the accumulation of indigestible granules such as lipofuscin in RPE or subsequent depositions toward Bruch's membrane may play a role in drusen biogenesis as a trigger of inflammation or via other mechanisms."
"Thereafter, lipid accumulation of Bruch's membrane begins to increase. Then drusen and basal deposits emerge. Therefore, lipofuscin accumulation is likely to play a role in the thickening of Bruch's membrane and, furthermore, the formation of drusen and basal deposits."
"In conclusion, the accumulation of lipofuscin, especially involving insoluble components increasing with age, may be implicated in the pathogenesis of AMD. Poorly digestible and insoluble components are likely to involve glycoxidation products." *Glycoxidized particles mimic lipofuscin accumulation in aging eyes- a new age-related macular degeneration model in rabbits* T. Yasukawa. Graefe's Arch Clin Exp Ophthalmol Oct, 2007; 245(10):1475-85

"Recently Zhou and associates demonstrated with an in vitro assay a link between inflammation, the complement system, oxidative damage, drusen, and RPE LF. They suggested that products of the photo-oxidation of RPE lipofuscin compounds could serve as a trigger for the complement system which could predispose the macula to pathologic alterations and could contribute to chronic inflammation over time."*Fundus Autofluorescence and Progression of Age-related Macular Degeneration.* Steffen Schmitz-Valckenberg, Monika Fleckenstein, Hendrik P.N. Scholl, and Frank G. Holz. Survey of Ophthalmology Jan-Feb 2009. 54(1): 96-117

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"The results of recent studies have implicated local inflammation and complement activation as the processes involved in the pathogenesis of age-related macular degeneration (AMD).... These results suggest that *AB* activates the complement system within drusen by blocking the function of factor I leading to a low-grade, chronic inflammation in subretinal tissues. These findings link four factors that have been suggested to be associated with AMD: inflammation, complement activation, *AB*deposition, and drusen." *Altered Function of Factor I Caused by Amyloid B: Implication for Pathogenesis of Age-Related Macular Degeneration from Drusen*, Jiying Wang, Kyoko Ohno-Matsui, Takeshi Yoshida, Ariko Kojima, Noriaki Shimada, Ken-ichi Nakahama, Olga Safranova, Nobuhisa Iwata, Takaomi C. Saido, Manabu Mochizuki and Ikuo Morita. The Journal of Immunology, 2008, 181: 712-720.

22. **The build up of soft drusen in the macular area creates a barrier that limits the ability of RPE cells to absorb oxygen and nutrients from the choriocapillaris and to expel waste material into the blood stream through the Bruch's membrane/chroroid complex. This contributes to the malfunctioning and death of RPE cells, and results in the death of overlying PCs and a corresponding deterioration of vision. This progressive degeneration of photoreceptor cells in the foveal region of the macula leads to the form of blindness known as geographic atrophy, or the "dry" form of AMD.**

"Oxidative stress causes retinal pigment epithelium (RPE) cell dysfunction and is a major risk factor leading to the development of dry-type age-related macular degeneration. Taking pharmacological and genetic approaches, we address the mechanisms by which sublethal oxidative stress inhibits RPE cell phagocytosis..... Sublethal oxidative stress dose-dependently inhibited RPE cell phagocytosis of photoreceptor outer segments (POS)" *alpha2 But not alpha1 AMP-activated protein kinase mediates oxidative stress-induced inhibition of retinal pigment epithelium cell phagocytosis of photoreceptor outer segments.* Qin S, De Vries GW. J Biol Chem. 2008 Mar 14;283(11):6744-51

"The purpose of this study was to investigate the impact of drusen on overlying cells of the retina... CONCLUSIONS: Retinal cells overlying both soft and hard drusen exhibit structural and molecular abnormalities indicative of photoreceptor degeneration and Muller glial activation. These abnormalities resemble the degenerative effects common to many forms of retinal degeneration, but are confined to areas directly overlying drusen. This suggests that photoreceptor cell function is compromised as a consequence of drusen formation." Johnson PT, Lewis GP, Talaga KC, Brown MN, Kappel PJ, Fisher SK, Anderson DH, Johnson LV. Drusen-associated degeneration in the retina. Invest Ophthalmol Vis Sci. 2003 Oct;44(10):4481-8.

