Photo-damage, photo-protection and age-related macular degeneration

Melisa D. Marquioni-Ramella and Angela M. Suburo*

Age-related macular degeneration (AMD) is a degenerative retinal disease that causes blindness in people 60–65 years and older, with the highest prevalence appearing in people 90 years-old or more. Epidemiological estimates indicate that the number of cases is increasing, and will almost double in the next 20 years. Preventive measures require precise etiological knowledge. This is quite difficult, since AMD is a multifactorial condition with intricate relationships between causes and risk factors. In this review, we describe the impact of light on the structure and physiology of the retina and the pigment epithelium, taking into account the continuous exposure to natural and artificial light sources along the life of an individual. A large body of experimental evidence demonstrates the toxic effects of some lighting conditions on the retina and the pigment epithelium, and consensus exists about the importance of photo-oxidation phenomena in the causality chain between light and retinal damage. Here, we analyzed the transmission of light to the retina, and compared the aging human macula in healthy and diseased retinas, as shown by histology and non-invasive imaging systems. Finally, we have compared the putative retinal photosensitive molecular structures that might be involved in the genesis of AMD. The relationship between these compounds and retinal damage supports the hypothesis of light as an important initiating cause of AMD.

1. Introduction

The clinical and social importance of age-related macular degeneration (AMD)

The retina is a photosensitive tissue that captures light and transforms it into electrical signals. The specialized cells that capture light include rods, specialized for nightlight vision,
and cones, which are in charge of daylight and color vision. Rods contain the visual pigment rhodopsin that is sensitive to blue-green light (500 nm). Cones, instead, respond to short wavelengths (S-cones, 420 nm), medium wavelengths (M-cones, 530 nm) and long wavelengths (L-cones, 560 nm).

Near the center of the retina, the macula lutea appears as a yellowish spot, including four anatomical regions: the perifovea, the parafovea, the fovea and the foveola. The perifovea shows a high density of retinal vessels and a high rod : cone ratio, although the density of cones and ganglion cells is higher than in the periphery. Almost 50% of the total ganglion cell population resides in the macula. Thus, the ganglion cell layer (GCL) shows more than one row, up to six cells deep, except at the foveola. The parafovea has a low density of retinal vessels and a rod : cone ratio close to 4 : 1. Cones become dominant in the slopes of the foveal pit, where vessels are restricted to a perifoveal capillary plexus. Narrowing and elongation of cones at the fovea are essential for visual acuity. Inner retinal layers are absent in the foveola, where photoreceptor cell bodies lie close to the vitreal surface.

AMD is a degenerative retinal disease that causes blindness in people 60–65 years and older, with the highest prevalence appearing in people 80 years-old or more. Vision loss is preceded by early asymptomatic stages characterized by the presence of medium-sized drusen (63–125 µm). The disease progresses to intermediate AMD with larger drusen and/or retinal pigment epithelium (RPE) alterations near the macula. The latter include hypo- or hyper-pigmentation and accumulation of autofluorescent material (lipofuscin). In addition, multifocal electoretinography, optical coherence tomography (OCT), and spectral domain OCT (SD-OCT) have shown the presence of various photoreceptor changes.

Early AMD lesions further develop into one of the two forms of late disease: geographic atrophy (GA) or dry AMD, characterized by loss of RPE cells and photoreceptors; and neovascular (or wet) AMD, where abnormally growing choroidal vessels invade the subretinal space between the RPE and the neural retina.

With a global prevalence been estimated in 8.69%,9 AMD has replaced cataracts and refractive errors as the leading cause of blindness and severe vision impairment in higher-income regions such as Western Europe, Australia, USA, Japan10 and Southern Latin America.11 Current estimates suggest that the 2.07 million cases recorded in 2010 will become more than 5.00 million in 2050.12

**Etiopathogenesis of AMD**

Chronic oxidative RPE injury, together with a low-level inflammatory response are important factors for development of early RPE lesions.8,13–15 Thus, a high risk for AMD is associated to cigarette smoking,16 which is a well-known oxidant.17,18 Age is the main risk factor for developing AMD, but in most cases, genetic factors explain the overall severity of the disease.19 That the most frequent factors associated to AMD are genetic variants facilitating inflammation,20 points at the existence of sustained stress in the retina and the RPE.

Two would be the most likely causes: exposure to environmental light and the visual transduction processes. Since both circumstances are unavoidable in ordinary life, the epidemiology of AMD arguments in favor of robust endogenous mechanisms quenching photo-oxidative stress. Light radiation reaching the retina and the RPE provoke oxidative stress, which is normally restrained by endogenous antioxidant systems and by mechanisms extinguishing the associated inflammation stress.15 The main risk factors for AMD probably reflect the failure of these systems and mechanisms but still, photo-oxidation would be the initial pathogenic factor. As previously expressed by others, “prevention or attenuation of the initial oxidative injury will reduce the risk of developing AMD, regardless of genetic background”.21

The association between this disease and environmental light is mainly based on epidemiological grounds, and on the physical evidence explaining the interaction of light with ocular tissues. Therefore, we will first analyze transmission of light to the retina, and then we will describe the diseased macula, as shown by modern imaging procedures. Finally, we will evaluate the putative retinal photo-sensitive molecular structures that might be involved in the genesis of AMD, which have been mainly identified by experimental studies in animal species or _in vitro_.

**2. Environmental light and AMD**

Effects of environmental light on the course of AMD must depend on the light wavelengths and intensities reaching the retina. Ocular structures can interact with a broad portion of the spectrum, ranging between 100 and 10 000 nm and including visible (750–400 nm), ultraviolet (UV-A, 400–320 nm; UV-B, 320–280 nm; UV-C, <280 nm) and infrared wavelengths.22,23 However, almost no UV-C reaches earth's surface,24 and various natural ocular filters restrict radiation transmission to photoreceptors.

