Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins

D Max Snodderly

ABSTRACT Epidemiologic data indicate that individuals with low plasma concentrations of carotenoids and antioxidant vitamins and those who smoke cigarettes are at increased risk for age-related macular degeneration (AMD). Laboratory data show that carotenoids and antioxidant vitamins help to protect the retina from oxidative damage initiated in part by absorption of light. Primate retinas accumulate two carotenoids, lutein and zeaxanthin, as the macular pigment, which is most dense at the center of the fovea and declines rapidly in more peripheral regions. The retina also distributes α-tocopherol (vitamin E) in a nonuniform spatial pattern. The region of monkey retinas where carotenoids and vitamin E are both low corresponds with a locus where early signs of AMD often appear in humans. The combination of evidence suggests that carotenoids and antioxidant vitamins may help to retard some of the destructive processes in the retina and the retinal pigment epithelium that lead to age-related degeneration of the macula. Am J Clin Nutr 1995;62(suppl):1448S-61S.

KEY WORDS Retina, age-related macular degeneration, aging, carotenoid, antioxidant, vitamin, fovea, lutein, zeaxanthin, α-tocopherol

INTRODUCTION

In the middle of the retina is a depression called the fovea (Figure 1) where the cone photoreceptors reach their maximum density and we have our highest visual acuity. The foveal depression also has a high density of yellow pigments, primarily the dihydroxy carotenoids lutein and zeaxanthin (1, 2, 7, 8). The yellow pigmentation has given the fovea and the immediately surrounding region the name macula lutea, or yellow spot. In clinical jargon, the name has been shortened simply to macula and the application of the term in the epidemiologic literature has been broadened to include the central retina surrounding the fovea for a radius of 3-4 mm extending approximately to the optic disk.

Age-related macular degeneration (AMD) is a degeneration of the retina and the retinal pigment epithelium (RPE) in the macular region; it occurs late in life and jeopardizes the acute vision of the fovea. In the United States it is the leading cause of new cases of legal blindness and no treatment is available for most patients (9). If macular vision is degraded, the ability to read, drive a car, and even recognize familiar faces can be lost. The effect on the quality of life of elderly people is severe, so there is high motivation to prevent this condition.

Fortunately, nutrition and lifestyle factors are emerging from epidemiologic studies as two components with promise for preventing AMD. In this article I review the evidence that antioxidant nutrients protect the retina and the RPE from damage and reduce the risk of developing AMD. I will concentrate on the evidence for protection by carotenoids and by vitamins C (ascorbic acid) and E (α-tocopherol). The influence of each of these nutrients has been studied epidemiologically in humans and experimentally in animals. Some of the protection conferred by these nutrients may be due to other types of biological activity (10, 11), but there is good evidence that antioxidant activity is an important component. References cited in this paper emphasize recent summary articles that can serve as introductions to the diverse literature relevant to AMD. For reviews of some of the other antioxidant systems that protect ocular tissues, see references 12 and 13.

The pathogenesis of AMD is still poorly understood. Multiple factors are probably involved, but the collective outcome appears to be a disturbance of the relation between the photoreceptors and the nourishing tissues beneath the retina (14, 15). The relations among these tissues are schematically illustrated in the bottom row of Figure 1. The tips of the photoreceptors, the cones and rods, contact processes of a melanin-containing cell layer, the RPE. The RPE cells sit in a monolayer on a basement membrane overlying a connective tissue meshwork called Bruch’s membrane. Immediately under Bruch’s membrane is a dense vascular bed in another melanin-rich layer, the choroid. There is a high flux of materials between the photoreceptors and the capillaries of the choroid through the RPE and through Bruch’s membrane. In addition, vitamin A is transported from the photoreceptors to the RPE and back to the photoreceptors as part of the visual cycle that regenerates the visual pigments after they have absorbed light and have triggered electrical signals. If this visual cycle and other metabolic traffic is disrupted by pathologic processes associated with AMD such as scar formation or accumulation of cellular debris, the photoreceptors degenerate (15, 16).

