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The Role of Oxidative Stress in the Pathogenesis of Age-Related Macular Degeneration

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Abstract. Age-related macular degeneration (AMD) is the leading cause of blind registration in the developed world, and yet its pathogenesis remains poorly understood. Oxidative stress, which refers to cellular damage caused by reactive oxygen intermediates (ROI), has been implicated in many disease processes, especially age-related disorders. ROIs include free radicals, hydrogen peroxide, and singlet oxygen, and they are often the byproducts of oxygen metabolism. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light. In vitro studies have consistently shown that photochemical retinal injury is attributable to oxidative stress and that the antioxidant vitamins A, C, and E protect against this type of injury. Furthermore, there is strong evidence suggesting that lipofuscin is derived, at least in part, from oxidatively damaged photoreceptor outer segments and that it is itself a photoreactive substance. However, the relationships between dietary and serum levels of the antioxidant vitamins and age-related macular disease are less clear, although a protective effect of high plasma concentrations of α-tocopherol has been convincingly demonstrated. Macular pigment is also believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching ROIs. Many putative risk-factors for AMD have been linked to a lack of macular pigment, including female gender, lens density, tobacco use, light iris color, and reduced visual sensitivity. Moreover, the Eye Disease Case-Control Study found that high plasma levels of lutein and zeaxanthin were associated with reduced risk of neovascular AMD. The concept that AMD can be attributed to cumulative oxidative stress is enticing, but remains unproven. With a view to reducing oxidative damage, the effect of nutritional antioxidant supplements on the onset and natural course of age-related macular disease is currently being evaluated. (**Surv Ophthalmol 45**:115–134, 2000. © 2000 by Elsevier Science Inc. All rights reserved.)

Key words. age-related macular degeneration • antioxidants • free radicals • macular pigment • oxidative stress • reactive oxygen intermediates

Age-related macular degeneration (AMD) is the leading cause of blind registration in the western world,[119] and its prevalence is likely to rise as a consequence of increasing longevity.[225] There is a general consensus that cumulative oxidative damage is responsible for aging, and may, therefore, play an important role in the pathogenesis of AMD. In this article, we review the literature germane to oxidative processes in the retina and examine the evidence for a causal link between oxidative stress and age-related macular degeneration.

I. Classification and Grading of Age-Related Macular Degeneration

We have adopted and modified the International Classification and Grading System for Age-Related

0039-6257/00/$–see front matter
PII S0039-6257(00)00140-5

Abbreviations Used in This Review
AMD: Age-related macular degeneration
ARM: Age-related maculopathy
ATP: Adenosine triphosphate
BDES: Beaver Dam Eye Study
BLSA: Baltimore Longitudinal Study of Aging
BMES: Blue Mountain Eye Study
CML: Carboxymethyl lysine
DHA: Docosahexanoic acid
EDCC: Eye Disease Case Control
NADH: reduced form of nicotinamide-adenine dinucleotide
NEI: National Eye Institute
NHANES: National Health and Nutrition Examination Survey
POLA: Pathologies Ooculaires Liees a l'age
PUFA: polyunsaturated fatty acids
Px: Peroxidase
ROI: Reactive oxygen intermediates
RPE: Retinal pigment epithelium
SOD: Superoxide dismutase

Maculopathy and Age-Related Macular Degeneration in order to avoid confusing terminology.[16] Age-related macular degeneration may be early or late. Early AMD is characterized by any of the following findings in the macular area: soft drusen; choroidal or outer retinal hyperpigmentation associated with drusen; and depigmentation of the retinal pigment epithelium (RPE). Late AMD may be atrophic or neovascular. Atrophic late AMD refers to any sharply demarcated area of hypopigmentation, depigmentation, or apparent absence of the RPE in the macular area. Neovascular late AMD describes any of the following findings in the macular area: RPE detachment; choroidal neovascularization; scar/glial tissue, whether epiretinal, intraretinal or sub-RPE; and macular hard exudates unrelated to any other retinal vascular disease.[16] Although there is no visual acuity component to this classification system, early AMD is typically associated with a Snellen acuity of 20/30 or better, whereas late AMD has a profound negative impact on central vision.

II. Basic Biochemistry

A. OXIDATIVE PROCESSES

Chemically, oxidation refers to the removal of electrons and reduction refers to the gain of electrons. Animals release energy from dietary carbohydrates, proteins, and lipids by oxidizing them to CO_2 and H_2O. A series of reactions known as the tricarboxylic acid (TCA) cycle is responsible for most of the oxidation of fuels, and the energy yielded is conserved in the form of the reduced electron-accepting coenzymes, NADH and FAD(2H). The electrons of these coenzymes can be used to reduce oxygen (O_2) to H_2O via the electron transport chain, and this reaction releases energy for the conversion of adenosine diphosphate and Pi to adenosine triphosphate (ATP) in a process known as oxidative phosphorylation. Oxidative phosphorylation occurs in the mitochondrion and is catalyzed by ATP synthase. The electron transport chain accounts for approximately 90% of our total O_2 consumption, the remainder being utilized by reactions involving oxidases or oxygenases.

B. REACTIVE OXYGEN INTERMEDIATES

Reactive oxygen intermediates (ROI) is an umbrella term used to describe free radicals, hydrogen peroxide, or singlet oxygen. Free radicals are molecules that contain one or more unpaired electrons in their outer orbits,[196] and examples include the superoxide anion ($O_2^-\bullet$), the hydroxyl free radical ($OH\bullet$), the hydroperoxyl radicals ($HO_2\bullet$) and the lipid peroxyl radicals[81] (Table 1). Singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) contain their full complement of electrons, but in an unstable or reactive state.

In the cell, ROI continually "leak" from the active sites of the enzymes involved in oxidative processes by inadvertently interacting with O_2 or other compounds. Stimuli known to increase the production of ROI include irradiation, aging, inflammation, raised partial pressure of O_2, air pollutants (O_3, NO_2), cigarette smoke, and reperfusion injury.[20,135]

TABLE 1

Reactive Oxygen Intermediates

Species	Comment
Superoxide anion (O_2^-)	Produced by electron transport chain and at other sites
Hydrogen peroxide (H_2O_2)	Contains no unpaired electrons; can generate a free radical through the Fenton reaction
Hydroxyl radical (OH^-)	The most reactive free radical
Lipid peroxyl radical ($ROO\bullet$)	An organic free radical
Singlet oxygen ($^1O_2$)	O_2 with antiparallel spins; damages molecules as it converts back to O_2

R = lipid; • = unpaired single electron in the outer orbital

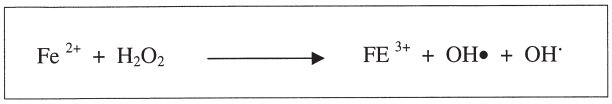

Fig. 1. Simplified version of the Fenton reaction. The hydroxyl radical is generated from hydrogen peroxide by the transfer of single electrons. OH• = hydroxyl radical. H_2O_2 = hydrogen peroxide.

In order to achieve a stable state, free radicals extract electrons from other molecules, which are themselves rendered unstable by this interaction, and a cytotoxic oxidative chain rection results. Hydrogen peroxide, although containing no unpaired electrons, can generate free radicals through the Fenton reaction (Fig. 1), and singlet oxygen can damage molecules as it converts back to normal oxygen.

C. REACTIVE OXYGEN INTERMEDIATES AND CELLULAR DAMAGE

Carbohydrates, membrane lipids, proteins, and nucleic acids are all vulnerable to damage caused by reactive oxygen species, and this damage is believed to contribute to the pathogenesis of many diseases (Table 2).[48,81]

1. Lipids

The formation of lipid free radicals and lipid peroxides, known as free radical auto-oxidation, is not subject to the kinetic barriers of spin restriction, which normally retard the oxidation of organic molecules. Polyunsaturated fatty acids (PUFAs) are particularly susceptible to free radical damage because their conjugated double bonds are convenient sources of hydrogen atoms, which contain one electron. The lipid radical then combines with oxygen to form lipid peroxyl radicals and lipid peroxides, which can achieve a steady state only by stealing electrons from other polyunsaturated fatty acids, thus creating a cytotoxic cascade of reactions that consume valuable PUFAs and produce damaged molecules. Degradation of the lipid eventually occurs, forming products such as malondialdehyde, which is found in the blood and urine and which can be used as a marker of free radical damage.

TABLE 2

Some of the Clinical Conditions Associated with Oxidative Injury

Ischemia/reperfusion injury
Atheroma
Cervical cancer
Diabetes
Chronic obstructive airway disease
Aging
Retinopathy of prematurity
Parkinson's disease

2. Protein

Fragmentation, cross-linking, and aggregation of proteins, as well as enhanced vulnerability to proteolysis, can result from oxidation of their amino acids.

3. Nucleic Acids

The oxidized bases of DNA arising from interactions with ROI contribute significantly to aging and age-related disorders.[6]

D. DEFENSE MECHANISMS AGAINST OXIDATIVE STRESS

Mechanisms used to protect against the effects of oxygen toxicity include cellular compartmentalization, repair, enzymantic removal of the ROI, and 'scavenging' of free radicals by vitamins and other compounds.[189]

1. Compartmentalization

Compartmentalization refers to the separation of ROI from cellular components that are susceptible to oxidative damage. For example, enzymes involved in the generation of H_2O_2 are found in peroxisomes which have a high content of antioxidant enzymes and which utilize the H_2O_2 for other oxidative reactions within the same organelle.

2. Repair

DNA repair involves replacing the distorted region of the helix by action of a DNA polymerase, followed by closure of the break by action of a ligase. Damage to a single base is repaired using DNA glycosylases. Oxidized amino acids of proteins are repaired by protein degradation and resynthesis of new proteins, and mechanisms to remove oxidized fatty acids from membrane lipids also exist.

3. Antioxidant Enzymes

There are several enzymes with antioxidant activity, and these include superoxide dismutase, catalase, and glutathione peroxidase (Fig. 2).

4. Antioxidant Vitamins

Vitamins C, E, and certain carotenoids can react directly and nonenzymatically with ROI, yielding harmless products and, thereby, terminating the free radical chain reaction.

5. Other Antioxidant Compounds

Other substances involved in the retinal antioxidant defense system include metallationein, melanin, and glutathione.

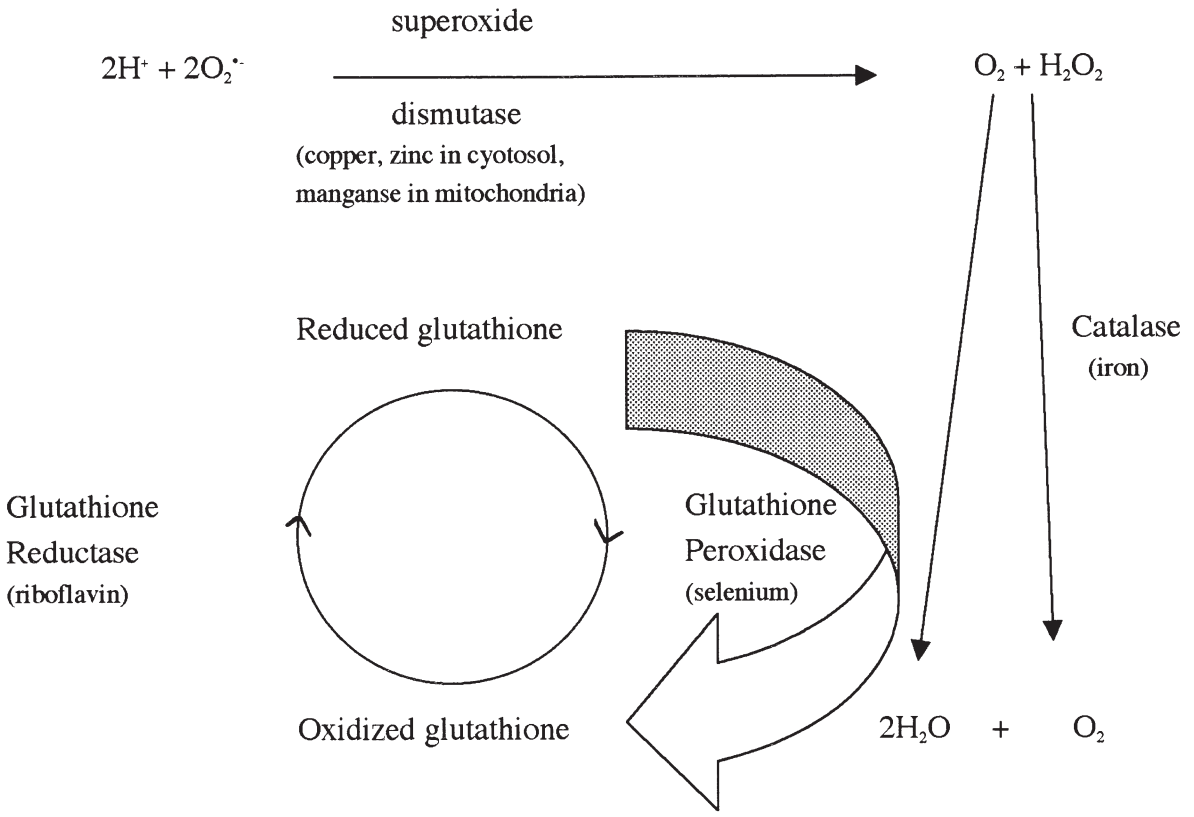

Fig. 2. The roles of key antioxidant enzymes in quenching hydrogen peroxide and the superoxide radical. The essential nutrient cofactors of the enzymes are indicated in brackets. $2O_2^{-}\bullet$ = superoxide radical. H_2O_2 = hydrogen peroxide.