**Sunlight**

Retinal irradiance in daylight is in the 0.01 mW cm⁻² to 0.1 mW cm⁻² range, depending on time of day, season, presence of snow or water surfaces, wearing a hat, etc. Additionally, squinting, which prevents the formation of a sun image on the inferior retina, and photophobia, serve as biological protections against sun exposure.25,26

The average human retina absorbs each day approximately 10¹² to 10¹³ photons,27 which can be increased by workplace exposure or activities in high light environments. The association between sun exposure and AMD is controversial, since it has been found in some studies28–31 but not in others.32–34 However, a meta-analysis based on 14 epidemiological studies strongly supports the notion that more sunlight exposure increases risk for AMD.35 Research has also emphasized the protective role of hats and eyeglasses and has suggested the possible relevance of individual differences in the reaction to sunlight exposure.36,37 Thus, the Age-related Maculopathy and
Macular Degeneration in the elderly European populations (EUREYE) study only reported significant associations between blue-light exposure and neovascular AMD for individuals in the quartile of lowest dietary antioxidant level—vitamin C, zeaxanthin, vitamin E, and zinc.48

Other light sources
Welding arcs emit a wide spectrum, ranging from infrared (IR) to ultraviolet (UV). The cornea and the lens absorb UV radiation, whereas water absorbs far-IR. Visible light and near-IR may reach the retina in the unprotected eye, producing an acute macular lesion that often results in a bilateral central scotoma accompanied by pigmentation alterations.49 With only partial protection, UV light may generate corneal epithelial injury, whereas blue light destroys the center of the macula.40 In a more recent study, macular lesions were demonstrated using OCT in 38% of welders \( (n = 80, \text{age mean} = 36.9 \text{ years}) \) that did not refer visual symptoms and showed no visual acuity problems.41 Thus, it would be of great interest to study the evolution of these lesions with age.

Concern has been raised about domestic and vehicular lighting, increasingly dependent on light-emitting diodes (LEDs). Dissemination of these devices poses a potential problem for the retina since billboards, and emergency lights extensively use blue LEDs. As it will be explained in the last part of this review, the retina is particularly vulnerable to blue-light. Current regulations establishes that for an exposure greater than \( 10,000 \text{ s} \), the exposure limit value (ELV) for blue-light radiance is about \( 100 \text{ W m}^{-2} \text{sr}^{-1} \) (or \( 1.0 \times 10^{6} \text{ J m}^{-2} \text{sr}^{-1} \)).42 Published spectral power distributions show that LEDs emit an intense blue-light component which is absent in the daylight spectra.41 Cold-white LEDs are particularly questionable, since they emit about 3–4 times as much energy in the blue-light risk portion of the spectrum as warm-white LEDs.43 Most important, due to their small size, it is relatively easy to produce LED sources of very high luminance that may generate visual discomfort.44 A publication from the Department of Energy, U.S. reported that “the proportion of blue-light in the spectrum is not significantly higher for LEDs than it is for any other light source at the same correlated color temperature (CCT)”.45 However, this report emphasized that safety could not be guaranteed for blue LEDs, nor for infants in close proximity to bright light sources.

Even though data from other species or \textit{in vitro} cultures cannot be directly extrapolated to humans, we cannot disregard the experimental studies suggesting that LED blue irradiation might produce greater damage than other wavelengths. After exposure to 750 lux, retinal damage in rats occurred earlier in those exposed to blue and white (CCT 6500 K) LEDs than in those exposed to white (CCT 6500 K) or yellow compact fluorescent lamps (CFLs).46 After 9 days under blue or white LEDs, the outer nuclear layer (ONL), containing photoreceptor nuclei, was reduced to about 1/3, whereas no significant changes appeared in rats exposed to CFLs.46 With a 3-day exposure, levels of superoxide anion in the retina were higher in those exposed to blue LEDs than in those exposed to white LEDs and CFLs. Using different commercially available blue LEDs, severe retinal damage was produced by radiances below the currently accepted ELV for blue-light.44 Experiments \textit{in vitro} also support the damaging potential of blue and white LED. Under the same illuminance (2500 lux) blue LED light damaged 661 W cells (a line derived from mouse cones) more severely than white and green LED lights.47 Only blue and white LED light significantly reduced cell viability when 661 W cultures were exposed under the same energy conditions (0.38 \text{ mW cm}^{-2}).47 The question of artificial light sources in AMD etiopathology still requires more evidence; however, we cannot presently exclude their potential role as a significant hazard. Regulations are required to control glaring from billboards and emergency lights because, in addition to their potential role in retinal photo-toxicity, they might also conspire against security.48

The normal eye filters UV and blue light
Cornea and lens. The cornea and lens absorb all UV-C light and most UV-B.44 However, some UV-A radiation is transmitted, since it is about 10 times more abundant than UV-B in the solar spectrum.44 Filtering in the human lens reflects the presence of tryptophan derivatives, the kynurenines, which block most of the incident light between 295 and 400 nm.50,52 Although kynurenines decrease with age, UV filtering properties of the human lens increase because these compounds form covalent bonds with crystallins.53 Advanced glycation end-products (AGE) also contribute to lens UV-filters.51 UV and blue-light transmission decrease linearly as a function of age.52 In contrast, a higher fraction of this region of the spectrum reaches the young retina (<8–10 years),43 determining the rapid formation of lipofuscin in children.54

A review, including a large number of studies (2003–2014), has shown clear-cut associations between cataract surgery and AMD. However, both increased transmission of short-wave-length light to the retina,50,55 and/or an inflammatory response,56,57 could explain the greater risk for AMD. Since the aphakic eye loses most UV- and blue-light filtering properties,56,55 it seems reasonable to replace cataractous lenses with intraocular lenses (IOLs) with those filtering properties. UV-filtering IOLs can be untinted or yellow-tinted. Compared with aphakic eyes, untinted IOLs allow a 60% reduction in blue-light irradiance, whereas yellow-tinted IOLs confer an additional reduction (17 to 56%).58