1 From The Schepens Eye Research Institute, Macular Disease Research Center, Boston, and the Department of Ophthalmology and Program in Neuroscience, Harvard Medical School, Boston.
2 Supported by grants from NIH (EY04911 and EY06591), Massachusetts Lions Eye Research Fund, and Hoffmann-La Roche, Inc.
3 Address reprint requests to DM Snodderly, The Schepens Eye Research Institute, Macular Disease Research Center, 20 Staniford Street, Boston MA, 02114.
FIGURE 1. Anatomic and metabolic relations in the foveal region of macaque monkey retinas. Left panels. A section through the fovea of a rhesus monkey (Macaca mulatta) photographed in green light (top row) and blue light (second row). The carotenoids of the yellow macular pigment appear as dark regions in blue, but not in green light (1, 2). Retinal layers are: GC, ganglion cell layer; IP, inner plexiform layer; IN, inner nuclear layer; PA, photoreceptor axons; ON, outer nuclear layer; IS/OS, inner segments and outer segments of the photoreceptors. Only a scattering of melanin from the retinal pigment epithelium (RPE) remains attached to the retina and the choroid has been removed entirely. The retinal capillary networks (3) and the capillaries of the choroid are indicated by the rows of circles to the right of the photos. The photoreceptors occupy the outer (deep) half of the retina; the retinal capillaries are restricted to the inner retina. Right panels. Oxygen tension in the fovea (second row) as a function of depth in the retina measured in the light and in the dark. An oxygen electrode was advanced through a retina of Macaca nemestrina to record this profile (4). Oxygen tension in the parafovea (first row) measured in the dark (heavy line) (4) compared with profiles of enzyme activities compiled from measurements of individual layers of retinas from Macaca mulatta (5). An indicator of oxidative metabolism was derived by assaying each layer for malic dehydrogenase, and an indicator of anaerobic glycolysis was obtained by assaying for lactic acid dehydrogenase. The maximum activity of malic dehydrogenase was normalized to 100 and all other enzyme activities were scaled accordingly on the horizontal axis. The right panels have been arbitrarily scaled vertically to match the dimensions of the retinal photographs. Bottom row. Relation between the photoreceptor outer segments and the tissues important for their sustenance, schematically shown at a larger scale. The central panel depicts an all-cone region at the center of the fovea, and the other panels depict regions with a mixture of rods and cones outside the foveal center (6). Rods and cones are present in equal numbers at about 0.5 mm from the foveal center. The apical surfaces of the cells of the retinal pigment epithelium (RPE) are studded with processes that ensheath the photoreceptor outer segments and pass between them. The choroid, with its dense vascular bed, is separated from the RPE cells by Bruch’s membrane. Both the choroid and the RPE contain dark melanin granules that reduce scattered light in the eye. The RPE also contains many other types of granules including abundant mitochondria and lipofuscin.
Near the foveal crest and slightly more eccentric, the retina and RPE appear to be especially vulnerable to age-related degeneration (15, 17). This area is where the greatest percentage of rod photoreceptors is lost during normal aging (18) and it is here that a local maximum of lipofuscin occurs in the RPE (19). The reasons for the local vulnerability are currently obscure. One possibility is that the foveal crest has low antioxidant protection. Vitamin E concentration goes through a local minimum in this region (20) and retinal carotenoid concentrations are much lower than at the foveal center (1, 2, 7, 8).

The biochemical and anatomical relations in the foveal region are complicated because of the lateral displacements that create the foveal depression and the rapid decline in numbers of cones with distance from the foveal center (21, 22). Along with these changes, the relation between the photoreceptors and the RPE alters as well (Figure 1, bottom row). In the foveal center, where there are only cones, the processes of the RPE cells extend only 2–3 μm to make contact with the cones before ensheathing the distal part of the outer segments. Outside the foveal center, the rods end near the surface of the RPE cells, but the cones end further away, so that the RPE cell processes must extend 10–20 μm to contact the cone outer segments before ensheathing them. These are only some of the differences that will have to be considered to identify the constellation of local factors that make the macula vulnerable to disease.

**Epidemiology of AMD**

Epidemiologic studies of AMD have been done using case-control designs and population studies; a clinical trial incorporating nutritional supplementation is currently under way. The results are summarized in three tables along with odds ratios and CIs or P values to indicate the strength of the effect. I have selected for presentation only those studies with a large number of subjects and a definite effect, either positive or negative. Primary consideration is given to variables that were examined in more than one investigation. Studies with small numbers of subjects and those showing no definite association between the variables examined are generally not discussed here. For a more exhaustive summary of the epidemiologic literature with consideration of the full range of studies, see reference 23.