III. Generation of Reactive Oxygen Intermediates in the Retina

In vivo, ROIs may occur as the byproducts of cellular metabolism[123] or as the result of photochemical reactions.[45] The retina is an ideal environment for the generation of reactive oxygen species for several reasons. First, oxygen consumption by the retina is much greater than by any other tissue.[188] Second, the retina is subject to high levels of cumulative irradiation. Third, photoreceptor outer segment membranes are rich in polyunsaturated fatty acids, which are readily oxidized and which can initiate a cytotoxic chain-reaction.[14] Fourth, the neurosensory retina and the retinal pigment epithelium (RPE) contain an abundance of photosensitizers.[55,75,180] Finally, the process of phagocytosis by the RPE is itself an oxidative stress and results in the generation of ROI.[205]

A. RETINAL IRRADIATION

Photochemical retinal injury was first described by Ham et al, who reported the histopathologic findings of 20 rhesus monkey retinas that had been exposed to blue light (441 nm) for 1000 seconds.[83] It was noted that short-wavelength light resulted in damage to the photoreceptor outer segments, cellular proliferation, and mitotic figures in the RPE and choroid, and hypopigmentation of the RPE, which resembled atrophic changes seen in AMD.[83] The sensitivity of the retina to light damage as a function of wavelength was then studied by the same investigators, who induced retinal injury in rhesus monkeys using eight different monochromatic wavelengths. It was found that the power required to cause photic damage was 70 to 1000 times lower for blue light (441.6 nm) than for the infrared wavelengths (1064 nm), depending on the duration of exposure (1 to 1000 seconds).

In 1983, Wiegand et al published their findings in albino rats exposed to constant illumination.[224] In brief, the main effect of light exposure was selective degeneration of photoreceptors, a reduction of the long-chain polyunsaturated fatty acid 22:6ω3 and an increase in the levels of lipid conjugated dienes. Both the loss of PUFAs and the increase in conjugated dienes, an accepted measure of lipid hydroperoxides,[22] provide compelling evidence that lipid peroxidation plays a role in retinal light damage.

Wu et al have recently confirmed that the mechanism of blue light-induced cell death is apoptosis,[228] and Organisciak et al have demonstrated that retinal light damage induces the expression of the antioxidative stress protein heme oxygenase-1 (HO-1).[154] In ad-

dition, photoreceptor loss is dramatically reduced by administration of a synthetic antioxidant agent. Again, these observations are consistent with the putative involvement of oxidative stress in retinal light damage.[154]

In 1990, Lam et al performed rhodopsin measurements and morphologic studies on 12 albino rats exposed to continuous illumination that ranged in wavelength from 490 to 580 nm after receiving intraperitoneal injections of dimethylthiourea, an antioxidant that scavenges hydrogen peroxide and the hydroxyl radical.[43,69,125] It was shown that dimethylthiourea-treated animals had significantly better preservation of photoreceptor nuclei and significantly higher levels of rhodopsin than control animals, indicating that the hydroxyl radical and hydrogen peroxide play an important role in mediating retinal photochemical damage. In 1999, these findings were corroborated by Ranchon et al, who also observed that the natural antioxidant *Ginkgo biloba*, which is known to scavenge superoxide, hydroxyl, and peroxyl radicals[76,137,167] and to inhibit the production of ROIs,[168] protected the retina from light-induced damage.[172]

B. POLYUNSATURATED FATTY ACIDS

The photoreceptor membranes of both rods and cones contain a lipid bilayer that provides a stable matrix that is passively permeable to ions, thereby accommodating subcellular compartmentalization and the stabilization of integral membrane proteins such as rhodopsin. Polyunsaturated fatty acids account for about 50% of the lipid bilayer of rod outer segment membranes, and proteins make up the remaining 50%. Docosahexanoic acid (DHA) (22:6ω-3), the most highly polyunsaturated fatty acid occurring in nature, makes up approximately 50% of the vertebrate rod photoreceptor phospholipids.[199] This very high proportion of long-chain PUFAs found in all phospholipid classes is a feature unique to retinal lipids.

Docosahexanoic acid and its precursor, the essential fatty acid α-linoleic acid, are entirely of dietary origin. As DHA contains six double bonds, and as the susceptibility of unsaturated fatty acids to oxidation correlates directly with the number of double bonds,[227] the retina is inherently susceptible to lipid peroxidation. Lipid peroxidation of membrane PUFAs results in loss of membrane function and structural integrity.[7,9]

The susceptibility of the human retina to lipid peroxidation is region- and age-dependent. De La Paz and Anderson compared in vitro lipid peroxidation of macular and peripheral retina in 15 human cadaver eyes. It was found that the susceptibility of the posterior pole retina to lipid peroxidation was positively related to age ($r^2 = 0.537$), but no such relationship was noted for peripheral retina.[49] Further, lower levels of DHA have been reported in the human macular region compared with the peripheral retina, suggesting that the macula is faced with a greater oxidant challenge than peripheral tissue.[212] It appears, therefore, that the ability to present an antioxidant defense at the macula, where it is most needed, diminishes with increasing age.

The susceptibility of rod outer segment membranes to lipid peroxidation can be altered by dietary modification. Bush et al reported on male rats that were reared in a cyclic (12 hr/12 hr) light/dark environment and fed a diet deficient in DHA and α-linoleic acid.[26] Retinal DHA levels were 65–75% lower in animals with restricted diets compared with control animals. DHA-deficient rats exhibited significantly less rod outer segment membrane disruptions, light-induced disk-shedding, and loss of rhodopsin, as compared with control animals. In a more recent study, Organisciak et al confirmed that a diet deficient in linoleic acid protects against retinal light damage in rats.[155]

C. RETINAL CHROMOPHORES

Chromophores, or photosensitizers, are molecules that absorb light to produce a chemical reaction that would not occur in their absence. Photochemical damage may be defined as injury arising from absorption of UV and visible light by a chromophore, which results in an electronic transition of the substrate to the excited state.[146] The retinal chromophores include rhodopsin, melanin, lipofuscin, and the mitochondrial respiratory enzymes, such as cytochrome c oxidase.

1. Rhodopsin

It has been shown that light-induced rod cell degeneration in the rat is rhodopsin-mediated, and that the severity of injury is related to the extent of rhodopsin bleaching and the pre-exposure rhodopsin content.[157,174,175] The oxidative nature of the rhodopsin-mediated light damage is supported by the findings of Organisciak et al, who have shown that augmented retinal antioxidant defenses can enhance rhodopsin preservation following exposure to visible light.[153,156,158] Furthermore, retinal photic injury associated with loss of rhodopsin induces expression of the antioxidative stress protein *heme oxygenase* and can be ameliorated by administration of dimethylthiourea.[154]

2. Lipofuscin

Lipofuscin is a lipid-protein aggregate that autofluoresces when excited with short wavelength light. It occurs within the lysosomes of a variety of metabolically active post-mitotic cells of neuronal and non-neuronal origin. Because it accumulates with age, lipofuscin is referred to as an "age pigment,"

and is considered a marker of cellular senescence. Furthermore, its presence correlates with a variety of age-related diseases.

In the RPE, lipofuscin granules are typically concentrated around the nucleus in the basal half of the cell. RPE cell lipofuscin is contained within granules of relatively uniform size,[64] and Wing et al have demonstrated a progressive accumulation of these granules with increasing age and noted that their concentration peaks in the posterior pole.[226] However, a focal reduction in RPE lipofuscin concentration at the fovea has been consistently demonstrated.[220,226] Unlike the lipofuscin of most tissues, which is derived from the incomplete autophagic degradation of spent intracellular organelles, RPE lipofuscin is also derived from phagocytosed photoreceptor outer segments.[113] Thus, RPE lipofuscin is likely to be both unique and heterogenous. Although its composition remains largely unknown, some lipids and the Schiff base reaction product *N*-retinylidene-*N*-retinylethanolamine (A2-E) have been identified.[61]

There is a growing body of evidence indicating that lipofuscin compromises RPE cellular function, and histopathological studies have demonstrated an association between high levels of lipofuscin and degeneration of RPE cells and the adjacent photoreceptors.[56] To our knowledge, three possible mechansims exist whereby lipofuscin may disrupt RPE cellular activities. First, metabolic processes may fail simply because of the reduction in functional cytoplasmic space and distortion of cellular architecture that results from the presence of intracellular lipofuscin.[230,231] Second, lipofuscin may actually induce oxidative damage of surrounding tissues, as it acts as a photosensitizer for generation of reactive oxygen intermediates.[21,75] And third, RPE lysosomal degradative functions are inhibited by *N*-retinylidine-*N*-retinylethanolamine, thus limiting the cell's capacity to digest intra- and extracellular material.[100] The current evidence supports the latter two options either alone or in combination.

Blue light-induced generation of reactive oxygen intermediates by lipofuscin has been demonstrated in vitro by Rozanowska et al.[180] First, these investigators evaluated blue light photoreactivity in human RPE cells and found a marked age-related increase in the rate of photo-dependent oxygen uptake, and demonstrated that the induced O_2 uptake was almost six times faster for lipofuscin (0.547 ± 0.110 mM/s) than for melanin (0.095 ± 0.022 mM/s). Next, the aerobic photoactivation of lipofuscin was shown to yield singlet oxygen, the superoxide anion, hydrogen peroxide, and lipid hydroperoxides. These findings established lipofuscin as the major chromophore of the RPE, and confirmed that aerobic photoactivation of lipofuscin forms several potentially cytotoxic ROIs.[180] Further study has revealed that the lipofuscin-mediated generation of ROIs in response to irradiation is wavelength-dependent, being greatest for the blue region of the visible spectrum.[181]

This photoinducible free radical generation by lipofuscin has been shown to result in lipid peroxidation and enzyme inactivation,[216] as well as RPE cellular dysfunction.[47] A2-E is a lysosomotropic agent that has the capacity to compromise both lysosomal function and integrity. Moreover, it is also capable of photoinducible generation of ROI, albeit to a lesser extent than RPE lipofuscin. Thus, it is probable that both mechanisms play a part in RPE dysfunction through lysosomal damage. To what extent these changes are implicated in the pathogenesis of AMD has yet to be determined.

3. Melanin

In an apparent contradiction, both photoprotective and phototoxic functions have been attributed to ocular melanin.[184] However, there is a general consensus that under typical in vivo conditions, melanin-mediated photooxidation is relatively unimportant.[170,180,184]

4. Cytochrome *c* Oxidase

Cytochrome *c* oxidase is an important mitochondrial enzyme involved in oxidative phosphorylation.[27] The absorbance of this enzyme varies according to its redox status,[149] and has a peak absorption of 440 nm in its reduced form.[134] It has been postulated that blue light-induced retinal damage is mediated by mitochondrial respiratory enzymes.[170,171] This hypothesis is supported by the observed reduction in the RPE content of cytochrome *c* oxidase content following exposure to blue light.[32] However, if cytochrome *c* oxidase is a major retinal chromophore, it is surprising that photochemical damage of the retina is not associated with gross structural alterations in the mitochondrial-rich ellipsoid regions of the photoreceptor inner segments.

5. Blood-borne Photosensitizers

Protoporphyrin IX (PP IX) is a precursor of hemoglobin found in erythrocytes and plasma that produces singlet oxygen and the superoxide anion when irradiated with blue light.[79] The authors postulated, therefore, that retinal damage seen in AMD may be induced by reactive oxygen intermediates generated by blood-borne photosensitizers of the highly vascular choriocapillaris. An accelerated pattern of age-related changes in Bruch's membrane in protoporphyric mice exposed to blue light has since been demonstrated.[78] To our knowledge, this line of enquiry has not been pursued further.