"Age-related macular degeneration (AMD) is a neurodegenerative disease of the eye and a leading cause of blindness among people over 60 years of age in many industrialized nations. AMD represents the late phase of age-related maculopathy with two subdivided groups: geographic atrophy (dry AMD) and choroidal neovascularization (CNV) (exudative AMD). The various histopathologic features of both types of AMD involve hard and soft drusen and basal laminar and linear deposits respectively), all of which are extracellular deposits located between the retinal pigment epithelium (RPE) and the underlying Bruch's membrane. The multiple hard drusen, soft drusen, and basal deposits are risk factors for geographic atrophy or CNV. ...
Geographic atrophy is severe atrophy of the RPE and photoreceptor cells, threatening central vision loss because of the lack of effective treatments" *Glycoxidized particles mimic lipofuscin accumulation in aging eyes - A new age-related macular degeneration model in rabbits.* T.Yasukawa. Graefe's Arch Clin Exp Ophthalmol 2007; 245(10):1475-85.

"Once definite geographic atrophy appears, it generally progresses contiguously from preexisting areas of involvement. Such a consumptive course is consistent with previously healthy areas of retinal pigment epithelium being affected by adjacent diseased tissue." *Toll-like Receptor 3 and Geographic Atrophy in Age-Related Macular Degeneration.* Yang et al. N Engl J Med. 2008 Oct 2;359(14):1456-63.

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"A2E inhibits hydrolytic activities in lysosomes and mediates blue light-induced damage to RPE cells... Loss of pigment epithelial cells in the retina may result in the so-called geographic atrophy, which is by far the most frequent form of AMD. To date, no cure or prevention of this disease, which affects a large number of elderly people, is available... So far, no efficient AMD therapy or prevention exists. Carotenoids and antioxidants, limiting exposure to light, or targeting of the precursors of A2E may be useful." *Age-Related Macular Degeneration. The Lipofusion Component N-retinyl-N-retinylidene ethanolamine (A2E) Detaches Proapoptotic Proteins from Mitochondria and Induces Apoptosis in Mammalian Retinal Pigment Epithelial Cells.* Suter M. et al Biol Chem 2000 Dec 15;275(50):39625-30

23. **Extensive accumulation of lipofuscin and increased oxidative stress within RPE cells in the macular region and the resulting basal deposits and drusen formation are also associated with the promotion of abnormal blood vessels growing from the choriocapillaris through Bruch's membrane, a condition known as choroidal neovascularization (CNV). This invasion of the retinal space by these abnormal immature blood vessels typically results in hemorrhage, exudation, scarring, and serous retinal detachment. Leakage from these small, weak abnormal blood vessels into the space between the RPE and the PCs results in a rapid death of PCs in the region and the rapid development of blindness, and is known as the "wet" form of AMD.**

"Early risk factors [of AMD] include the macular deposition of debris (drusen) on Bruch's membrane, the extracellular matrix separating the choriocapillaris from the retinal pigment epithelium (RPE). Later stages of "dry" AMD involve the degeneration of photoreceptor and RPE cells resulting in geographic atrophy. In "wet" AMD, abnormal blood vessels grow from the choriocapillaris through Bruch's membrane (choroidal neovascularization or CNV)."
"Oxidative stress has long been associated with AMD pathology... A direct molecular link between oxidative damage and AMD was established by the finding that carboxyethylpyrrole (CEP), an oxidative protein modification generated from docosahexaenoate (DHA)-containing phospholipids, was elevated in Bruch's membrane and drusen from AMD patients. Subsequently, CEP adducts as well as CEP autoantibodies were found to be elevated in plasma from AMD donors and CEP adducts were found to stimulate neovascularization *in vivo,* suggesting a role in the induction of CNV. From such observations, oxidative protein modifications were hypothesized to serve as catalysts of AMD pathology. .."*Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers.* Jiayin Gu, Gayle J. T. Paeur, Xiuzhen Yue, Umadevi Narendra, Gwen M Sturgill, James Bena, Xiaorong Gu, Neal S. Peachey, Robert G. Salomon, Stephanie A. Hagstrom, John W. Crabb, and Clinical Genomic and Proteomic Study Group. Molecular and Cellular Proteomics Papers in Press. Published on February 6, 2009