Several recent large population studies of AMD have been done in the United States. Those reviewed here include the following:

1) NHANES-I, the first National Health and Nutrition Examination Survey, data from 3082 people between the ages of 45 and 74 y, examined by an ophthalmologist in 1971–1972 (the relatively low age limit is a serious limitation for the data from this study).
2) Framingham, the Framingham Eye Study, data from 2675 residents or former residents between the ages of 52 and 85 y of the town of Framingham, MA, examined in 1973–1975.
3) Beaver Dam, the Beaver Dam Eye Study, data from 4926 residents or former residents between the ages of 43 and 86 y of the town of Beaver Dam, WI, examined in 1988–1990.
4) Baltimore, the Baltimore Longitudinal Study of Aging, ocular data from 827 study participants, plasma data from 668 participants aged ≥ 40 y, examined in 1988–89 (blood samples were taken 2 y before the eye exam for 69% of participants, 4 y before for 13%, and concurrently for the rest), and
5) Watermen, data from 782 fishermen aged 30–95 y from the Chesapeake Bay region, MD.

The remaining entries in the tables are case-control studies.

**Diagnostic criteria**

Epidemiologic studies have relied heavily on fundus photography to diagnose AMD and categorize the disease processes (23). (“Fundus” means the floor of the eye, which refers to the back of the eye including the region around the fovea.) From fundus photographs, subjects are classified as having early AMD if there is evidence of substantial changes in the RPE. These changes can be large deposits of cellular debris (drusen) between the RPE and Bruch’s membrane, or diminished or uneven melanin pigmentation. Subjects are considered to have late, advanced, or severe AMD if there are regions of atrophy of the RPE or if new vessels are growing from the choroid under the RPE (neovascularization) (24–26). The atrophic changes are sometimes called dry AMD, whereas neovascularization is termed wet AMD. The occurrence of neovascularization is judged by elevation of the retina as the result of leakage of fluid from the new vessels or by the presence of vascular membranes, hemorrhages, or subretinal scars.

Often, visual acuity remains unaffected for many years because degeneration starts outside the center of the fovea and does not reach the region of maximum acuity until late in the evolution of the disease (15, 27). In part for this reason, only a subset of the studies have required a loss of visual acuity or a distortion of the visual field as a diagnostic criterion for AMD (28–33). Other functional deficits, such as reduced vision adjacent to the fovea, are not usually quantitatively assessed.

**Identification of risk factors probably related to oxidative processes**

**Cigarette smoking**

Three known risk factors for AMD are probably related to oxidative processes: cigarette smoking, sunlight exposure, and low ocular melanin (Table 1). Males show stronger effects than females, but this may be due to their higher consumption of cigarettes (36). When neovascular AMD and atrophic AMD are analyzed separately, smoking appears to be preferentially linked with the neovascular form (35, 36).

Cigarette smoking exposes the smoker to large quantities of prooxidants (40). Products of lipid peroxidation are elevated in the blood (41) and in the breath (42) of smokers. Erythrocytes of smokers are more susceptible to lipid peroxidation in vitro, perhaps because of reduced activity of protective enzymes (42).

Most authors have concluded that blood vitamin E concentrations are little affected by smoking, whereas carotenoids are substantially reduced (43–45). Although smokers have lower dietary carotenoid intake than do nonsmokers, their blood carotenoid concentrations are even lower than would be predicted from the differences in diet (43, 44, 46). This suggests that the oxidant load from smoking depletes the blood of carotenoids.