D. RESPIRATORY BURST

ROIs are generated during phagocytosis, and RPE phagocytosis of photoreceptor outer segments has

been shown to increase extracellular H_2O_2 production ninefold.[205] Furthermore, significantly greater amounts of catalase and metallothionein, and gene expression of these antioxidant substances, have been observed on exposure of cultured RPE cells to bovine photoreceptor outer segments or to H_2O_2, as compared to control. In other words, RPE phagocytosis of photoreceptor outer segments is an oxidative stress, and H_2O_2 is probably the ROI involved. As phagocytosis is a primary function of the RPE, it is possible that it plays a role in the pathogenesis of AMD.

IV. Oxidative Stress and Age-Related Macular Degeneration: The Evidence

In this section, we examine the evidence in support of the view that oxidative stress contributes to the development of AMD. First, we discuss the relationship between oxidative damage and the process of aging. Next, we review the literature germane to antioxidant status and AMD. Finally, we explore the relationships between pro-oxidants and AMD.

A. OXIDATIVE STRESS AND AGING

Aging has been defined as " the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age."[95] The free radical theory of aging and the evolutionary theory of aging are of particular relevance to our discussion.

1. The Free Radical Theory of Aging

The free radical theory of aging proposes that aging and age-related disorders are the result of cumulative damage arising from reactions involving ROIs. This theory is particularly enticing, as it explains many age-related phenomena, such as the relationship between longevity and basal metabolic rate, the clustering of degenerative disorders in the end-stages of life, the beneficial effect of caloric restriction on life-span and the greater longevity of females.[95] Age-related oxidative damage has been demonstrated in collagen, elastin, mucopolysaccharides and nuclear and mitochondrial DNA, and lipid peroxidation has been shown to contribute to lipofuscinogenesis.[39,124,143,203] Furthermore, there is an age-related rise in systemic oxidant load, and age-related morbidity is associated with low antioxidant defenses.[109,122,164,179]

2. The Evolutionary Theory of Aging

The evolutionary theory of aging proposes that there is a decline in the force of natural selection with increasing age, and that we may have evolved with genes which promote senescence once we have passed our period of procreation.[84] In other words, we do not eliminate genes that have a detrimental effect in later life if they have a beneficial effect, or no effect, in early life. The genetic basis of longevity has been dramatically demonstrated in *C. elegans*, where a mutation of the *age-1* locus results in a 70% increase in mean life-span and a 110% increase in maximum life span.[72,73]

The free radical and evolutionary theories of aging are compatible. Transgenic flies (*Drosophilia melanogaster*) with simultaneous overexpression of the copper-zinc superoxide dismutase (Cu/Zn SOD) and catalase genes, the main defense systems against oxidative stress, exhibited as much as one-third extension of life-span, a prolonged mortality rate doubling time and delayed loss of physical performance, as compared with control flies.[159] These findings represented the first direct evidence of a causal link between oxidative stress and aging and age-related disorders.

3. Aging and Ocular Senescence

The eye is not an isolated organ, but rather one of many systems subject to the processes of aging. It is known that mortality, blindness, and other conditions tend to increase exponentially with age.[217] It does not follow, however, that morbidity is the inevitable result of normal aging, as the former is sporadic, whereas the latter is universal.

If the free radical theory of aging applies to the eye, an altered antioxidant/oxidant balance should be evident for age-related ocular diseases, such as AMD, cataract and glaucoma.[218] The Lens Opacities Case-Control Study found multivitamin supplements and/or higher levels of dietary antioxidant index to be protective for all types of cataract,[129,130] and higher plasma levels of vitamin E were inversely related to risk of nuclear opacities in the Longitudinal Study of Cataract.[128] Oxidative damage has also been hypothesized to play a role in the pathogenesis of glaucoma, as the trabecular meshwork is exposed to high levels of oxidative stress arising from aerobic metabolism, high aqueous concentrations of hydrogen peroxide and photochemical reactions in the anterior segment. A recent study has demonstrated a decline in the specific activity of human trabecular superoxide dismutase, but not catalase, with increasing age, thus supporting the view that oxidative stress may be etiologically involved in primary open angle glaucoma.[50]

B. ANTIOXIDANT STATUS AND AMD

Much of the research into the relationship between oxidative stress and AMD has focused on the the antioxidant status of subjects with and without the disease, and the limitations of these studies warrant comment. The cross-sectional epidemiologic studies investigating dietary intake of antioxidants do not lend themselves to consistent and reliable data because of bias and confounding.[33,197] Sources of bias include selection of subjects and recall bias

with respect to nutritional intake. Confounding refers to the mixing of effects, which results in masking of true associations. For example, the National Health and Nutrition Examination Survey (NHANES) finding that a diet rich in fruit and vegetables protects against age-related macular disease may simply reflect the fact that people with better diets take better care of themselves.[77] In other words, the observational nature of the epidemiological studies does not allow us to establish whether or not the observed association is causative. Furthermore, evaluation of dietary intake by questionnaire takes no account of the digestive and absorptive idiosyncracies of the individual or of tissue availability of the nutrient under investigation. Serum levels of antioxidant substances of dietary origin are also of limited value, because they reflect recent nutritional intake only, and this is especially important for substances that have a low biological turnover, such as macular pigment.

With full appreciation of these limitations, each antioxidant will be discussed in relation to AMD and/or retinal light damage. In order to present a balanced view, we take care to refer to all studies that have investigated the antioxidant under discussion.

1. Vitamin C

Ascorbate is the most effective aqueous-phase antioxidant in human blood,[71] and it is thought to be essential for protection against disease processes and degenerative disorders caused by oxidative stress.[95]

a. Vitamin C and Light Damage

Organisciak et al injected cyclic light and dark–reared weanling albino rats with L-ascorbic acid, ascorbate derivatives, or a water vehicle (control animals), and then exposed the animals to intense visible light.[158] In terms of pre-exposure rhodopsin levels, L-ascorbate, Na-ascorbate, and dehydroascorbate were found to protect cyclic light and dark–reared rats against rod cell loss (57–62% versus 38% of control) in a dose-dependent manner, but no such protective effect was found for D-ascorbate. Further, vitamin C supplements resulted in raised retinal ascorbate levels and were protective only if given prior to light exposure. And finally, ascorbate supplements were associated with preservation of rod outer segement docosahexaenoic acid, suggesting that it is the antioxidant properties of the vitamin C that account for its protective effect.[158]

b. Dietary Vitamin C and AMD

The NHANES reported that a diet containing high quantities of foodstuffs rich in vitamins A and C was negatively associated with AMD.[77] The Eye Disease Case Control Study (EDCC) and the Beaver Dam Eye Study (BDES) also reported a protective effect associated with high intake of oral vitamin C, but the association was not statistically significant in these latter studies.[187,213] The Blue Mountains Eye Study (BMES), however, found no such protective effect for dietary ascorbate.[192] The inconsistency of the findings suggests that the putative protective effect of dietary ascorbate may be too weak to identify in the sample sizes reported, and such an effect cannot be dismissed until larger studies are undertaken.

c. Plasma Vitamin C and AMD

The EDCC Study reported that low plasma levels of vitamin C were associated with increased risk of AMD, but high levels were not found to be protective. Further, an antioxidant index, comprising plasma carotenoids, selenium, ascorbate, and vitamin E, was inversely related to AMD.[1] The Baltimore Longitudinal Study of Aging (BLSA) reported a nonsignificant protective effect associated with the highest quintile of plasma vitamin C levels versus the lowest quintile (odds ratio: 0.55), and observed a significant protective effect for an antioxidant index which included α-tocopherol, ascorbate, and β-carotene.[222] These findings were not reproduced in the POLA Study, a population-based investigation of antioxidant status and age-related ocular disease among 2157 subjects aged 60 years or older, which failed to identify an inverse association between plasma ascorbate and AMD.[54]

2. Vitamin E

Vitamin E is the major chain-breaking antioxidant of cellular membranes.[35,161,162] It exists in four common forms, including α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol.[57] Of these, α-tocopherol is the most effective scavenger of free radicals[25] and the most predominant tocopherol in human retina and plasma.[5,93] Selenium, a micronutrient, complements the antioxidant function of vitamin E.[35]

a. Vitamin E and Retinal Light Damage

The retina contains high quantities of α-tocopherol (in the rod outer segments) and RPE,[74,101] and the concentrations within these tissues are very sensitive to dietary intake of the vitamin.[198]

Organisciak et al found that the RPE content of vitamin E, which was 4 to 7 times that of the neural retina, rose with increasing age, and speculated that this rise was in response to increasing oxidative stress.[152] It has also been noted that the central neural retina closely regulates its vitamin E content,[41] and analogies with the carotenoids of macular pigment are inescapable.[94,193,194] Interestingly, carotenoids and α-tocopherol can act synergistically as radical scavengers.[163]

The concentration of vitamin E in the rhesus monkey retina-RPE-choroid reaches a maximum at the fovea and at eccentricities of 1.0 mm or more and declines to a minimum near the foveal crest.[40] It has been postulated that this minimum α-tocopherol concentration in the region of the foveal crest results in an area of vulnerability, thus accounting for the frequent occurrence of atrophic AMD at this site.[183]

Evidence in support of the concept that vitamin E protects against retinal oxidative damage includes: 1) vitamin E deficiency results in retinal degeneration,[96] excessive RPE lipofuscin,[96] and a decrease in the PUFA content of rod outer segments and the RPE;[63] 2) in vitro experiments have shown that bovine rod outer segments challenged with oxygen exhibit substantial destruction of membrane structure, and that this oxidative damage can be limited by high levels of endogenous vitamin E;[62] and 3) dark-rearing, which results in increased vulnerability to retinal light damage,[15] is associated with reduced ascorbate and vitamin E levels in the rat retina.[166] It should be noted, however, that some researchers have failed to demonstrate that vitamin E and selenium protect against photochemical damage of the retina.[111,200]

In brief, if retinal vitamin E does protect against photochemical damage, its role is likely to be a complex one. Further study, which takes into account the interrelationships between vitamin E and other retinal antioxidants, such as ascorbate, lutein, and zeaxanthin, as well as vitamin A, is indicated.

b. Dietary Vitamin E and AMD

Dietary intake of vitamin E is very difficult to estimate, because the long-term consumption of oils, in which the α-tocopherol concentration varies considerably, is difficult to determine by questionnaire. For example, people frequently change their brand of cooking oil, and are unaware of the type used in the processed and ready-made foods they purchase. Further, the bioavailability of vitamin E in supplements varies considerably.[4] Future studies should endeavor to minimize the impact of such measurement error by including large numbers of subjects, and they should address other important issues, such as the source of the vitamin compound (α-tocopherol is more commonly found in supplements than γ-tocopherol), interactions between the various tocopherols,[93] and consumption of polyunsaturated fatty acids.[44]

To our knowledge, only the BDES and the EDCC study have investigated the relationship between dietary intake of vitamin E and AMD. The BDES reported a significantly increased risk of large macular drusen associated with the lowest versus highest quintile of past dietary intake of vitamin E (odds ratio: 0.4; P = 0.04).[213] However, the significance of this relationship was lost if total vitamin E intake was considered (diet and supplements) or if recent dietary intake (within 5 years of onset of disease) was studied. The EDCC found no significant inverse association between dietary intake of vitamin E, with or without supplements, and neovascular AMD.[187]

c. Plasma Vitamin E and AMD

The POLA Study represents the most recently published and the most extensive investigation of the relationship between plasma α-tocopherol and age-related macular degeneration.[54] Fasting blood levels of vitamin E, after multivariate adjustment, showed a weak negative association with AMD (P = 0.07). Lipid standardized plasma α-tocopherol had a significant inverse relationship with early (P = 0.04) and late (P = 0.003) AMD, representing a risk reduction for AMD of 82% for those in the highest quintile versus the lowest quintile. In order to correct for confounding, which arises from the fact that atherosclerosis is related to α-tocopherol[169] and is probably also related to age-related macular disease,[215] the investigators adjusted for variables related to cardiovascular disease, and the significant inverse association remained unchanged. The POLA study findings are consistent with those of the BLSA and the BDES, although the statistical significance of the protective effect of serum α-tocopherol in the BDES was lost after adjusting for serum lipids.[139,222] Although the EDCC and the BMES found no significant associations between plasma levels of vitamin E and AMD, it should be noted that the former did detect a significant protective effect for a serum antioxidant index which included α-tocopherol and the latter study comprised only a small number of patients (N = 156).[1,191]