The directly photo-sensitive retinal ganglion cells involved in the circadian rhythm are maximally sensitive to light at \(~480 \text{ nm}\),59 therefore, concerns about both the sleep–wake cycle following implantation of filtering IOLs have been raised.60 Decrease in the transmission of 480 nm light may occur, but it is very small compared to that of the aged lens. By the age of 80 years, transmission of 480 nm light is only 28% of the transmission in 10 year-old children.61 A prospective study, including 961 participants has shown that sleep quality improves after removal of cataract, notwithstanding the type of IOL used.62 Most important, the beneficial effect of blue-light filtering IOLs is strongly supported by a very recent study.
measuring the enlargement of the atrophic area in patients with dry AMD. After implantation of a non-blue filter IOL, this enlargement was almost twice as after implantation of a blue-blocking IOL.\textsuperscript{63}

**Macular pigment.** Macular pigment (MP) is composed of the xanthophyll carotenoids: lutein, zeaxanthin, and meso-zeaxanthin.\textsuperscript{64} Xanthophylls gradually increase towards the center of the macula, and in the human fovea, they reach concentrations greater than 1 mM.\textsuperscript{65} The cause of this elevated concentration might be explained by the low activity of β,β-carotene-9',10'-dioxygenase, the only known mammalian enzyme that cleaves xanthophylls, which is much weaker in humans and primates than in other mammals.\textsuperscript{66} Xanthophylls accumulate preferentially in the outer and inner plexiform layers (ONL and INL) where they may be inserted in the plasma membrane, or associated with specific binding proteins.\textsuperscript{65} The lutein : zeaxanthin : meso-zeaxanthin ratio changes progressively from 1 : 1 : 1 at the fovea to a ratio approaching 3 : 1 : 0 in the periphery. Since their peak absorbance is at 460 nm, and because they are located in the anterior (vitreal) portion of individual photoreceptors, macular pigments attenuate the amount of blue-light incident on the photoreceptors in the most sensitive region of the retina.\textsuperscript{67}

Macular pigment optical density (MPOD), which may be measured \textit{in vitro} or \textit{in vivo},\textsuperscript{68,69} is positively related to visual performance.\textsuperscript{65,70} Blue-light filtering improves the visibility of distant objects, most likely because scattered light from haze aerosols suspended on the horizon is predominantly blue.\textsuperscript{71} The implantation of blue-light filtering IOLs after cataract surgery is associated with augmentation of MPOD in the presence of raised serum concentrations of lutein and zeaxanthin,\textsuperscript{57} highlighting the efficacy of these molecules as blue filters. By contrast, in a sample of healthy volunteers (\(n = 828\)), MPOD levels were significantly and independently reduced by age, current and past smoking and AMD family history.\textsuperscript{72}

After the Age-related Eye Disease Study (AREDS) provided level 1 evidence that supplementation with vitamins C and E, β-carotene and zinc resulted in a 25% risk reduction of progression from intermediate to advanced AMD,\textsuperscript{73} numerous clinical and epidemiological studies have tried to ascertain the putative protecting role of macular xanthophylls. Addition of lutein + zeaxanthin to the AREDS formulation did not further reduce the risk of progression to advanced AMD,\textsuperscript{74} and only a mild beneficial effect on visual acuity has been observed after a one-year lutein supplementation.\textsuperscript{75} However, functional abnormalities of the central retina in early AMD can be ameliorated by lutein and zeaxanthin supplementation, an effect attributed to elevations in MPOD.\textsuperscript{76} A recent review concluded that supplementation with macular carotenoids is probably the best available measure to strengthen the antioxidant defenses of the macula, thus reducing the risk of AMD and/or its progression.\textsuperscript{57} Xanthophyll carotenoid supplementation in AMD would not only be significantly associated with improvements in visual acuity and contrast sensitivity, but also with a concomitant increase of MPOD.\textsuperscript{77} Results of carotenoid supplementation may depend on previous nutritional conditions and genetic risk status. Thus, in the Blue Mountains Eye and the Rotterdam studies, an interaction between lutein/zeaxanthin intake and early AMD incidence was only found in participants with high genetic risk (carriers of \(≥2\) risk alleles of CFH or ARMS2).\textsuperscript{78} The impact of supplements containing different combinations of lutein, zeaxanthin and meso-zeaxanthin on visual function in normal subjects and subjects with early AMD is under investigation.\textsuperscript{79}

Independently of their filtering function in the macula, carotenoids could serve as antioxidants in the macula and in the RPE. They protect against singlet oxygen mediated photo-oxidation reactions and can also react with free radicals.\textsuperscript{80} Thus, they would also reduce photo-oxidation of retinyl derivatives (such as A2-phosphatidylethanolamine and A2E, see below).\textsuperscript{81,82} Cultured RPE cells actively uptake lutein and zeaxanthin and these xanthophylls prevent photo-oxidative inactivation of the proteasome, and photo-oxidation induced changes in the expression of MCP-11, IL-8, and CFH.\textsuperscript{83} Zeaxanthin has direct anti-oxidant actions on RPE cells, including the induction of Nrf2-mediated phase II enzymes such as heme-oxygenase-1, NAD(P)H:quinone oxidoreductase and γ-glutamyl-cysteine ligase.\textsuperscript{84}

**Melanin.** Melanins, the heterogeneous polymers formed by tyrosinase (TYR) oxidation products of tyrosine, and L-DOPA (1-3,4-dihydroxyphenylalanine), are essential instruments for defense against UV exposure.\textsuperscript{85} Uveal melanocytes and RPE cells contain eumelanin and trace amounts of pheomelanin.\textsuperscript{86} Eumelanin, which has a broadband absorption spectrum smoothly decaying to the lower-energy end, can rapidly dissipate UV and blue-light energy as heat.\textsuperscript{87} Thus, eumelanin light absorption followed by rapid thermal relaxation could quench potentially harmful photo-chemical reactions. Melanin can also scavenge free radicals and reduce the oxidative stress resulting from lipid peroxidation and reactive oxygen species (ROS) production.\textsuperscript{88}