In British and US populations, smokers have lower blood concentrations of vitamin C (41, 46, 47). This is partly due to lower dietary intake (46), but even after adjusting for dietary
TABLE 1
Risk factors for age-related macular degeneration (AMD) probably related to oxidative processes

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio (CI) or P value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td>Smokers lose vision earlier than nonsmokers</td>
</tr>
<tr>
<td>Paetkau et al (34), n = 114</td>
<td>6</td>
<td>Males affected; females not</td>
</tr>
<tr>
<td>Hyman et al (32), n = 162</td>
<td>2.6 (1.15, 5.75)</td>
<td>Current smokers, neovascular AMD</td>
</tr>
<tr>
<td>Hyman et al (35)</td>
<td>6</td>
<td>Current smokers, atrophic AMD</td>
</tr>
<tr>
<td>n = 182^2</td>
<td>2.2</td>
<td>Smokers versus those who never smoked</td>
</tr>
<tr>
<td>n = 227</td>
<td></td>
<td>Females, current smokers versus all others</td>
</tr>
<tr>
<td>EDCC (28), n = 346^6</td>
<td>2.2 (1.4, 3.5)</td>
<td>Males, current smokers versus all others</td>
</tr>
<tr>
<td>Klein et al (36), Beaver Dam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 36^2</td>
<td>2.5 (1.01, 6.2)</td>
<td>Females, current smokers versus all others</td>
</tr>
<tr>
<td>n = 17^2</td>
<td>3.29 (1.03, 10.5)</td>
<td>Low sunlight exposure</td>
</tr>
<tr>
<td>Liu et al (37), NHANES I, n = 6^6</td>
<td>2.0 (1.44, 2.78)</td>
<td>Aphakic patients versus general population</td>
</tr>
<tr>
<td>Taylor et al (25), Watermen, n = 8^5</td>
<td>1.36 (1.0, 1.85)</td>
<td>Estimated exposure for previous 20 y.</td>
</tr>
<tr>
<td>Cruickshanks et al (24), Beaver Dam, n = 77^6</td>
<td>2.26 (1.06, 4.81)</td>
<td>P = 0.015 if welders excluded</td>
</tr>
<tr>
<td>Low ocular melanin</td>
<td></td>
<td>Both sexes, leisure time outside in summer</td>
</tr>
<tr>
<td>Hyman et al (32), n = 162</td>
<td>3.6 (1.6, 8.4)</td>
<td>White population, males larger effect, genetic</td>
</tr>
<tr>
<td>Weider et al (38), n = 650</td>
<td>P = 0.0001</td>
<td>Lighter iris = less choroidal melanin</td>
</tr>
<tr>
<td>Sandberg et al (39), n = 132^8</td>
<td>P = 0.011</td>
<td>Lighter iris, worse disease in fellow eye</td>
</tr>
</tbody>
</table>

^1 For case-control studies, the number of case subjects included to analyze the specific factor in the most complete analysis (e.g., multivariate) is listed.
Population studies are identified by the name of the population; the number of people with the relevant stage of AMD is indicated.

^2 Neovascular AMD only.

^3 All case subjects had some loss of acuity attributed to AMD; approximately one-half of case subjects had neovascular AMD.

^4 Eye disease case-control study group. Neovascular AMD only; required some loss of acuity.

^5 AMD defined as loss of macular reflex, pigment dispersion and clumping, and drusen; some loss of acuity attributed to AMD.

^6 Neovascular or atrophic AMD, both considered advanced AMD.

^7 AMD defined as presence of macular drusen.

^8 Neovascular AMD in one eye.

intake the US population still showed an inverse relation between smoking and blood vitamin C concentrations (47).

There is little information about the effects of smoking on tissue concentrations of antioxidants in the retina or RPE. Our laboratory has evidence that the retinal carotenoids, lutein and/or zeaxanthin, are reduced in smokers (48). The low retinal densities may be a consequence of lower blood concentrations (44), there may be a direct effect on the retina, or both.

Sunlight exposure and low ocular melanin

The other two risk factors listed in Table 1, sunlight exposure and low ocular melanin, share at least one biological mechanism. Sunlight damages as well as stimulates the retina (49) and low ocular melanin traps less light. Light damage results at least in part from oxidative insult, as described in detail later.

A sudden increase in light exposure results if a cataractous lens is surgically removed and the patient remains aphakic. Data from NHANES I indicate that these patients are more likely to develop AMD than are people with intact lenses (37). The odds ratios in that paper apparently were computed with subjects showing no retinal abnormality as the control group, rather than with patients whose cataracts were not removed, so this effect may be overestimated.