3. Vitamin A

Vitamin A (retinol) is essential for vision, as it must be available in the retina as a precursor of 11-*cis*-retinal for the regeneration of rhodopsin. Vitamin A exists in the following three oxidation states: an alcohol (retinol), an aldehyde (retinal), and an acid (retinoic acid). Until recently, there was no evidence of retinol's antioxidant activity in photoreceptor cells. In an experiment reported by Keys et al, physiological concentrations of retinol added to liposomes composed of rod cell phospholipids were found to protect the lipids from oxidation.[114] Therefore, as the chain reaction of lipid peroxidation within the membrane is broken, a small amount of retinol may protect large concentrations of membrane lipids. Vitamin A is also involved in the repair of cells that have been oxidatively damaged.[35] Of note, in the retina, vitamin E is believed to protect vitamin A from oxidative degeneration.[177]

a. Dietary Vitamin A and AMD

The NHANES reported a 40% reduction in risk for AMD in persons who consumed foods rich in vitamin A at least once per day, as compared with those who ate these foods less than once per week (Odds ratio: 0.59; confidence interval: 0.37–0.99).[77] However, this protective effect of dietary vitamin A has not been confirmed in subsequent reports, and[17,187,192,213] it is possible that the NHANES finding simply represents a protective effect of dietary carotenoids, which are found in the same foods.[195] This hypothesis is consistent with the findings of the BDES, which failed to detect a significant association between total dietary vitamin A (pro-vitamin A carotenoids plus retinol), or simply dietary retinol, and early AMD, but did detect a weak inverse relationship between past consumption of pro-vitamin A carotenoids and early AMD (odds ratio: 0.29) and a significant inverse relationship between pro-vitamin A carotenoids and the presence of large drusen.[213] In other words, the protective effect of pro-vitamin A carotenoids could not be attributed to vitamin A per se.

b. Plasma Vitamin A and AMD

With respect to plasma retinol and AMD, the POLA study failed to detect a significant association.[54]

4. Carotenoids

Carotenoids are naturally occurring pigments that are essential to photosynthetic organisms, as they capture radiant energy.[120] In mammals, these compounds are entirely of dietary origin. There are between 40 and 50 carotenoids in a typical Western diet,[115,116] of which 34 have been identified in human milk and serum.[118] Some carotenoids can be converted to vitamin A and are, therefore, said to have pro-vitamin A activity.

The antioxidant properties of the carotenoids are now well established and include the ability to quench singlet oxygen and triplet sensitizers,[38,68,121,151,210] interact with free radicals[18,24,99,108,121] and prevent lipid peroxidation.[7,132,160,214,232] It has been shown that a carotenoid's antioxidant activity is enhanced at low oxygen tension[108] and that the carotenoids interact with other antioxidants.[59] For example, the carotenoids appear to protect or repair α-tocopherol[59] and to act synergistically with vitamin C in protecting against oxidative damage.[160]

Of the 34 carotenoids identified in human serum, only lutein and zeaxanthin are found in the retina where they are collectively known as *macular pigment*.[193] The concentration of lutein and zeaxanthin peaks at the center of the fovea, diminishes with eccentricity and is optically undetectable a distance of 1.2 to 1.5 mm from the foveola.[90,194] Macular pigment is contained mainly within the photoreceptor axons of the receptor axon layer, but is also seen in relatively high concentrations in the interneurons of the inner plexiform layers.[193] In common with all mammalian carotenoids, macular pigment is entirely of dietary origin and can be augmented with appropriate dietary modification.[88,126,127]

Direct oxidation products of lutein and zeaxanthin have been reported in human retina, indicating that these carotenoids do act as antioxidants in the macula.[117] The inhibition of lipid peroxidation is desirable in the retina, not least because of the high concentration of polyunsaturated fatty acids in the photoreceptor membranes.

Macular pigment is also believed to limit retinal oxidative damage by filtering out blue light. We have already discussed photochemical damage of the retina, and we have emphasized that the threshold for damage is lowest for the blue region of the visible spectrum. The absorbance spectrum of macular pigment peaks at 460 nm,[165] and it has been calculated that the macular carotenoids reduce the amount of blue light incident on the photoreceptors of the fovea by approximately 40%.[193] This filtering effect reduces chromatic aberration,[176] and short wavelength sensitivity. It is also believed to be responsible for the relative preservation of foveal short-wavelength cone sensitivity with age.[80]

a. Dietary Carotenoids and AMD

It has been shown that dietary intake of lutein and zeaxanthin is positively related to the optical density of macular pigment for males only and to serum levels of these carotenoids for both genders.[86] However, studies investigating dietary carotenoid intake should be interpreted with caution, because there are so many confounding variables, such as the individual's absorptive and digestive characteristics. Furthermore, there is still a lack of sufficient data regarding the carotenoid content of foods.

The EDCC study found that a high dietary intake of carotenoids protected against AMD.[187] After adjusting for other risk-factors, the highest quintile of carotenoid intake was associated with a 43% reduction in risk for AMD (odds ratio: 0.57; confidence interval: 0.35–0.92; P for trend: 0.02). Interestingly, of the carotenoids, lutein and zeaxanthin were found to be the most protective. The BDES, which included a prospective arm, reported that past intake of α-carotene, β-carotene and pro-vitamin A carotenoids, as well as baseline intake of pro-vitamin A carotenoids, was associated with a significantly reduced risk of large drusen at 5 years follow-up. However, no such protective effect for dietary intake of lutein and zeaxanthin was detected.[213]

The two other studies investigating carotenoid consumption and AMD considered β-carotene only,

and failed to detect a significant protective effect associated with this compound.[192,222] It should be emphasized here that β-carotene is not found in human retina.

b. Serum Carotenoids and AMD

Serum levels of carotenoids are less problematic for epidemiologic studies because they obviate the need to calculate the carotenoid content of foods,[138] and they circumvent the problem of interindividual variability in digestive and absorptive characteristics.[29] However, the main limitation of such blood levels rests on the fact that the concentrations at the time of sampling reflect only recent nutritional intake. As macular pigment has a very slow turnover,[126] recent dietary intake and current serum levels of these carotenoids are of limited value.

The EDCC study reported a significantly decreased risk of neovascular AMD associated with medium and high serum levels of carotenoids, which was calculated as the sum of lutein, zeaxanthin, α-carotene, β-carotene, cryptoxanthin, and lycopene, compared with those in the low group.[2] Further, high levels of the individual retinal carotenoids, lutein, and zeaxanthin, were also found to be protective.[1] The BDES, which included a smaller number of patients (BDES: N = 167; EDCC: N = 421), did not corroborate these findings, but did report that low levels of lycopene were associated with increased risk of AMD.[139] The BLSA and the BMES measured only serum β-carotene, which is not found in the retina, and failed to detect a protective effect associated with high serum levels of this carotenoid.[191,222]

c. Macular Pigment and AMD

Unfortunately, investigations into the relationship between macular pigment and AMD have been limited to observational data correlating measurements of the pigment to risk of developing the disease.

In 1988, Bone et al quantified macular lutein and zeaxanthin by high-performance liquid chromatography in 87 human eyes from donors ranging in age from 3 to 95 years and observed no dependence on age for either carotenoid.[19] These findings were consistent with those of Werner et al.[221] However, it has since been shown that several factors influence the optical density of macular pigment, including smoking habits,[92] iris color,[87] lens density,[91] and gender.[86] In a recent study in which we have corrected for these confounding variables, we found a significant inverse relationship between macular pigment optical density and age in 46 healthy caucasian subjects ranging in age from 21 to 80 years ($r = 0.446$; $P = 0.003$; unpublished data). Our finding, therefore, supports the view that declining macular pigment may be associated with increasing risk for AMD.

Of the other putative risk-factors for age-related macular disease, light iris color, tobacco use, female gender, and increasing lens density are all associated with low macular pigment optical density.[86,87,91,92] Interestingly, an increased oxidant load and reduced antioxidant defenses have been linked to cigarette smoking,[147] and light iris color transmits substantially more light to the retina.[211] The inverse relationship between lens density and macular pigment optical density suggests that oxidative stress may represent a common pathogenesis for cataract and AMD, as the crystalline lens and the macula both accumulate lutein and zeaxanthin to the exclusion of all other carotenoids and, therefore, may share an uptake mechanism.[106,129,229] In other words, an individual who accumulates large quantities of lutein and zeaxanthin in the crystalline lens and the retina is less likely to develop cataract and AMD. This hypothesis is supported by observations that cataract is associated with increased risk of AMD.[30,133]

Further evidence that macular pigment may protect against AMD was provided by Weiter et al, who demonstrated that the pattern and extent of foveal sparing in annular macular degeneration, such as atrophic AMD, closely matched the distribution of the pigment.[219] Moreover, it has been shown that the expected age-related decline in photopic sensitivity, which is also an early feature of AMD,[8,60] is not seen in older subjects with high quantities of macular pigment.[89]

And finally, Landrum et al used HPLC to measure lutein and zeaxanthin in 22 eyes with early AMD and 15 healthy human donor eyes; they found significantly lower quantities of these carotenoids in the macula and whole retina of the diseased eyes.[127] The investigators speculated that, since the decrease in lutein and zeaxanthin was noted across the retina and not simply at the site of disease, low macular pigment is causally important in the pathogenesis of AMD and not simply the consequence of the pathological process.

5. Antioxidant Enzymes

Superoxide dismutase, catalase, and glutathione peroxidase are antioxidant enzymes that form part of the complex system that protects the retina from oxidative damage, and all three enzymes are found in the photoreceptors and in the RPE.[10,11,173]

a. Superoxide Dismutase

Superoxide dismutase (SOD) catalyzes the quenching of the superoxide anion to produce hydrogen peroxide and oxygen (Fig. 2).[145] The SODs are metalloproteins, some that contain manganese (Mn-SOD) and others that contain copper and zinc (CuZn-SOD). In the POLA study, no association could be detected between systemic SOD activity and AMD.[53]

De La Paz et al measured SOD activity in central and peripheral neurosensory retina of healthy human eyes from donors ranging in age from 7 to 85 years and found no age-related decline in the activity of this enzyme at the macula.[51] Frank et al, using a polyclonal antibody to bovine erythrocyte CuZn-SOD, measured superoxide dismutase immunoreactivity in the macular RPE of 19 human donor eyes without pathology and a similar number of eyes with choroidal neovascular membranes (CNVM).[70] A positive relationship between age and cytoplasmic and lysosomal CuZn-SOD immunoreactivity was demonstrated for eyes in both groups, but this relationship achieved statistical significance only in the case of cytoplasmic levels of the enzyme in eyes with a CNVM. Liles et al found that total RPE-SOD activity was statistically comparable for eyes with and without AMD, and was unrelated to age.[131] It appears, therefore, that retinal SOD does not play an overtly important role in protection against AMD beyond its contribution to the overall local antioxidant defense system.

b. Glutathione, Glutathione Peroxidase and Glutathione Reductase

Glutathione is a water-soluble tripeptide endogenous to mammalian photoreceptor outer segment, which scavenges oxidizing agents by reacting with them. Glutathione is found in bovine retina,[97] where it has been shown to protect photoreceptor PUFAs from oxidation independently of vitamin E or retinol.[114] The POLA Study has failed to detect a significant inverse association between red blood cell glutathione and AMD.[54]

Glutathione peroxidase (glutathione-Px) uses glutathione as an electron donor to reduce organic hydroperoxides (Fig. 2).[13] It is found in human retina and is dependent on selenium as a cofactor.[190] The POLA study analyzed the relationship between antioxidant enzymes and age-related macular disease in 2156 subjects and found that higher plasma levels of glutathione-Px were significantly associated with a nine-fold increase in the prevalence of late AMD, but were unassociated with early AMD.[53] This finding suggests that glutathione-Px is one of the strongest indicators of AMD ever identified. As plasma glutathione-Px consists of the extracellular form of the enzyme,[136] the biologic meaning of the POLA study's finding has yet to be determined. It is worth noting, however, that extracellular glutathione-Px is found in retina, ciliary epithelium, and aqueous humor, and is believed to act as an extracellular antioxidant.[36] It has been postulated, therefore, that increased oxidative stress associated with AMD results in upregulation of plasma glutathione-Px activity.