The function of melanin in sun photo-protection seems to be undeniable.\textsuperscript{86} Therefore, if sunlight is a stressing factor involved in the etiopathogenesis of AMD, melanin might be one of the anti-AMD defense mechanisms. In line with this hypothesis, AMD is more frequent in white persons than in persons of black African inheritance.\textsuperscript{89} Most studies also agree that white subjects with light blue-colored irises have a higher AMD prevalence than those with dark-colored irises.\textsuperscript{89} In the Beaver Dam Eye Study, increased risk of early AMD was found for persons with high sunlight exposure and light colored eyes (gray/blue), or light colored hair (blond/red).\textsuperscript{90} Remarkably, initial recovery of patients with neovascular AMD after anti-VEGF treatment shows a seasonal oscillation that is inversely correlated with global radiation intensity,\textsuperscript{91} and functional improvement is significantly higher in patients with dark-colored eyes than in those with light-colored eyes.\textsuperscript{91} In addition, a recent report suggests a possible relationship between early AMD and TYR single nucleotide polymorphisms (SNPs) previously associated with skin and eye pigmentation.\textsuperscript{92} Quantitative observations in eyes from human cadaveric donors indicate a decrease in RPE melanin with age, most
likely related to photo-oxidation.\textsuperscript{93,94} Aging also affects melano- 
nosomes, and by age 90, most RPE melanin appears as mela- 
nolipofuscin.\textsuperscript{95} The latter can generate ROS upon excitation 
with blue light.\textsuperscript{96} Studies \textit{in vitro} have shown that melanin may 
reduce the accumulation of lipofuscin in RPE cells,\textsuperscript{97} and the 
photo-oxidation of its components.\textsuperscript{98}

**Pupillary diameter.** The pupil modulates retinal illumina-
tion; consequently, it would also regulate retinal susceptibility 
to photo-toxicity. In eyes with little pigmentation, light might 
reach the retina by transmission through the iris and the 
sclera, possibly increasing the risk of light-induced damage.\textsuperscript{99}

Light sensitivity of the pupil constriction reflex seems to be 
unaffected by age;\textsuperscript{100} however, AMD patients confronted by a 
navigation task display larger pupillary diameters than con-
trols of the same age and sex.\textsuperscript{101} A larger pupillary diameter 
under the same luminance conditions might increase retinal 
light exposure and contribute to progression of the disease.

**Light-induced damage**

Electromagnetic radiation in the 100 nm–1 mm range is widely 
known as "optical radiation".\textsuperscript{42} Light absorption by biological 
material implies energy transfer, which may be damaging for 
absorbing tissues. Light-inflicted damage will depend on the 
specific combination of radiation wavelength, exposure time, 
tissue properties and volume.\textsuperscript{22}

Photo-chemical damage arises when a chromophore, or 
photo-sensitive molecule, undergoes physico-chemical 
changes after the absorption of a photon. In the eye, chromo-
phores include visual pigments in the photoreceptors, the 
macular pigments, absorbing in the 400–530 nm range, and 
the broadband absorbers melanin and lipofuscin in the RPE 
and choroid.\textsuperscript{23} Effects of chromophore excitation may be trans-
mitted to neighboring molecules, dissipating extra energy in 
various ways, including chemical bond splitting, hydrogen 
exchange and ROS production, such as singlet oxygen, super-
oxide, hydrogen peroxide and hydroxyl radicals. In turn, these 
radicals react with nearby molecules, inducing diverse photo-
oxidative changes. Thus, photo-chemical damage is almost 
synchronous with photo-oxidative damage.\textsuperscript{22,102} Cells may or 
may not repair these lesions depending on the irradiation 
intensity and the exposure time.\textsuperscript{22,23,27,103} Spreading of photo-
oxidative effects is particularly damaging in tissues with high 
concentration of cell membranes, such as photoreceptor outer 
segments.\textsuperscript{104} Oxidative stress contributes to photoreceptor cell 
death in animal models of retinal degeneration, including 
light-induced retinopathy.\textsuperscript{105,106}

3. Macular damage in aging and AMD

**Photoreceptors**

Eyes from 40-year or older persons without significant ocular 
disease show loss of photoreceptor nuclei in the macular ONL, 
together with disappearance of outer segments, but without 
defects in the RPE or the choriocapillaris.\textsuperscript{107} Quantitative 
microscopy studies in donor eyes demonstrated a steady 
decline in central rod number with age, without concomitant 
changes in cone numbers.\textsuperscript{108} Cones, however, displayed some 
morphological abnormalities, including lipofuscin depo-
sition.\textsuperscript{109,110} More recent studies have detected a significant 
thinning of the RPE and the choroid, together with an increase 
in the thickness of the OPL,\textsuperscript{3} which in the macular region is 
known as the fiber layer of Henle. Greater OPL thickness most 
likely reflects activation and hypertrophy of Müller cells follow-
ing photoreceptor loss.\textsuperscript{111} Aging eyes also exhibited a reduced 
thickness of the retinal nerve fiber layer (RNFL), GCL, inner 
plexiform layer (IPL), INL and photoreceptor inner segments, 
except at the fovea.\textsuperscript{3,112} By contrast, width of the photoreceptor 
outer segment layer correlated positively with age, presumably 
reflecting the age-related decrease in RPE phagocytosis.\textsuperscript{112} The 
amount of parafoveal rods significantly decreased in aging 
retinas.\textsuperscript{106} Although changes in foveal cone numbers were not 
detected histologically,\textsuperscript{108} adaptive optics have shown that, in 
old age, cone packing density decreases up to 25% within 
0.45 mm of the foveal center, but not in peripheral 
regions.\textsuperscript{113–115}