Most attempts to assess the importance of sunlight as a risk factor for developing AMD result in the formidable task of estimating the exposure of individuals over long portions of their lifespan. In the Watermen study, the exposure to sunlight over a 20-y period was estimated for a group of fishermen in the Chesapeake Bay region (25). There was a positive association between the amount of exposure and the occurrence of advanced AMD. The P value for the relation was improved to 0.015 by excluding those who had potentially confounding exposure to high-intensity illumination from welding. Two other confounding influences were minimized by the design of the study: the outdoor work was in an unobstructed environment and most watermen did not use sunglasses because they get covered by spray.

In the Beaver Dam Eye Study, the exposure to sunlight was estimated from the amount of time spent outdoors in summer and the use of hats with brims and sunglasses. Advanced AMD was positively associated with the amount of leisure time spent outside in summer for both sexes (24). Early AMD (indistinct drusen) was less frequent among those who used hats and sunglasses (odds ratio 0.61). It may be important that many people in this region of the United States are descendants of Nordic and Northern European immigrants who have light-colored irises and therefore would be especially sensitive to the effects of sunlight. Eyes with light-colored irises transmit 100 times as much light as do those with dark brown irises (50). In addition, light-colored irises are associated with low choroidal melanin (38, 51). The melanin of the iris and choroid have been shown in animal experiments to confer substantial protection against light damage by reducing retinal irradiance (52). The melanin of the RPE probably has a different role, that of protecting the RPE itself, and not the overlying retina (53).

The Beaver Dam results have a striking parallel in animal experiments. It is relatively easy to show that light damages the
retinas of albino rats, which have no ocular melanin. However, the susceptibility to damage is dependent on the light history of the animal. Rats raised in dim illumination have higher amounts of rhodopsin (the visual pigment in the rods) than do animals raised in bright light (54), but the animals raised in bright light have higher retinal concentrations of protective factors: the glutathione-related antioxidant enzymes and vitamins E and C. When animals raised under the two different conditions are exposed to the same intense, constant light, the ones raised in dim light suffer much more severe damage to the photoreceptors (55). The human analogue to this experiment may be the Beaver Dam residents who, after a cold, dark winter indoors, suddenly begin to spend time outdoors in the bright sunshine of spring and summer. Because everyone is exposed to sunlight, it is somewhat puzzling that the epidemiologic results linking AMD with sunlight exposure are not stronger. One major study failed to find any association between AMD and iris color or light exposure (28). However, the light received by the retina and RPE is a joint function of iris color and environmental exposure, which apparently was not evaluated. Furthermore, the estimation of environmental exposure is difficult; hence, a null outcome could easily result from inaccurate exposure estimates.

**Risk factors possibly related to oxidative processes**

**Sex-related risk factors**

Many other potential risk factors for AMD have been studied, and the results that may be related to antioxidant considerations are summarized in **Table 2**. Sex is one of the factors that has been difficult to evaluate. There is a pervasive clinical impression in the Boston area that AMD is more frequent among women—a conclusion that has been difficult to prove (23).

In the Framingham study, women were found to have a higher prevalence of AMD than men when loss of visual acuity was included as a criterion (30). Unfortunately, in the eye exam, the contribution of cataracts to visual acuity loss was not separated from the effects of AMD. Because women had more cataracts, it has been suggested that some women with acuity loss due to cataracts might have been diagnosed erroneously as having AMD, thus exaggerating the prevalence of AMD in women (60). However, data from the Watermen study suggest