With respect to retinal glutathione-Px, De La Paz et al found no effect of age on the specific activity of this enzyme in normal human retinas,[51] although reduced activity of glutathione-Px has been observed in retinal homogenates of cynomolgus monkeys with early onset AMD.[148]

Glutathione reductase does not act directly as an antioxidant, but it is required for regeneration of glutathione (Fig. 2). AMD is associated with significantly reduced levels of plasma glutathione reductase,[37] although there is no correlatlion between severity of disease and RBC glutathione-reductase activity.[52]

c. Catalase

Catalase is an iron (Fe)-dependent enzyme that scavenges H_2O_2 either catalytically or peroxidatively.[31,82] Catalase has been demonstrated in human neurosensory retina and RPE.[51,70,131]

RPE catalase activity is significantly reduced in eyes with AMD, and there is an age-related decline in its activity.[178] Tate et al observed an indistinguishable two-fold increase in RPE catalase activity in response to a challenge with rod outer segments or exogenous H_2O_2, suggesting that phagocytosis of the rod outer segments by the RPE is an oxidative stress which probably produces H_2O_2.[205] This ROI is believed to act as an intracellular signal that induces an increase in activity of key antioxidant enzymes, such as catalase.

Significantly lower catalase activity has been reported in the retinal homogenates of cynomolgus monkeys with early onset macular degeneration, as compared with normal relatives and unrelated controls, but, consistent with human studies,[52] no significant difference was observed between the two groups in terms of systemic catalase activity.[149] Frank et al confirmed the inverse relationship between age and activity of RPE catalase, but found no difference in the RPE content of this enzyme for eyes with and without AMD. They concluded that it is probably unrelated to the pathogenesis of age-related macular disease.[70] Of note, there is no age-related decline in catalase activity of the central or peripheral neurosensory retina.[51]

In brief, therefore, the published literature regarding the relationship between retinal and systemic catalase activity and age-related macular disease is inconsistent, although it appears that this enzyme is unlikely to play a disproportionately important role beyond its function as a component of the antioxidant defense system of the retina. Of note, there is no characteristic ocular pathology associated with homozygous catalase deficiency.[12]

6. Zinc

Zinc (Zn) is the most abundant trace element in the human eye.[110] It is thought to play an important role in mammalian antioxidant defenses, because it

acts as a cofactor for CuZn-SOD[142] and is involved in the regulation of catalase activity.[204] Furthermore, zinc induces the synthesis of metallothionein, a known scavenger of hydroxyl radicals,[185] and stabilizes membrane lipids against oxidation.[209]

Although there is a plausible rationale in support of the view that zinc protects against age-related macular disease, the clinical and epidemiologic evidence is less convincing. The EDCC and the BDES failed to detect a significant relationship between serum zinc levels and the risk of AMD.[2,139] The retrospective arm of the BDES did, however, detect a weak protective effect for the highest quintile of dietary zinc intake, as compared with the lowest quintile (odds ratio: 0.6),[141] but this was confirmed only for pigmentary macular changes in the prospective arm of that study.[213] The BMES found no significant relationship between AMD and dietary intake of zinc among 2900 participants.[192]

Stur et al conducted a 2-year, double-blind, randomized, placebo-controlled trial of zinc supplements in 112 subjects with neovascular AMD in one eye.[201] Although zinc supplements were associated with raised serum levels of the trace element at final follow-up, they were not found to affect the clinical course of the condition in a benefical way.

7. Metallothionein

Metallothionein is an acute-phase stress protein that participates in the detoxification of heavy metals and the maintenance of zinc and copper homeostasis[218] and scavenges hydroxyl radicals.[98,185] It has been demonstrated in human RPE, where it is in lower concentration in the macular region than in the periphery.[206] Further, there is an age-related decline in the macular RPE content of metallothionein.[206]

In 1995, Tate et al induced metallothionein gene expression in cultured RPE cells by addition of H_2O_2 and by challenge with photoreceptor outer segments, thus confirming that phagocytosis is an oxidative stress and that mettalothionein acts as an antioxidant.[205] The authors postulated, therefore, that the age-related decline in macular RPE metallothionein may result in an inability to mount a sufficient defense against oxidative stress and, thus, contribute to AMD. This hypothesis is supported by the observed reduction in metallothionein synthesis in retinal homogenates of cynomolgus monkeys with early AMD.[148]

C. PRO-OXIDANTS AND AMD

1. Fatty Acids

To our knowledge, there are only three published studies that have investigated the relationship between dietary intake of fats and age-related macular disease.[140,182,186] The BDES and the EDCC found that a high intake of polyunsaturated fatty acids was associated with a higher risk of early and late AMD, respectively, but the relationship was significant only in the EDCC.[140,186] A case-control study comparing plasma levels of polyunsaturated fatty acids in 65 patients with AMD and 65 control subjects found no statistically significant difference between the two groups.[182]

2. Light and AMD

To our knowledge, there have been five epidemiological studies investigating the relationship between exposure to sunlight and the risk of age-related macular disease.[2,42,46,102,208,223] Of these, the three case-control studies failed to establish a significant association.[2,46,102] The cohort studies, however, did detect a significantly increased risk of AMD in association with higher cumulative lifetime exposure to sunlight.[42,46,207,208] The consistency of the cohort studies does suggest a causal link for sunlight, because cohort epidemiologic studies are less prone to bias than case-control studies.[144] As the entire population is potentially exposed to sunlight, the odds ratios of 1.36 and 2.19 for high exposure to blue or visible light and AMD represent quite robust evidence in support of the sunlight/AMD hypothesis.[42,208]

3. Lipofuscin and AMD

It has been shown that age, the strongest risk factor for ARM and AMD, correlates strongly with RPE lipofuscin content.[65] Indeed, 19% of RPE intracellular space is occupied by lipofuscin in 81–90-year-old subjects, compared with just 1% in the first decade of life.[67] Also, massive quantities of lipofuscin have been demonstrated in the RPE cells of eyes with atrophic AMD.[183] Furthermore, RPE cells that contain excessive amounts of *N*-retinylidene-*N*-retinylethonalamine exhibit cell membrane blebbing and cytoplasm extrusion into Bruch's membrane,[61] processes that may contribute to drusen formation[104,105,220] and the development of AMD.[66]

4. Waste Products of Oxidative Processes in AMD

Olin et al measured plasma thiobarbituric acid reactive substances, an estimate of lipid peroxides, in the plasma of 62 elderly rhesus macaques, and found that its circulating levels were significantly higher in monkeys with > 10 drusen, compared with those without drusen.[150] Further direct evidence of a role for oxidative process in age-related macular disease has been recently published by Hammes et al.[85] N^{ϵ}-(carboxymethyl) lysine (CML), which is a product of either lipoprotein peroxidation or sequential glycation and oxidation, is a biomarker of oxidant stress in a tissue. CML was found in 11 of 11 human subfoveal age-related neovascular membranes, but it was not identified in the healthy retina of a donor eye. Further, CML is present in soft drusen and adjacent RPE cells before the development of choroidal

neovascularization, but not in control eyes.[103] Although the implication of these findings warrants further exploration, it is consistent with an etiologic role for oxidative stress in the pathogenesis of AMD.

V. Current Research and Future Perspectives

The National Eye Institute has set up the Age-Related Eye Disease Study, which is evaluating the role of antioxidant supplements on the natural history of AMD in a randomized, placebo-controlled clinical trial involving 4753 patients in 11 centers.[33] Recruitment ended in August 1997 and a minimum follow-up of 5 years is required.[33] The Physicians Health Study, sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health, is to utilize funding from the National Eye Institute to evaluate the effect of β-carotene (50 mg on alternate days) in a randomized, placebo-controlled trial involving 22,071 male physicians with a minimum follow-up of 12 years.[3] The Women's Health Study will also evaluate the impact of antioxidant supplements on the risk of developing age-related macular disease, but in postmenopausal female healthcare workers.[23]

Although the Age-Related Eye Disease Study, the Physicians Health Study, and the Women's Health Study will provide valuable information regarding the benefits and toxic effects of antioxidant supplements with respect to age-related macular disease, they will not directly investigate the putative role of oxidative stress in the pathogenesis of AMD. For example, a failure of antioxidant supplements to protect against age-related macular disease may simply reflect the poor diet–plasma correlation of carotenoids that has recently been identified in healthy persons aged over 65 years.[28] Also, the various antioxidants have different chemical properties and interact in a synergistic fashion, suggesting that the maintenance of an effective antioxidant environment in a nonhomogenous biological state depends on adequate concentrations of several antioxidants in the relevant tissues.[58] In other words, the absence of a beneficial effect of dietary antioxidant supplements does not exclude an etiopathogenic role for oxidative stress in age-related macular disease.

Beyond the enhancement of antioxidant defenses, the oxidant/antioxidant balance may also be altered favorably by reducing oxidative stress. In a recent study, for example, urinary indices of lipid peroxidation increased significantly in 10 healthy, nonsmoking male volunteers (mean age ± SD: 32.6 ± 1.7 years) after consumption of a diet high in PUFAs, and decreased significantly after a diet deficient in PUFAs, indicating that dietary restriction of polyunsaturated fatty acids may limit oxidative stress.[107] In theory, prereceptorial absorption of blue light by incorporating narrow-band yellow filters into spectacles, contact lenses, or intraocular lenses may be another means of limiting retinal oxidative stress. Clearly, however, such measures cannot be justified on the basis of current evidence.

VI. Summary

There is no shortage of research into the relationship between oxidative stress and AMD, but firm evidence of a causal link is still lacking. Certainly, the concept that AMD is the result of oxidative damage is provocative, because it is biologically plausible. To date, research has focused on either the antioxidant status of subjects with age-related macular disease or the pathologic findings associated with laboratory-based, experimentally-induced oxidative stress.

The published experimental data provide firm evidence of oxidative damage occurring in the retina and RPE, but the relationship between these events and the onset of AMD remains unclear. The main limitation of the in vitro techniques rests on the fact that these studies tend to focus on too few variables, and we are hopeful that the development of in vitro models of retinal oxidative processes will make a substantial contribution to this area of research.[34,202] The identification of benzidine-reactive substances by Kayatz et al also represents an advance in this field and will enable investigators to study the formation, decomposition, and transportation of lipid peroxides in the retina at an ultrastructural level for the first time.[112]

In conclusion, the role of oxidative stress in the pathogenesis of AMD is biologically plausible, but remains unproven. The task of confirming or refuting a causative link between tissue damage by ROI and the onset or progression of AMD, or indeed any disease, is a challenging one. Ultimately, complex models simulating in vivo conditions will be required to investigate the relative importance of the various reactions involving free radicals, hydrogen peroxide and singlet oxygen.

Method of Literature Search

References for this review were identified through a comprehensive literature search of the electronic MEDLINE database (1966–1999), and included where appropriate. The literature search was not confined to the English language, and relevant non-English language publications were translated. Additional articles, textbooks, and abstracts, which were unavailable on electronic archives, were selected from review of the bibliographies of the articles generated from the above search. To ensure the up-to-date nature of our review article, current issues of

Archives of Ophthalmology, *Survey of Ophthalmology*, *American Journal of Ophthalmology*, *Ophthalmology*, *British Journal of Ophthalmology*, *Experimental Eye Research*, *Current Eye Research*, and *Investigative Ophthalmology and Visual Sciences* were regularly reviewed throughout the period of writing. The following key words and combinations of these words were used in compiling the search: *age-related macular degeneration; antioxidants; catalase; free radicals; glutathione; hydrogen peroxide; light damage; lutein; macular pigment; metallothionein; oxidative stress; retinal pigment epithelium; singlet oxygen; superoxide dismutase; zeaxanthin; zinc.*