Histological evaluation of dry AMD showed RPE irregulari-
ties and atrophy, whereas wet AMD samples displayed both 
RPE defects and fibrovascular scars.\textsuperscript{108} Foveal cone numbers 
showed few changes, but rods were almost completely lost in 
the parafovea. In the wet AMD samples, photoreceptors surviv-
ing in the neighborhood of disciform scars were largely 
cones.\textsuperscript{108} Since external light is focused on the cone-rich fovea, 
sparing of foveal cones suggests that they may be more resist-
ant than rods to light-induced damage. Nevertheless, since 
they depend on the rod-derived cone viability factor 
(RdCVF),\textsuperscript{116} they would disappear after demise of parafoveal 
rods. It has been demonstrated that RdCVF protects 661 W 
cells from photooxidative damage\textsuperscript{117} and, most important, 
that RdCVF-deficient mice are extraordinarily sensitive to light-
induced damage.\textsuperscript{118}

Histological findings are supported by analysis of rod func-
tion,\textsuperscript{119} and adaptive optics scanning laser ophthalmoscopy.\textsuperscript{120} 
Besides, AMD retinas also displayed reduced cone reflectivity, 
suggesting mild structural abnormalities.\textsuperscript{120} Additionally, 
scanning laser polarimetry studies indicated that the number 
of central cone photoreceptors may be lower, and/or structural 
alterations of their axons significantly higher, than in non-
AMD eyes of the same age.\textsuperscript{121} Reduction of the RPE/photore-
ceptor and ONL layers overlying drusen has been consist-
ently found,\textsuperscript{6,122,123} but reports about their thinning in drusen-
free areas\textsuperscript{122} need confirmation.

Loss of rod photoreceptors, with cone sparing, resembles 
the consequences of white light-induced damage in rodents, 
where cones remained after complete disappearance of 
rods.\textsuperscript{124} Remarkably, in rats maintained under cyclic lighting, 
the retinas of older animals suffered more damage from 
exposure to intense light than those of younger animals.\textsuperscript{125}

**Retinal pigment epithelium and lipofuscin**

The outer segments of rods and cones are under constant 
renewal, with old discs being shed from the apical tip and
phagocytosed by RPE cells. A current development is the in vivo study of disc renewal in human cones through changes in their reflectance.

Daily shed outer segments are phagocytosed by the RPE and processed using a combination of phagocytic and autophagic mechanisms where lysosomes are fused with autophagosomes. Since some autophagy characteristic proteins (LC3 and Atg5) appear in the membrane of phagosomes, the process is known as non-canonical autophagy or LC3-associated phagocytosis. Most of the material is recycled to the photoreceptors; however, the RPE accumulates lipofuscin, a non-digested heterogeneous substance, within the residual bodies of the lysosomal compartment.

Lipofuscin distribution in the RPE shows a defined pattern, increasing from the equator to the posterior pole with a consistent dip at the macula. Curiously, melalin follows a contrasting distribution, decreasing from the equator to the posterior pole, but with a regular peak at the macula. This polarization fades by the age of 50, presumably because most melalin becomes incorporated into melanolipofuscin granules.

Lipofuscin has a broad excitation range (300–600 nm) and a broad emission spectrum (480–800 nm), allowing histological and non-invasive studies of fundus autofluorescence. Wholemount studies of human donor retinas have shown that the topography of RPE autofluorescence follows the distribution of rod photoreceptors, being highest in the vicinity of the rod-rich perifoveal annulus. The highest autofluorescence levels were found in 80 years or more retinas. Older retinas displayed an increase of non-hexagonal shapes, without changes in RPE cell density. Degranulation of RPE cells appeared in healthy and AMD aged eyes, whereas granule aggregation was only observed in AMD eyes. In the latter, some RPE cells were greatly enlarged and displayed cytoskeletal alterations. In GA patients, the atrophic patches were usually surrounded by a junctional region of abnormal autofluorescence. Distinct patterns have been described and some of them may have a genetic basis.

4. Main targets of photo-toxicity

Understanding the role of light exposure in the course of AMD requires identification of the molecular targets that initiate photo-oxidation reactions, which we may call primary targets. Some other molecules, the secondary targets, would not be directly affected by light, but they would become the immediate targets of ensuing photo-oxidation. Some compounds could be both primary and secondary targets, for example all-trans-retinal or lipofuscin. Available information about light molecular targets results from a large amount of experimental work that has been mainly done in animals or in vitro.

Early work in albino rats showed photoreceptor damage after light exposure through blue (360–530 nm) and green (490–580 nm) filters. Electroretinogram (ERG) alterations, however, were most efficiently induced by exposure to 500 nm. The RPE was sometimes involved, depending on age of the animal, temperature, previous illumination conditions and the intensity and duration of the damaging light. Results suggested that these lesions depended, directly or indirectly, on rhodopsin excitation. Indirect damage would require the activation of other photoreceptor molecules appearing under light adaptation conditions, perhaps including products of the rhodopsin bleaching process, such as vitamin A derivatives. By contrast, experiments using blue light (441 nm), done in monkeys, showed an initial damage of the RPE, followed by alteration of the photoreceptor outer segments and remarkable recovery 10–11 days after exposure.

Available data for monochromatic-induced retinal damage support the existence of at least two damage action spectra. Irradiation in the 320–440 nm range predominantly affected photoreceptors, whereas in the 440–550 nm range injured the RPE and/or the photoreceptors. Rhodopsin, and also other chromophores such as lipofuscin, intermediate products of the visual cycle, and even melanin, could be the photo-sensitive targets converting light into retinal damage. Rhodopsin, however, is not only affected by 500 nm light, but can also be a target for blue light-induced photoreversal of bleaching. This phenomenon increases the photon-catch capacity of the retina and its susceptibility to light damage, thus explaining why blue light has a greater damage potential than green light.

Of note, most spectral data for retinal damage has been obtained in anesthetized animals, often using funduscopic visible changes as threshold damage. Therefore, lesions described in these experiments do not resemble aging or AMD changes, but those found in welders and laser or sun-gazing accidents. Their relationship with AMD is conceivable, but is far from proven, particularly since these experiments provide little information about the damage spectra of very long exposures in freely moving subjects. Curiously, exposure of albino mice or rats to diffuse white light induces photoreceptor death, without overt RPE damage.