"Our work also provides an insight into the mechanism of CNV (choroidal neovascularization) development. When RPE cells migrate from Bruch's membrane, the RPE-Bruch's membrane complex is dismantled. It seems that the bare Bruch's membrane devoid of RPE is no longer recognized as a barrier and it becomes vulnerable to CNV invasion. Our data therefore emphasize the importance of RPE as a barrier to CNV invasion."
"RPE cells are housekeepers for photoreceptors. They not only are essential for photoreceptor metabolism, but also participate in the RPE-Bruch's membrane complex to form the blood-retinal barrier... In summary, our present work demonstrates that the presence of a subretinal deposit induces RPE cell translocation, which in turn generates pathological features characteristic of AMD, including formation of the sub-RPE deposit and CNV. These findings indicate a subretinal source of sub-RPE deposits and a key role of RPE translocation in the formation of sub-RPE deposits. Our data also provide evidence that the presence of sub-RPE deposits is sufficient to induced CNV to penetrate Bruch's membrane" *Translocation of the Retinal Pigment Epithelium and Formation of Sub-Retinal Pigment Epithelium Deposit Induced by Subretinal Deposit.* Lian Zhao, Zhenfang Wang, Yun Liu, Ying Song, Yiwen Li, Alan M. Laties, Rong Wen Molecular Vision 2007; 13:873-80

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"Neovascular AMD is characterized by abnormal growth of capillaries from the choroid into the Bruch's membrane and RPE and by subsequent exudation of fluid, lipid and blood . It results ultimately in a disciform scar in the macula and is responsible for severe, sudden visual loss." *Age and Disease-Related Structural Changes in the Retinal Pigment Epithelium.* Vera L Bonilha, Clinical Ophthalmology Feb 2008; 2(2):413-424

"Lipofuscin results from an incomplete degradation of altered material trapped in lysosomes and the accumulation of lipofuscin is related to an increased risk of choroidal neovascularization (CNV) due to age-related macular degeneration (AMD).
RPE lipofuscin is a byproduct of the phagocytosis of lipid-rich photoreceptor outer segments and consists of a complex mixture of pigments. A major fluorophore is A2E.... A2E affects normal RPE functions by causing membrane permeabilization inhibiting lysosomal function, inhibiting cytochrome c oxigenase , acting as a detergent inhibiting the ATP-driven proton pump and partly mediating light damage by acting as a photosensitizer, targeting DNA. ...This study demonstrates that A2E is an endogenous ligand for retinoic acid receptor (RAR). The data suggest that A2E accumulation results in the pro-angiogenic conversion of retinal pigment epithelial cell phenotype predisposing the environment to CNV development via RAR activation. The data in the present study support that the accumulation of A2E induces the enhancement of VEGF expression, suggesting a role of A2E in the progression of exudative AMD. ... These results suggest that A2E accumulation results in the phenotypic alteration of retinal pigment epithelial cells, predisposing the environment to choroidal neovascularization development." *A2E, a Pigment of the Lipofuscin of Retinal Pigment Epithelial Cells, Is an Endogenous Ligand for Retinoic Acid Receptor* Aya Iriyama, Ryoji Fujiki, Yuji Inoue, Hidenori Takahashi, Yasuhiro Tamaki, Shinichiro Takezawa, Kenichi Takeyama, Woo-Dong Jang, Shigeaki Kato, and Yasuo Yanagi J. Biol. Chem. May 2, 2008; Vol. 283, Issue 18, 11947-11953

"CNV grows through the break in Bruch's membrane from the underlying choriocapillaris and invades the sub-RPE, the subretinal space, or both. These immature vessels leak serum and blood that can induce a fibrotic reaction known as a disciform scar. Despite recent encouraging advances in pharmaceutical, photochemical, and surgical treatments of CNV, the long-term prognosis of exudative AMD is poor in many cases, especially once vision is impaired." *Glycoxidized particles mimic lipofuscin accumulation in aging eyes - A new age-related macular degeneration model in rabbits.* T.. Graefe's Arch Clin Exp Ophthalmol 2007; 245(10):1475-85.