References

1. Antioxidant status and neovascular age-related macular degeneration. The Eye Disease Case-Control Study Group [published errata appear in Arch Ophthalmol 1993 Sep; 111(9):1228, 1993 Oct;111(10):1366 and 1993 Nov; 111(11):1499]. Arch Ophthalmol 111:104–9, 1993
2. Risk factors for neovascular age-related macular degeneration. The Eye Disease Case-Control Study Group. Arch Ophthalmol 110:1701–8, 1992
3. Final report on the aspirin component of the ongoing Physicians Health Study. Steering Committee of the Physicians Health Study Research Group [see comments]. N Engl J Med 321:129–35, 1989
4. Acuff RV, Thedford SS, Hidiroglou NN, et al: Relative bioavailability of RRR- and all-rac-alpha-tocopheryl acetate in humans: studies using deuterated compounds [see comments]. Am J Clin Nutr 60:397–402, 1994
5. Alvarez RA, Liou GI, Fong SL, et al: Levels of alpha- and gamma-tocopherol in human eyes: evaluation of the possible role of IRBP in intraocular alpha-tocopherol transport. Am J Clin Nutr 46:481–7, 1987
6. Ames BN, Gold LS: Endogenous mutagens and the causes of aging and cancer. Mutat Res 250:3–16, 1991
7. Anderson SM, Krinsky NI: Protective action of carotenoid pigments against photodynamic damage to liposomes. Photochem Photobiol 18:403–8, 1973
8. Applegate RA, Adams AJ, Cavender JC, Zisman F: Early color vision changes in age-related maculopathy. Appl Opt 26:1455–7, 1987
9. Arstila AU, Smith MA, Trump BF: Microsomal lipid peroxidation: morphological characterization. Science 175:530–3, 1972
10. Atalla LR, Sevanian A, Rao NA: Immunohistochemical localization of glutathione peroxidase in ocular tissue [published erratum appears in Curr Eye Res 1989 Feb;8(2): 229]. Curr Eye Res 7:1023–7, 1988
11. Attala F, Fernandez MA, Rao NA: Immunohistochemical localization of catalase in ocular tissue. Curr Eye Res 6: 1181–7, 1997
12. Ayebi, HE,. Wyss SR. Acatalasemia, in Stanbury JB, Wyngaarden JB, Frederickson DS (eds): The Metabolic Basis of Inherited Disease. New York, McGraw-Hill, 1978, pp 1792–1807
13. Balin AK, Allen RG: Mechanisms of biologic aging. Dermatol Clin 4:347–58, 1986
14. Bazan NG: The metabolism of omega-3 polyunsaturated fatty acids in the eye: the possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. Prog Clin Biol Res 312:95–112, 1989
15. Birch DG, Jacobs GH: Light-induced damage to photopic and scotopic mechanisms in the rat depends on rearing conditions. Exp Neurol 68:269–83, 1980
16. Bird AC, Bressler NM, Bressler SB, et al: An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 39:367–74, 1995
17. Blumenkranz MS, Russell SR, Robey MG, et al: Risk factors in age-related maculopathy complicated by choroidal neovascularization. Ophthalmology 93:552–8, 1986
18. Bohm F, Tinkler JH, Truscott TG: Carotenoids protect against cell membrane damage by the nitrogen dioxide radical [letter]. Nat Med 1:98–9, 1995
19. Bone RA, Landrum JT, Fernandez L, et al: Analysis of the macular pigment by HPLC: retinal distribution and age study. Invest Ophthalmol Vis Sci 29:843–9, 1988
20. Borish ET, Prior WA, Venuugopal S, et al: DNA synthesis is blocked by cigarette tar-induced DNA single-strand breaks. Carcinogenesis 8:1517–20, 1987
21. Boulton M, Dontsov A, Jarvis-Evans J, et al: Lipofuscin is a photoinducible free radical generator. J Photochem Photobiol B 19:201–4, 1993
22. Buege JA, Aust SD: Microsomal lipid peroxidation, in Fleischer S, Packer L (eds.), Methods in Enzymnology. New York, Academic Press, 1978, pp 302–10
23. Buring JE, Hennekens CH: The Womens Health Study: summary of the study design. J Myocardiol Ischaemia 4:27–9, 1989
24. Burton GW, Ingold KU: beta-Carotene: an unusual type of lipid antioxidant. Science 224:569–73, 1984
25. Burton GW, Ingold KU: Autoxidation of biological molecules: 1: The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J Am Chem Soc 103:6472–7, 1981
26. Bush RA, Reme CE, Malnoe A: Light damage in the rat retina: the effect of dietary deprivation of N-3 fatty acids on acute structural alterations. Exp Eye Res 53:741–52, 1991
27. Capaldi RA: Structure and function of cytochrome c oxidase. Annu Rev Biochem 59:569–96, 1990
28. Carroll YL, Corridan BM, Morrissey PA: Carotenoids in young and elderly healthy humans: dietary intakes, biochemical status and diet-plasma relationships. Eur J Clin Nutr 53:644–53, 1999
29. Carughi A, Hooper FG: Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture. Am J Clin Nutr 59:896–9, 1994
30. Chaine G, Hullo A, Sahel J, et al: Case-control study of the risk factors for age related macular degeneration. France-DMLA Study Group. Br J Ophthalmol 82:996–1002, 1998
31. Chance B, Sies H, Boveris A: Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605, 1979
32. Chen E, Soderberg P, Qian W, et al: Inhibition of cytochrome c oxidase by blue light (404 nm). A factor that causes retinal injury? Invest Ophthalmol Vis Sci 33:919–26, 1992
33. Chew EY: Nutritional supplement use and age-related macular degeneration. Curr Opin Ophthalmol 6:19–24, 1995
34. Chida M, Suzuki K, Nakanishi-Ueda T, et al: In vitro testing of antioxidants and biochemical end-points in bovine retinal tissue. Ophthalmic Res 31:407–15, 1999
35. Chow CK: Vitamin E and oxidative stress. Free Radic Biol Med 11:215–32, 1991
36. Chu FF, Esworthy RS, Doroshow JH, et al: Expression of plasma glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. Blood 79:3233–8, 1992
37. Cohen SM, Olin KL, Feuer WJ, et al: Low glutathione reductase and peroxidase activity in age-related macular degeneration. Br J Ophthalmol 78:791–4, 1994
38. Conn PF, Schalch W, Truscott TG: The singlet oxygen and carotenoid interaction [published erratum appears in J Photochem Photobiol B 1993 Jan;17(1):89]. J Photochem Photobiol B 11:41–7, 1991
39. Cortopassi GA, Shibata D, Soong NW, et al: A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. Proc Natl Acad Sci USA 89:7370–4, 1992
40. Crabtree DV, Adler AJ, Snodderly DM: Radial distribution of tocopherols in rhesus monkey retina and retinal pigment epithelium-choroid. Invest Ophthalmol Vis Sci 37: 61–76, 1996
41. Crabtree DV, Adler AJ, Snodderly DM: Vitamin E, retinyl

palmitate, and protein in rhesus monkey retina and retinal pigment epithelium-choroid. Invest Ophthalmol Vis Sci 37: 47–60, 1996
42. Cruickshanks KJ, Klein R, Klein BE: Sunlight and age-related macular degeneration. The Beaver Dam Eye Study. Arch Ophthalmol 111:514–8, 1993
43. Curtis WE, Muldrow ME, Parker NB, et al: N,N-dimethylthiourea dioxide formation from N,N-dimethylthiourea reflects hydrogen peroxide concentrations in simple biological systems. Proc Natl Acad Sci USA 85:3422–5, 1988
44. Dam, H: Interrelations between vitamin E and polyunsaturated fatty acids in animals. Vitam Horm 20:527–31, 1962
45. Dargel R: Lipid peroxidation—a common pathogenetic mechanism? Exp Toxicol Pathol 44:169–81, 1992
46. Darzins P, Mitchell P, Heller RF: Sun exposure and age-related macular degeneration. An Australian case-control study. Ophthalmology 104:770–6, 1997
47. Davies S, Boulton ME: The phototoxicity of lipofuscin in vitro. Exp Eye Res 102 (Suppl), Abstract 349.98. Proceedings of the XIII International Congress of Eye Research, Paris, 1998
48. Davis KJA: Oxidative damage and repair: Chemical, biological and medical aspects. 99. 91. Oxford/New York, Pergamon Press, 1991, pp 99–109
49. De La Paz M, Anderson RE: Region and age-dependent variation in susceptibility of the human retina to lipid peroxidation. Invest Ophthalmol Vis Sci 33:3497–9, 1992
50. De La Paz MA, Epstein DL: Effect of age on superoxide dismutase activity of human trabecular meshwork. Invest Ophthalmol Vis Sci 37:1849–53, 1996
51. De La Paz MA, Zhang J, Fridovich I: Antioxidant enzymes of the human retina: effect of age on enzyme activity of macula and periphery. Curr Eye Res 15:273–8, 1996
52. De La Paz MA, Zhang J, Fridovich I: Red blood cell antioxidant enzymes in age-related macular degeneration. Br J Ophthalmol 80:445–50, 1996
53. Delcourt C, Cristol JP, Leger CL, et al: Associations of antioxidant enzymes with cataract and age-related macular degeneration. The POLA Study. Pathologies Oculaires Liees a l'Age. Ophthalmology 106:215–22, 1999
54. Delcourt C, Cristol JP, Tessier F, et al: Age-related macular degeneration and antioxidant status in the POLA study. POLA Study Group. Pathologies Oculaires Liees a l'Age. Arch Ophthalmol 117:1384–90, 1999
55. Delmelle M: Retinal sensitized photodynamic damage to liposomes. Photochem Photobiol 28:357–60, 1978
56. Dorey CK, Wu G, Ebenstein D, et al: Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. Invest Ophthalmol Vis Sci 30:1691–9, 1989
57. Drevon CA: Absorption, transport and metabolism of vitamin E. Free Radic Res Commun 14:229–46, 1991
58. Eastwood MA: Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? QJM 92:527–30, 1999
59. Edge R, McGarvey DJ, Truscott TG: The carotenoids as anti-oxidants—a review. J Photochem Photobiol B 41:189–200, 1997
60. Eisner A, Fleming SA, Klein ML, et al: Sensitivities in older eyes with good acuity: eyes whose fellow eye has exudative AMD. Invest Ophthalmol Vis Sci 28:1832–7, 1987
61. Eldred GE, Lasky MR: Retinal age pigments generated by self-assembling lysosomotropic detergents [see comments]. Nature 361:724–6, 1993
62. Farnsworth CC, Dratz EA: Oxidative damage of retinal rod outer segment membranes and the role of vitamin E. Biochim Biophys Acta 443:556–70, 1976
63. Farnsworth CC, Stone WL, Dratz EA: Effects of vitamin E and selenium deficiency on the fatty acid composition of rat retinal tissues. Biochim Biophys Acta 552:281–93, 1979
64. Feeney-Burns L, Berman ER, Rothman H: Lipofuscin of human retinal pigment epithelium. Am J Ophthalmol 90: 783–91, 1980
65. Feeney-Burns L, Eldred GE: The fate of the phagosome: conversion to age pigment and impact in human retinal pigment epithelium. Trans Ophthalmol Soc UK 103:416–21, 1983
66. Feeney-Burns L, Gao CL, Tidwell M: Lysosomal enzyme cytochemistry of human RPE, Bruchs membrane and drusen. Invest Ophthalmol Vis Sci 28:1138–47, 1987
67. Feeney-Burns L, Hilderbrand ES, Eldridge S: Aging human RPE: morphometric analysis of macular, equatorial, and peripheral cells. Invest Ophthalmol Vis Sci 25:195–200, 1984
68. Foote CS, Denny RW: Chemistry of singet oxygen. VII Quenching by β-carotene. J Am Chem Soc 90:6233–5, 1968
69. Fox RB: Prevention of granulocyte-mediated oxidant lung injury in rats by a hydroxyl radical scavenger, dimethylthiourea. J Clin Invest 74:1456–64, 1984
70. Frank RN, Amin RH, Puklin JE: Antioxidant enzymes in the macular retinal pigment epithelium of eyes with neovascular age-related macular degeneration. Am J Ophthalmol 127:694–709, 1999
71. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377–81, 1989
72. Friedman DB, Johnson TE: A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 118:75–86, 1988
73. Friedman DB, Johnson TE: Three mutants that extend both mean and maximum life span of the nematode, Caenorhabditis elegans, define the age-1 gene. J Gerontol A Biol Sci Med Sci 43:102–109, 1988
74. Friedrichson T, Kalbach HL, Buck P, van Kuijk FJ: Vitamin E in macular and peripheral tissues of the human eye. Curr Eye Res 14:693–701, 1995
75. Gaillard ER, Atherton SJ, Eldred G, et al: Photophysical studies on human retinal lipofuscin. Photochem Photobiol 61:448–53, 1995
76. Gardes-Albert M, Ferradini C, Sekaki A, Droy-Lefaix MT: Oxygen-centred free radicals and their interactions with EGb 761 or CP 202, in Ferradini C, Droy-Lefaix MT, Christen Y (eds): Advances in Gingko Biloba Extract Research. Paris, Elsevier, 1993, pp 1–11
77. Goldberg J, Flowerdew G, Smith E, et al: Factors associated with age-related macular degeneration. An analysis of data from the first National Health and Nutrition Examination Survey. Am J Epidemiol 128:700–10, 1988
78. Gottsch JD, Bynoe LA, Harlan JB, et al: Light-induced deposits in Bruchs membrane of protoporphyric mice. Arch Ophthalmol 111:126–9, 1993
79. Gottsch JD, Pou S, Bynoe LA, Rosen GM: Hematogenous photosensitization. A mechanism for the development of age- related macular degeneration. Invest Ophthalmol Vis Sci 31:1674–82, 1990
80. Haegerstrom-Portnoy G: Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? J Opt Soc Am A Opt Image Sci Vis 5: 2140–4, 1988
81. Halliwell B: Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91(Suppl):14–22, 1991
82. Halliwell B, Gutteridge JM: The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med 8:89–193, 1985
83. Ham WT Jr, Ruffolo JJ Jr, Mueller HA, et al: Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. Invest Ophthalmol Vis Sci 17:1029–35, 1978
84. Hamilton WD: The moulding of senescence by natural selection. J Theor Biol 12:12–45, 1966
85. Hammes HP, Hoerauf H, Alt A, et al: N(epsilon)(carboxymethyl)lysin and the AGE receptor RAGE colocalize in age-related macular degeneration. Invest Ophthalmol Vis Sci 40:1855–9, 1999
86. Hammond BR Jr, Curran-Celentano J, Judd S, et al: Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. Vision Res 36:2001–12, 1996
87. Hammond BR Jr, Fuld K, Snodderly DM: Iris color and