Rhodopsin

White light did not cause photoreceptor degeneration in mice lacking functional rhodopsin, thus, rhodopsin must play an essential role in the retinal response to excessive lighting. This role is further supported by the correlation between rates of visual pigments regeneration and light-induced damage thresholds. Moreover, white light did not induce retinal damage in mice and rats under halothane anesthesia, which blocks rhodopsin metabolic regeneration. In these animals, however, exposure to blue light (403 nm) induced photoreceptor apoptosis and RPE swelling.

The absence of functional transducin, which blocks signaling from light-activated rhodopsin, did not protect from bright light-induced degeneration. However, mutant mice with persistent rhodopsin activation, which are extremely sensitive to low-intensity cyclic light, were protected.
Photo-transduction and oxidative stress in the outer segments

Oxidative metabolism, which is required to support the light pathway, could also induce or aggravate photoreceptor damage.\(^{150}\) Since a significant fraction of the O\(_2\) used by cells is converted to ROS, excessive activation of photo-transduction might determine a higher activity of the respiratory complexes, and a higher oxidative stress. These phenomena might occur within the outer segments, which contain their own machinery for ATP synthesis, including mitochondrial-like electron transport chains, F1-ATP synths and the TCA cycle enzymes, as has been demonstrated in bovine retinas using proteomic procedures and immunogold transmission electron microscopy.\(^{151-153}\) Remarkably, bovine and mouse outer segments are selectively stained with mitochondrial dyes.\(^{151,154}\)

The nicotinamide adenine dinucleotide phosphate (NADPH) oxides (NOX) are also involved in light-induced oxidative stress. The primary function of NOX enzymes is the reduction of oxygen into superoxide anion using NADPH as an electron donor and oxygen as an electron acceptor.\(^{155}\) Increase of these species is observed in mouse outer segments when whole eyeball cultures are irradiated with blue light (405 nm), and can be prevented by the NOX inhibitor apocynin.\(^{154}\)

The visual cycle and the retinoids

Photo-excitation of rhodopsin and other visual pigments leads to isomerization of their chromophore 11-cis-retinal to all-trans-retinal, which dissociates from the opsin protein. Generation of the visual pigments requires the restoration of 11-cis-retinal. Visual pigments in rods and cones recover at very different rates, about 40 min in rods but only 2–3 min in cones.\(^{156}\) These are the times required by their specific regeneration processes: the rod and the cone visual cycles.\(^{156,157}\)

The rod visual cycle. Both 11-cis- and all-trans-retinal form Schiff base adducts with phosphatidylethanolamine (PE). The ATP-binding cassette subfamily member 4 (ABCA4) flips this N-retinylidene-PE to the disk cytoplasmic leaflet,\(^{158}\) and cytoplasmic dehydrogenases (RHs) reduce it to all-trans-retinal.\(^{156}\) This retinoid is released and bound by the interphotoreceptor retinoid-binding protein (IRBP). It is then captured by RPE cells, becoming bound to a retinoldehyde-binding protein (CRALBP). All-trans-retinal is esterified by lecithin-retinol acyltransferase (LRAT) and turned into 11-cis-retinol by the isomerase (RPE-specific 65 kDa protein; Rpe65). Further oxidation produces 11-cis-retinal, which abandons the RPE and is taken up by photoreceptors, regenerating a functional visual pigment.\(^{156}\)

The cone visual cycle. The cone visual cycle is intraretinal. Instead of trafficking to the RPE, all-trans-retinal diffuses from cones to the Müller cells, where it is isomerized to 11-cis-retinol, probably by dihydroceramide desaturase-1 (DESI).\(^{159}\) This is a type 2 isomerase that, at difference with Rpe65, acts directly on all-trans-retinol.\(^{159}\) 11-cis-Retinol is rapidly sterified by multifunctional O-acyltransferase (MFAT).\(^{160}\) CRALBP plays an important role in the cone visual cycle, since its absence desensitizes cone-driven vision in humans and mice.\(^{161}\) Both cone and rod dark adaptation depend on the presence of CRALBP.\(^{161}\)

Photoxicity of all-trans-retinal. The possible role of all-trans-retinal as the agent of light-induced damage, initially discussed by Noell (1966),\(^{141}\) has been extensively described.\(^{132,149}\) Remarkably, the instantaneous concentration of this retinoid in the light-exposed outer segment could be as high as 5 mM.\(^{162,163}\) Bleaching less than 0.5% of all rhodopsin would still generate toxic levels of all-trans-retinal.\(^{164}\) Thus, this molecule could either be an indirect damage target of rhodopsin activation and/or the direct target of short-wavelength light. Peak absorption of all-trans-retinal is at 380 nm, which is almost completely filtered by the human lens. However, this retinoid still shows substantial absorption at >410 nm wavelengths.\(^{165}\) UV-A (355 nm) and blue (422 nm) light excitation of all-trans-retinal in the presence of oxygen generates singlet oxygen, which can in turn oxidize all-trans-retinal.\(^{166}\) The degradation products, including several endoperoxides, shorter-chain aldehydes and epoxides, significantly increase all-trans-retinal cytotoxic effects on RPE cells in vitro.\(^{166}\)

Rod photoreceptors would be the primary site of all-trans-retinal attack.\(^{167}\) Damage is induced through different mechanisms, including photo-damage of its own transporter ABCA4,\(^{163}\) impairment of mitochondrial function, increase in the production of superoxide through the activation of NOX enzymes,\(^{168}\) and/or the activation of Toll-like-receptor 3 (TLR-3),\(^{169}\) followed by microglial activation.\(^{170}\) In addition, in vitro irradiation (400–700 nm) of rod outer segments in the presence of all-trans-retinal impairs the ability of rhodopsin to regenerate,\(^{171}\) indicating another probable cause of photoreceptor degeneration.