"Basal laminar and linear deposits (BLD) are associated with the development of choroidal neovascularization (CNV)."
"Conclusions: Diffuse deposits such as BLD appear consistently with the development of CNV in AMD. ... The results of the current study may support the hypothesis that inflammatory processes are involved in the pathogenesis of BLD and CNV in AMD." *.Are low inflammatory reactions involved in exudative age-related macular degeneration? Morphological and immunhistochemical analysis of AMD associated with basal deposits.* A. Lommatzsch, P. Hermans, K. D. Muller, N. Bornfeld, A. C. Bird, and D. Pauleikhoff. Graefe's Archive for Clinical and Experimental Ophthalmology. April 15, 2008:803-810

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"Choroidal neovascularization (CNV) is the defining characteristic of late stage ‘wet’ or neovascular AMD. Classical neovascular AMD is mainly illustrated with CNV and subretinal neovascular fibrous tissue. The neovascularization has two etiologic patterns: (1) new vessels sprouting from the choroidal vessels, penetrating Bruch’s membrane and growing into the subretinal space are the classical descriptions of wet AMD" *Molecular Pathology of Age-Related Macular Degeneration.* X Ding et al. Progress in Retinal and Eye Research 2009 (1):1-18]

24. **While there are some who claim that this causality is yet to be proven, a growing number of experts in AMD pathology are now recommending that it would be beneficial to limit exposure to blue light wavelengths over a lifetime.**

"It should be noted that broad-spectrum white light, traditionally used for bright light therapy, also contains blue light of potential concern particularly for very high intensity, long-duration exposure. Clearly, the safety of bright light therapy for people needs investigating. In the meantime it would be suggested that light in the 500 to 530 nm wavelength range (blue–green) should still be effective while avoiding the putative blue hazard". *Clinical Management of Delayed Sleep Phase Disorder.* Leon C. Lack. Behavioral Sleep Medicine 2007, Vol. 5, No. 1, Pages 57-76.

"Lowering retinal exposure to blue light and ensuring that intake of key antioxidant nutrients is sufficient are the main recommendations from our study....Although it is not possible to establish causality between sunlight exposure and neovascular AMD, our results suggest that people in the general population should use ocular protection and follow dietary recommendations for the key antioxidant nutrients. *Sunlight Exposure, Antioxidants, and Age-Related Macular Degeneration*. AE Fletcher; GC. Bentham; M Agnew; IS Young, C Augood, U Chakravarthy, P TVM de Jong, M Rahu, J Seland, G Soubrane, L Tomazzoli, F Topouzis; JR Vingerling, J Vioque. Arch Ophthalmol. Oct 22, 2008; 126:1396-1403.

"A wide spectrum of pharmacotherapeutic agents ranging from antibiotics, psychoactive drugs, antiarrhythmic drugs and diuretics to dye for intraocular surgery have been implicated in causing drug-induced RPE disturbances, impaired visual acuity and defective visual fields, highlighting the importance of eliciting a thorough drug history before subjecting patients to unprotected light exposure. This is of particular relevance in intraocular surgery where prolonged and direct illumination of the retina with strong light source may be used, and light therapy for seasonal affective disorder for which neuroleptics and antidepressants are often concomitantly prescribed." *Toxicology of the Retina: Advances in Understanding the Defence Mechanisms and Pathogenesis of Drug- and Light-Induced Retinopathy.* Siu et al. Clinical and Experimental Ophthalmology 2008; 36:176-185.