macular pigment optical density. Exp Eye Res 62:293–7, 1996
88. Hammond BR Jr, Johnson EJ, Russell RM, et al: Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 38:1795–801, 1997
89. Hammond BR Jr, Wooten BR, Snodderly DM: Preservation of visual sensitivity of older subjects: association with macular pigment density. Invest Ophthalmol Vis Sci 39:397–406, 1998
90. Hammond BR Jr, Wooten BR, Snodderly DM: Individual variations in the spatial profile of human macular pigment. J Opt Soc Am A Opt Image Sci Vis 14:1187–96, 1997
91. Hammond BR Jr, Wooten BR, Snodderly DM: Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. Optom Vis Sci 74:499–504, 1997
92. Hammond BR Jr, Wooten BR, Snodderly DM: Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. Vision Res 36:3003–9, 1996
93. Handelman GJ, Machlin LJ, Fitch K, et al: Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. J Nutr 115:807–13, 1985
94. Handelman GJ, Snodderly DM, Krinsky NI, et al: Biological control of primate macular pigment. Biochemical and densitometric studies. Invest Ophthalmol Vis Sci 32:257–67, 1991
95. Harman D: The aging process. Proc Natl Acad Sci USA 78: 7124–8, 1981
96. Hayes KC: Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. Invest Ophthalmol Vis Sci 13: 499–510, 1978
97. Hermann H, Moses SG: Content and state of glutathione in the tissues of the eye. J Biol Chem 158:33–45, 1945
98. Hidalgo J, Giralt M, Garvey JS, et al: Physiological role of glucocorticoids on rat serum and liver metallothionein in basal and stress conditions. Am J Physiol 254:E71–8, 1988
99. Hill TJ, Land EJ, McGarvey DJ, et al: Interactions between carotenoids and the CCl3O2*radical. J Am Chem Soc 117: 8322–6, 1995
100. Holz FG, Schutt F, Kopitz J, et al: Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. Invest Ophthalmol Vis Sci 40:737–43, 1999
101. Hunt DF, Organisciak DT, Wang HM, et al: alpha-Tocopherol in the developing rat retina: a high pressure liquid chromatographic analysis. Curr Eye Res 3:1281–8, 1984
102. Hyman LG, Lilienfeld AM, Ferris FL 3d, et al: Senile macular degeneration: a case-control study. Am J Epidemiol 118: 213–27, 1983
103. Ishibashi T, Murata T, Hangai M, et al: Advanced glycation end products in age-related macular degeneration. Arch Ophthalmol 116:1629–32, 1998
104. Ishibashi T, Patterson R, Ohnishi Y, et al: Formation of drusen in the human eye. Am J Ophthalmol 101:342–53, 1986
105. Ishibashi T, Sorgente N, Patterson R, et al: Pathogenesis of drusen in the primate. Invest Ophthalmol Vis Sci 27:184–93, 1986
106. Jacques PF, Chylack LT Jr, McGandy RB, et al: Antioxidant status in persons with and without senile cataract. Arch Ophthalmol 106:337–40, 1988
107. Jenkinson A, Franklin MF, Wahle K, et al: Dietary intakes of polyunsaturated fatty acids and indices of oxidative stress in human volunteers. Eur J Clin Nutr 53:523–8, 1999
108. Jorgensen K, Skibsted LH: Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. Z Lebensm Unters Forsch 196:423–9, 1993
109. Julius M, Lang CA, Gleiberman L, et al: Glutathione and morbidity in a community-based sample of elderly. J Clin Epidemiol 47:1021–6, 1994
110. Karcioglu ZA: Zinc in the eye. Surv Ophthalmol 27:114–22, 1982
111. Katz ML, Eldred GE: Failure of vitamin E to protect the retina against damage resulting from bright cyclic light exposure. Invest Ophthalmol Vis Sci 30:3090–9, 1989
112. Kayatz P, Heimann K, Schraermeyer U: Ultrastructural localization of light-induced lipid peroxides in the rat retina. Invest Ophthalmol Vis Sci 40: 2314–21, 1999
113. Kennedy CJ, Rakoczy PE, Constable IJ: Lipofuscin of the retinal pigment epithelium: a review. Eye 9:262–74, 1995
114. Keys SA, Zimmerman WF: Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Res 68:693–702, 1999
115. Khachik F, Beecher GR, Goli MB, et al: Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromotography. Pure Appl Chem 63:71–80, 1991
116. Khachik F, Beecher GR, Goli MB, et al: Separation and quantification of carotenoids in foods, in Packer L (ed): Methods in Enzymology. San Diego, Academic Press, 1992, pp 347–59
117. Khachik F, Bernstein PS, Garland DL: Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest Ophthalmol Vis Sci 38:1802–11, 1997
118. Khachik F, Spangler CJ, Smith JC Jr, et al: Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 69:1873–81, 1997
119. Klein R, Wang Q, Klein BE, et al: The relationship of age-related maculopathy, cataract, and glaucoma to visual acuity. Invest Ophthalmol Vis Sci 36:182–91, 1995
120. Krinsky NI: The protective function of carotenoid pigments, in Giese AL (ed), Photophysiology. New York, Academic Press, 1968, pp 123–95
121. Krinsky NI, Deneke SM: Interaction of oxygen and oxy-radicals with carotenoids. J Natl Cancer Inst 69:205–10, 1982
122. Kritchevsky SB, Muldoon MF: Oxidative stress and aging: still a hypothesis [editorial; comment]. J Am Geriatr Soc 44:873–5, 1996
123. Kukreja RC, Hess ML: The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovasc Res 26: 641–55, 1992
124. LaBella FS, Vivian S, Thornhill DP: Amino acid composition of human aortic elastin as influenced by age. J Gerontol 21:550–5, 1966
125. Lam S, Tso MO, Gurne DH: Amelioration of retinal photic injury in albino rats by dimethylthiourea. Arch Ophthalmol 108:1751–7, 1990
126. Landrum JT, Bone RA, Joa H, et al: A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res 65:57–62, 1997
127. Landrum JT, Bone RA, Kilburn MD: The macular pigment: a possible role in protection from age-related macular degeneration. Adv Pharmacol 38:537–56, 1997
128. Leske MC, Chylack LT Jr, He Q, et al: Antioxidant vitamins and nuclear opacities: the longitudinal study of cataract. Ophthalmology 105:831–6, 1998
129. Leske MC, Chylack LT Jr, Wu SY: The Lens Opacities Case-Control Study. Risk factors for cataract [see comments]. Arch Ophthalmol 109:244–51, 1991
130. Leske MC, Wu SY, Hyman L, et al: Biochemical factors in the lens opacities. Case-control study. The Lens Opacities Case-Control Study Group. Arch Ophthalmol 113:1113–9, 1995
131. Liles MR, Newsome DA, Oliver PD: Antioxidant enzymes in the aging human retinal pigment epithelium. Arch Ophthalmol 109:1285–8, 1991
132. Lim BP, Nagao A, Terao J, et al: Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. Biochim Biophys Acta 1126:178–84, 1992
133. Liu IY, White L, LaCroix AZ: The association of age-related macular degeneration and lens opacities in the aged. Am J Public Health 79:765–9, 1989
134. Lubart R, Wollman Y, Friedmann H, et al: Effects of visible and near-infrared lasers on cell cultures. J Photochem Photobiol B 12:305–10, 1992
135. Machlin LJ, Bendich A: Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1:441–5, 1987

136. Maddipati KR, Marnett LJ: Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. J Biol Chem 262:17398–403, 1987
137. Maitra I, Marcocci L, Droy-Lefaix MT, Packer L: Peroxyl radical scavenging activity of Ginkgo biloba extract EGb 761. Biochem Pharmacol 49:1649–55, 1995
138. Mangels AR, Holden JM, Beecher GR, et al: Carotenoid content of fruits and vegetables: an evaluation of analytic data [published erratum appears in J Am Diet Assoc 1993 May;93(5):527]. J Am Diet Assoc 93:284–96, 1993
139. Mares-Perlman JA, Brady WE, Klein R, et al: Serum antioxidants and age-related macular degeneration in a population-based case-control study. Arch Ophthalmol 113:1518–23, 1995
140. Mares-Perlman JA, Brady WE, Klein R, et al: Dietary fat and age-related maculopathy [see comments]. Arch Ophthalmol 113:743–8, 1995
141. Mares-Perlman JA, Klein R, Klein BE, et al: Association of zinc and antioxidant nutrients with age-related maculopathy. Arch Ophthalmol 114:991–7, 1996
142. Marklund SL, Westman NG, Lundgren E, et al: Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 42:1955–61, 1982
143. Matsumura G, Herp A, Pigman W: Depolymerization of hyalouronic acid by autoxidants and radiations. Radiat Res 28:735–52, 1966
144. McCarty C, Taylor HR: Light and risk for age-related eye diseases, in Taylor A, Nutritional and Environmental Influences on the Eye. Boca Raton, CRC Press, 1999, pp 135–150
145. McCord JM, Fridovich I: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–55, 1969
146. Mellerio J: Light effects on the retina, in Albert D, Jakobiec F (eds): Principles and Practice of Ophthalmology: Basic Sciences. Philadelphia, W B Saunders Co, 1994, pp 1326–45
147. Mezzetti A, Lapenna D, Pierdomenico SD, et al: Vitamins E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. Atherosclerosis 112:91–9, 1995
148. Nicolas MG, Fujiki K, Murayama K, et al: Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. Exp Eye Res 62:399–408, 1996
149. Novak RL, Stefansson E: Measurements of retina and optic nerve oxidative metabolism in vivo via dual wavelength reflection spectrophotometry of cytochrome a, a3, in Master B (ed): Non-invasive Diagnostic Techniques. New York, Springer-Verlag, 1990, pp 499–509
150. Olin KL, Morse LS, Murphy C, et al: Trace element status and free radical defense in elderly rhesus macaques (Macaca mulatta) with macular drusen. Proc Soc Exp Biol Med 208:370–7, 1995
151. Oliveros E, Braun AM, Aminian-Saghafi T, et al: Quenching of singlet oxygen ($^1\Delta_g$) by carotenoid derivatives: kinetic analysis by near infra-red luminescence. New J Chem 18:535–9, 1994
152. Organisciak DT, Berman ER, Wang HM, Feeney-Burns L: Vitamin E in human neural retina and retinal pigment epithelium: effect of age. Curr Eye Res 6:1051–5, 1987
153. Organisciak DT, Bicknell IR, Darrow RM: The effects of L- and D-ascorbic acid administration on retinal tissue levels and light damage in rats. Curr Eye Res 11:231–41, 1992
154. Organisciak DT, Darrow RM, Barsalou L, et al: Light history and age-related changes in retinal light damage. Invest Ophthalmol Vis Sci 39:1107–16, 1998
155. Organisciak DT, Darrow RM, Jiang YL, et al: Retinal light damage in rats with altered levels of rod outer segment docosahexaenoate. Invest Ophthalmol Vis Sci 37:2243–57, 1996
156. Organisciak DT, Jiang YL, Wang HM, et al: The protective effect of ascorbic acid in retinal light damage of rats exposed to intermittent light. Invest Ophthalmol Vis Sci 31: 1195–202, 1990
157. Organisciak DT, Noell WK: The rod outer segment phospholipid/opsin ratio of rats maintained in darkness or cyclic light. Invest Ophthalmol Vis Sci 16:188–90, 1977
158. Organisciak DT, Wang HM, Li ZY, et al: The protective effect of ascorbate in retinal light damage of rats. Invest Ophthalmol Vis Sci 26:1580–8, 1985
159. Orr WC, Sohal RS: Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophilia melanogaster. Science 263:1128–30, 1994
160. Packer L: Antioxidant action of carotenoids in vitro and in vivo and protection against oxidation of human low-density lipoproteins. Ann NY Acad Sci 691:48–60, 1993
161. Packer L: Interactions among antioxidants in health and disease: vitamin E and its redox cycle. Proc Soc Exp Biol Med 200:271–6, 1992
162. Packer L, Landvik S: Vitamin E in biological systems. Adv Exp Med Biol 264:93–103, 1990
163. Palozza P, Krinsky NI: beta-Carotene and alpha-tocopherol are synergistic antioxidants. Arch Biochem Biophys 297: 184–7, 1992
164. Paolisso G, Tagliamonte MR, Rizzo MR, et al: Oxidative stress and advancing age: results in healthy centenarians. J Am Geriatr Soc 46:833–8, 1998
165. Pease PL, Adams AJ, Nuccio E: Optical density of human macular pigment. Vision Res 27:705–10, 1987
166. Penn JS, Naash MI, Anderson RE: Effect of light history on retinal antioxidants and light damage susceptibility in the rat. Exp Eye Res 44:779–88, 1987
167. Pincemail J, Dupuis M, Nasr C, et al: Superoxide anion scavenging effect and superoxide dismutase activity of Ginkgo biloba extract. Experientia 45:708–12, 1989
168. Pincemail J, Thirion A, Dupuis M, et al: Ginkgo biloba extract inhibits oxygen species production generated by phorbol myristate acetate stimulated human leukocytes. Experientia 43:181–4, 1987
169. Price JE, Fowkes FG: Antioxidant vitamins in the prevention of cardiovascular disease. The epidemiological evidence. Eur Heart J 18:719–27, 1997
170. Putting BJ, Van Best JA, Vrensen GF, et al: Blue-light-induced dysfunction of the blood-retinal barrier at the pigment epithelium in albino versus pigmented rabbits. Exp Eye Res 58:31–40, 1994
171. Putting BJ, van Best JA, Zweypfenning RC, et al: Spectral sensitivity of the blood-retinal barrier at the pigment epithelium for blue light in the 400–500 nm range. Graefes Arch Clin Exp Ophthalmol 231: 600–6, 1993
172. Ranchon I, Gorrand JM, Cluzel J, et al: Functional protection of photoreceptors from light-induced damage by dimethylthiourea and Ginkgo biloba extract. Invest Ophthalmol Vis Sci 40:1191–9, 1999
173. Rao NA, Thaete LG, Delmage JM, et al: Superoxide dismutase in ocular structures. Invest Ophthalmol Vis Sci 26: 1778–81, 1985
174. Rapp LM, Williams TP: The role of ocular pigmentation in protecting against retinal light damage. Vision Res 20: 1127–31, 1980
175. Rapp LM, Williams TP: Damage to the albino rat retina produced by low intensity light. Photochem Photobiol 29: 731–3, 1979
176. Reading VM, Weale RA: Macular pigment and chromatic aberration. J Opt Soc Am 64:231–4, 1974
177. Robison WG, Kuwabara T, Bieri JG: The roles of vitamin E and unsaturated fatty acids in the visual process. Retina 2: 263–81, 1982
178. Robison WG Jr, Kuwabara T, Bieri JG: Deficiencies of vitamins E and A in the rat. Retinal damage and lipofuscin accumulation. Invest Ophthalmol Vis Sci 19:1030–7, 1980
179. Rondanelli M, Melzi dEril GV, Anesi A, Ferrari E: Altered oxidative stress in healthy old subjects. Aging Clin Exp Res 9:221–3, 1997
180. Rozanowska M, Jarvis-Evans J, Korytowski W, et al: Blue light-