All-trans-retinal arrives to the RPE together with phagocytosed outer segment discs, but can also be synthesized in the RPE from β,β carotene or all-trans-retinol.\(^{172}\) All-trans-retinal is highly cytotoxic to human RPE cells in primary cultures, and potentiates the effect of light irradiation.\(^{172}\)

The pharmacological control of visual chromophore biosynthesis has been proposed as a preventive method for retinal diseases depending on light-induced damage, increase of retinoid byproducts and hyperoxia.\(^{173}\) Emixustat hydrochloride, presently in clinical trial for dry AMD is an RPE65 inhibitor and retinal scavenger. This drug has significant adverse effects; however, it has shown that all-trans-retinal sequestration is a crucial function for photo-toxicity protection.\(^{174}\)

Lipofuscin and A2E. Although lipofuscin has been extensively described, its composition is still poorly understood and might differ between the diverse regions of the retina. Lipofuscin, which contains little protein, would mainly derive from all-trans-retinal, docosahexaenoic acid (DHA), and other components from outer segments.\(^{175-177}\) Its best known constituents are the bisretinoids, a complex mixture of autofluorescent compounds.\(^{132,176}\) Retinal isomers, including all-trans and 11-cis, covalently react with the amine group of PE forming N-retinylidene-PE. The addition of a second retinal molecule produces N-retinylidene-N-retinylphosphatidylethanolamine.
(A2PE). A2E forms after removal of the A2PE phospholipid moiety. Lipofuscin also contains all-trans-retinal dimers, which are more abundant than A2E in the retina of Abca4 mice.27 RPE bisretinoids exhibit diverse excitation maxima, but they all emit fluorescence centered around 600 nm, which is similar to the maximum emission of the fundus autofluorescence.176

Numerous experiments, in vivo and in vitro, support the role of lipofuscin, all-trans-retinal and A2E as targets for blue light. In primates, visible light (488 and 568 nm) may photo-bleach RPE cells autofluorescence at levels previously considered safe. Experiments in vitro suggested that A2E might be involved in this response.27 At higher intensity irradiation levels, but still not higher than the maximum permissible exposure, the RPE developed long-term structural disruption. At present, it is unclear whether these lesions represent a lipofuscin- or photopigment-dependent damage mechanism.27 However, damage induced in RPE cell cultures fed isolated lipofuscin granules, and exposed to short-wavelength visible light (390–550 nm),97 but in the absence of photoreceptors, cannot be attributed to a rhodopsin effect.

A2E may be less damaging than retinaldehydes,172 and it has been postulated that the formation of A2E and its precursor A2PE would reduce the photo-reactivity of all-trans-retinal.172 In contrast with this hypothesis, a damage spectrum has been described for A2E-loaded porcine RPE cells, with lesions occurring between 390 and 552 nm (maximal at 420–450 nm).178 This apparent contradiction can be explained by the increased photo-toxicity of A2E oxidation products,179 which would contribute to RPE photo-damage in rat retinas exposed to blue light.180 Photo-oxidation and photo-degradation of bisretinoids release small carboxyls involved in the formation of Advanced Glycation End-products, which may accumulate in drusen and laminar deposits.181 It has also been suggested that photo-activation and cleavage of bisretinoids promote complement attack on RPE cells.182 These similarities in the cytotoxicity of lipofuscin and A2E, plus the fact that both molecules show the same distribution in mice RPE,184 were taken as an indication that A2E might be the target triggering and maintaining the course of AMD.185

A2E and its oxides have been studied in situ using high-resolution matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). Whereas the RPE central area displayed the highest lipofuscin fluorescence intensities, the highest A2E densities were found in the far periphery.186,187 Comparison of A2E distribution in human and mouse retinas suggests that this bisretinoid is characteristic of rod-rich areas. Low levels in the cone-rich area macular area suggest that the cone visual cycle does not favor the transformation of all-trans-retinal into A2E. Thus, light-induced damage in the central retina would not depend on A2E. Nevertheless, the distribution of lipofuscin in a perifoveal ring corresponds to the localization of perifoveal rods,134 which are the first photoreceptors to perish in aging and AMD.108,119,120

On the other hand, photo-oxidative damage (448 nm) of lipofuscin-loaded primary human RPE cells and ARPE-19 cells activated the inflammasome, suggesting a link between photo-oxidative damage and innate immune activation.188

The ABCA4 gene and clearance of all-trans-retinal. Clearance of all-trans-retinal is delayed when certain variants of the ABCA4 gene are present, as in recessive Stargardt’s disease (STGD1), a juvenile form of macular degeneration.189 Two variants of the human gene have been associated with increased risk for AMD.190

STGD1 patients show a distinctive fundus autofluorescence pattern, the granular pattern with peripheral punctate spots (GPS+), that also appears in 2–3% of GA AMD patients.137 About half of the GPS+ patients carried a monoallelic ABCA4 variant, whereas only 10% of the GPS-patients carried these variant alleles.137 Of note, light deprivation might contribute to reduced progression of decreased autofluorescence in STGD1 patients.191 Although the vast majority of AMD cases are not related to ABCA4 gene variants, the aforementioned associations support a role of all-trans-retinal in AMD development.

Data obtained in mice carrying Abca4 mutations suggest a complex and still controversial scenario. Abca+ mice, which are more vulnerable to light-induced retinal degeneration, accumulate RPE lipofuscin and A2E.192 Rdh8+/− Abca4−/− mice, with a delayed all-trans-retinal clearance, develop retinal lesions resembling human AMD (RPE/photoreceptor dystrophy, lipofuscin, drusen-like deposits under the RPE and chorioidal neovascularization), and show an acute retinopathy under irradiation levels harmless for Rdh8+/− Abca4−/− mice.149

Abca4−/− mice increased the expression of proteins activating the complement system, and downregulated the complement regulatory proteins. Besides, they showed basal laminar deposits along the Bruch’s membrane.193 Moreover, all-trans-retinal sensitized human RPE cells in vitro to alternative complement pathway attack,194 suggesting another likely link between light exposure, the visual cycle and AMD.

Retinal lipids

DHA is the most abundant fatty acid in whole retinas (22–24%).195 Prolonged light exposure and high-light rearing environments reduce DHA levels in rod outer segments.196 Interestingly, acute exposure to bright light did not damage photoreceptor outer segments in rats with dietary DHA or linoleic acid deprivation.196,197

Involvement of N-retinylidene-PE in the clearance of all-trans-retinal probably explains the extraordinarily high content of PE and its long-chain DHA in photoreceptor membranes. In the disc membranes, PE would act as a sink preventing diffusion of 11-cis-retinal.198

As a precursor of neuroprotectin D1, DHA may also shield the disc membranes, PE would act as a sink preventing diffusion of 11-cis-retinal.198

As a precursor of neuroprotectin D1, DHA may also shield retinal cells from oxidative stress.199 Importantly, photo-activation of rhodopsin may be regulated by the relative proportion of polyunsaturated lipids, such as DHA, and cholesterol, in the disc membranes. Thus, quantum yield of all-trans-retinal depends on the availability of DHA in the retina.196
Lipid peroxidation significantly increases in the retina of rats exposed to light. Moreover, it has been shown that the oxidative potential of the posterior region of the human eye, including the macula, increases with age. Exposure to light induces phospholipid oxidation and immunoreactivity for oxidized phosphatidylethanolamine appears in photoreceptors and RPE cells at the healthy human macular area. Its levels increase with age and eyes with AMD show stronger immunoreactivity than age-matched normal eyes. Increase of oxidized phospholipids multiplies the expression of monocyte chemoattractant protein-1 (MCP-1), followed by macrophage accumulation, and these effects are prevented by antioxidants. Moreover, subretinal application of oxidized phospholipids induces choroidal neovascularization, typical of the wet-type AMD.

### Carboxyethylpyrrole adducts

Carboxyethylpyrrole protein (CEP)-adducts are oxidative products derived from fragmentation of DHA-containing lipids. They are elevated in ocular tissues and plasma in AMD patients, where they can be detected in drusen. Purified lipofuscin granules also contain CEP-adducts. Likewise, CEP adducts are found in the retina of rodents exposed to intense light. The photo-oxidative processes that generate CEP-adducts could occur in photoreceptor cells, but may also take place after disc shedding in the RPE autophagosomes and lysosomal bodies. CEP-adducts may well be another pathway to macular degeneration, since autoantibodies are present in the blood of AMD patients. Moreover, mice immunization with CEP-seroalbumin induced, after 12–14 months, numerous sub-RPE deposits and accumulation of complement proteins in the Bruch’s membrane.

### Isolevuglandins

Levuglandins (Lgs) and isolevuglandins are γ-keto-aldehydes derived from the oxidation of arachydonyl phospholipids. These molecules are highly reactive toward free primary amines such as the ε-amine of lysine residues in proteins and the primary amino groups of phosphatidylethanolamines. They also react with mitochondrial cytochrome P450 27A1 (CYP27A1), impairing its function in sterol elimination. Isolevuglandins are highly abundant in the human retina, where immunoreactivity is mainly localized in photoreceptor inner segments. They are not detected in retinas of mice reared under dim light, but can be found in inner segments and RPE cells after exposure to a bright light source (10,000 lux 2 h). Isolevuglandin E2 adducts have been found in purified lipofuscin granules.

## 5. Concluding remarks

Evidence presented here supports the concept that light reaching the retina and the RPE provokes oxidative stress, leading to a buildup of toxic compounds that induce inflammation and cell death. Experimental and clinical findings indicate that light can affect oxidative homeostasis in the outer retina, either by excessive activation of photo-transduction processes or by the impairment of waste disposal mechanisms. All-trans-retinal and its subproducts appear as the major offenders in the retinal degeneration circuit.

In experimental models, accumulations of all-trans-retinal in photoreceptors, and bisretinoids and lipofuscin in the RPE, are light-dependent processes. In addition, these compounds are both photo-reactive, and photo-toxicity inducers as well. Experimental evidence indicates that all-trans-retinal accumulation in photoreceptors suffices for the initiation of their degeneration. Therefore, early AMD might represent the direct effect of all-trans-retinal on photoreceptors, perhaps reinforced by lipofuscin accumulation in cones. In a second stage, lipofuscin, A2E, and related compounds, would increasingly accrue in the RPE, giving rise to a new target site for phototoxicity. The course of the disease would then accelerate, since the light attack becomes possible at two different fronts. Differences between early and late AMD could perhaps be explained by this temporal pattern. In addition, all-trans-retinal photo-toxicity includes disruption of ABCA4, the N-retinylidene-PE transporter. Since photoreceptor PE molecules are highly enriched in DHA, all-trans-retinal photo-toxicity might be involved in the formation of CEP-adducts that appear in drusen and lipofuscin granules.

The availability of precise and fast analytic tools has also brought to light that rods and cones follow different death pathways. Most important, both histological and modern imaging procedures have shown that perifoveal rods die before foveal cones. This sequence could be associated to differences in the management of visual pigment regeneration, which requires an RPE step for rods, but is mainly intraretinal for cones. Moreover, cones seem to be more resistant to light-damage than rods. Since their light-resistance and survival depends on RdCVF availability, loss of perifoveal rods predicts the future demise of foveal cones. Of note, experiments suggest that a replacement therapy might extend cone survival.

Ample evidence shows that light-induced photoreceptor and/or RPE injury would trigger the inflammatory component, amplifying the initial damage. These processes explain the importance of certain gene variants for complement regulatory proteins as risk factors for AMD development.

As shown by data presented in this review, the hypothesis of light as an initiation cause of AMD is mainly supported by the existence of molecular targets in the retina and the pigment epithelium which light can transform into photoreceptor toxics.

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Photochemical & Photobiological Sciences

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