induced reactivity of retinal age pigment. In vitro generation of oxygen-reactive species. J Biol Chem 270:18825–30, 1995
181. Rozanowska M, Wessels J, Boulton M, et al: Blue light-induced singlet oxygen generation by retinal lipofuscin in non-polar media. Free Radic Biol Med 24:1107–12, 1998
182. Sanders TAB, Haines AP, Wormald R, et al: Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. J Clin Nutr 57:428–34, 1993
183. Sarks JP, Sarks SH, Killingsworth MC: Evolution of geographic atrophy of the retinal pigment epithelium. Eye 2: 552–77, 1988
184. Sarna T: Properties and function of the ocular melanin—a photobiophysical view. J Photochem Photobiol B 12:215–58, 1992
185. Sato M, Bremner I: Oxygen free radicals and metallothionein. Free Radic Biol Med 14:325–37, 1993
186. Seddon J, Ajani U, Sperduto R: Dietary fat intake and age-related macular degeneration (abstract). Invest Ophthalmol Vis Sci 35(Suppl):2003, 1994
187. Seddon JM, Ajani UA, Sperduto RD, et al: Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group [see comments] [published erratum appears in JAMA 1995 Feb 22;273(8):622]. JAMA 272:1413–20, 1994
188. Sickel W: Retinal metabolism in dark and light, in Fuortes MGF (ed): Handbook of Sensory Physiology. Berlin, Springer-Verlag 1972, pp 667–727
189. Sies H: Oxidative stress: from basic research to clinical application. Am J Med 91(Suppl):31–7, 1991
190. Singh SV, Dao DD, Srivastava SK, et al: Purification and characterization of glutathione S-transferases in human retina. Curr Eye Res 3:1273–80, 1984
191. Smith W, Mitchell P, Rochester C: Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. Am J Ophthalmol 124:838–40, 1997
192. Smith W, Mitchell P, Webb K, et al: Dietary antioxidants and age-related maculopathy: the Blue Mountains Eye Study. Ophthalmology 106:761–7, 1999
193. Snodderly DM, Auran JD, Delori FC: The macular pigment. II. Spatial distribution in primate retinas. Invest Ophthalmol Vis Sci 25:674–85, 1984
194. Snodderly DM, Handelman GJ, Adler AJ: Distribution of individual macular pigment carotenoids in central retina of macaque and squirrel monkeys. Invest Ophthalmol Vis Sci 32:268–79, 1991
195. Sommerburg O, Keunen JE, Bird AC, et al: Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br J Ophthalmol 82:907–10, 1998
196. Southorn PA, Powis G: Free radicals in medicine. I. Chemical nature and biologic reactions. Mayo Clin Proc 63:381–9, 1988
197. Sperduto RD, Ferris FL 3d, Kurinij N: Do we have a nutritional treatment for age-related cataract or macular degeneration? [editorial]. Arch Ophthalmol 108:1403–5, 1990
198. Stephens RJ, Negi DS, Short SM, et al: Vitamin E distribution in ocular tissues following long-term dietary depletion and supplementation as determined by microdissection and gas chromatography-mass spectrometry. Exp Eye Res 47:237–45, 1988
199. Stone WL, Farnsworth CC, Dratz EA: A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segments. Exp Eye Res 28:387–97, 1979
200. Stone WL, Katz ML, Lurie M, et al: Effects of dietary vitamin E and selenium on light damage to the rat retina. Photochem Photobiol 29:725–30, 1979
201. Stur M, Tittl M, Reitner A, et al: Oral zinc and the second eye in age-related macular degeneration. Invest Ophthalmol Vis Sci 37:1225–35, 1996
202. Tappel AL, Tappel AA, Fraga GG, et al: Application of simulation modelling to lipid peroxidation. Free Radic Biol Med 7:361–8, 1989
203. Tas S, Tam CF, Walford RL: Disulfide bonds and the structure of the chromatin complex in relation to aging. Mech Ageing Dev 12:65–80, 1980
204. Tate DJ Jr, Miceli MV, Newsome DA: Zinc induces catalase expression in cultured fetal human retinal pigment epithelial cells. Curr Eye Res 16:1017–23, 1997
205. Tate DJ Jr, Miceli MV, Newsome DA: Phagocytosis and H2O2 induce catalase and metallothionein gene expression in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 36:1271–9, 1995
206. Tate DJ Jr, Newsome DA, Oliver PD: Metallothionein shows an age-related decrease in human macular retinal pigment epithelium. Invest Ophthalmol Vis Sci 34:2348–51, 1993
207. Taylor HR, Munoz B, West S, et al: Visible light and risk of age-related macular degeneration. Trans Am Ophthalmol Soc 88:163–73, 1989
208. Taylor HR, West S, Munoz B, et al: The long-term effects of visible light on the eye [see comments]. Arch Ophthalmol 110:99–104, 1992
209. Thomas JP, Bachowski GJ, Girotti AW: Inhibition of cell membrane lipid peroxidation by cadmium- and zinc- metallothioneins. Biochim Biophys Acta 884:448–61, 1986
210. Tinkler JH, Bohm F, Schalch W, Truscott TG: Dietary carotenoids protect human cells from damage. J Photochem Photobiol B 26:283–5, 1994
211. van den Berg TJ, Ijspeert JK, de Waard PW: Dependence of intraocular straylight on pigmentation and light transmission through the ocular wall. Vision Res 31:1361–7, 1991
212. van Kuijk FJ, Buck P: Fatty acid composition of the human macula and peripheral retina. Invest Ophthalmol Vis Sci 33:3493–6, 1992
213. VandenLangenberg GM, Mares-Perlman JA, Klein R, et al: Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. Am J Epidemiol 148:204–14, 1998
214. Vile GF, Winterbourn CC: Inhibition of adriamycin-promoted microsomal lipid peroxidation by beta- carotene, alpha-tocopherol and retinol at high and low oxygen partial pressures. FEBS Lett 238:353–6, 1988
215. Vingerling JR, Dielemans I, Bots ML, et al: Age-related macular degeneration is associated with atherosclerosis. The Rotterdam Study. Am J Epidemiol 142:404–9, 1995
216. Wassell J, Davies S, Bardsley W, Boulton M: The photoreactivity of the retinal age pigment lipofuscin. J Biol Chem: 20: 23828–32, 1999
217. Weale R: The eye within the framework of human senescence: biological decline and morbidity. Ophthalmic Res 30:59–73, 1998
218. Webb M: Toxicological significance of metallothionein. Experientia Suppl 52:109–34, 1987
219. Weiter JJ, Delori F, Dorey CK: Central sparing in annular macular degeneration. Am J Ophthalmol 106:286–92, 1988
220. Weiter JJ, Delori FC, Wing GL, et al: Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. Invest Ophthalmol Vis Sci 27:145–52, 1986
221. Werner JS, Donnelly SK, Kliegl R: Aging and human macular pigment density. Appended with translations from the work of Max Schultze and Ewald Hering. Vision Res 27: 257–68, 1987
222. West S, Vitale S, Hallfrisch J, et al: Are antioxidants or supplements protective for age-related macular degeneration? [see comments]. Arch Ophthalmol 112:222–7, 1994
223. West SK, Rosenthal FS, Bressler NM, et al: Exposure to sunlight and other risk factors for age-related macular degeneration [see comments]. Arch Ophthalmol 107:875–9, 1989
224. Wiegand RD, Giusto NM, Rapp LM, Anderson RE: Evidence for rod outer segment lipid peroxidation following constant illumination of the rat retina. Invest Ophthalmol Vis Sci 24:1433–5, 1983
225. Williams RA, Brody BL, Thomas RG, et al: The psychosocial impact of macular degeneration. Arch Ophthalmol 116:514–20, 1998
226. Wing GL, Blanchard GC, Weiter JJ: The topography and age relationship of lipofuscin concentration in the retinal pigment epithelium. Invest Ophthalmol Vis Sci 17:601–7, 1978

227. Witting LA: Lipid peroxidation in vivo. J Am Oil Chem Soc 42:908–13, 1965
228. Wu J, Seregard S, Spangberg B, et al: Blue light induced apoptosis in rat retina. Eye 13:577–83, 1999
229. Yeum KJ, Taylor A, Tang G, Russell RM: Measurement of carotenoids, retinoids, and tocopherols in human lenses. Invest Ophthalmol Vis Sci 36:2756–61, 1995
230. Young RW: Solar radiation and age-related macular degeneration. Surv Ophthalmol 32:252–69, 1988
231. Young RW: Pathophysiology of age-related macular degeneration. Surv Ophthalmol 31:291–306, 1987
232. Zhang LX, Cooney RV, Bertram JS: Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–14, 1991

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The authors have no proprietary or commercial interest in any product or concept discussed in this article.

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