**Table 2**

<table>
<thead>
<tr>
<th>Risk factor and reference</th>
<th>Odds ratio (CI) or P value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kahn et al (30), Framingham, n = 218&lt;sup&gt;2&lt;/sup&gt;</td>
<td>P &lt; 0.01</td>
<td>Women of all ages more; required acuity loss, may have cataract</td>
</tr>
<tr>
<td>Klein et al (56), Beaver Dam, n = 3263</td>
<td>P = 0.11</td>
<td>Females ≥ 55 y had more drusen area than did men</td>
</tr>
<tr>
<td>Klein et al (56, 57), Beaver Dam, n = 30</td>
<td>P = 0.02</td>
<td>Females ≥ 75 y had more neovascular AMD and hypertension</td>
</tr>
<tr>
<td>EDCC (28), n = 236&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.2 (1.3, 3.9)</td>
<td>Women with children versus childless women</td>
</tr>
<tr>
<td>Kahn et al (51), Framingham, n = 151-163&lt;sup&gt;3&lt;/sup&gt;</td>
<td>P &lt; 0.05</td>
<td>Left ventricular hypertrophy, history of lung infection, diastolic blood pressure, for men and women &lt; 75 y</td>
</tr>
<tr>
<td>Hyman et al (32), n = 162&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.1 (1.1, 2.7)</td>
<td>Combines heart disease, stroke, and hypertension</td>
</tr>
<tr>
<td>Goldberg et al (58), NHANES I, n = 12&lt;sup&gt;s&lt;/sup&gt;</td>
<td>4.64 (1.41, 15.27)</td>
<td>Specifically cerebrovascular disease</td>
</tr>
<tr>
<td>Hyman et al (35), n = 182&lt;sup&gt;s&lt;/sup&gt;</td>
<td>3.2</td>
<td>Diastolic blood pressure &gt; 95 mm Hg</td>
</tr>
<tr>
<td>Serum lipids</td>
<td></td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>EDCC (28), n = 415&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.1 (2.3, 7.3)</td>
<td>Serum HDL, neovascular AMD</td>
</tr>
<tr>
<td>Hyman et al (35)</td>
<td></td>
<td>Serum HDL, atrophic AMD</td>
</tr>
<tr>
<td>n = 182&lt;sup&gt;s&lt;/sup&gt;</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>n = 227</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Hyperopia</td>
<td></td>
<td>Females affected, not males</td>
</tr>
<tr>
<td>Hyman et al (32), n = 162&lt;sup&gt;4&lt;/sup&gt;</td>
<td>P = 0.009</td>
<td>Suggest protection from wearing glasses</td>
</tr>
<tr>
<td>Weiter (59), n = 142</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Goldberg et al (58), NHANES I, n = 178&lt;sup&gt;s&lt;/sup&gt;</td>
<td>1.43 (1.04, 1.97)</td>
<td>Neovascular AMD</td>
</tr>
<tr>
<td>EDCC (28), n = 346&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.5 (0.9, 2.4)</td>
<td></td>
</tr>
<tr>
<td>Family history of AMD</td>
<td></td>
<td>Parents reported by child, siblings by eye exam</td>
</tr>
<tr>
<td>Hyman et al (32), n = 162&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.9 (1.5, 5.5)</td>
<td></td>
</tr>
<tr>
<td>Hyman et al (35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 182&lt;sup&gt;s&lt;/sup&gt;</td>
<td>5.3</td>
<td>Neovascular AMD</td>
</tr>
<tr>
<td>n = 227</td>
<td>4.5</td>
<td>Atrophic AMD</td>
</tr>
</tbody>
</table>

<sup>1</sup> For case-control studies, the number of case subjects included to analyze the specific factor in the most complete analysis (eg, multivariate) is listed. Population studies are identified by the name of the population: the number of people with the relevant stage of AMD is indicated.

<sup>2</sup> AMD defined as pigment disturbance, drusen formation, or neovascular changes.

<sup>3</sup> Neovascular AMD only; required some loss of acuity.

<sup>4</sup> All case subjects had some loss of acuity attributed to AMD; approximately one-half of case subjects had neovascular AMD.

<sup>s</sup> AMD defined as loss of macular reflex, pigment dispersion and clumping, and drusen; some loss of acuity attributed to AMD.

<sup>s</sup> Neovascular AMD only.
that cataracts make it more difficult to view the retina and to identify AMD (61). Hence, it is also possible that the prevalence of AMD in women with cataracts could, in fact, have been underestimated.

Later data, from the Beaver Dam study, do not rely on measures of visual acuity. They show that women aged > 75 y have a higher probability than do men of developing neovascular AMD \((P = 0.02)\). Furthermore, women aged > 55 y had more retinal area covered by drusen than did men, although this difference, after adjustment for age, had a \(P\) value of only 0.11.

Another sex-related influence on the risk for AMD was identified by the Eye Disease Case Control (EDCC) Study. Women with children had a higher risk for neovascular AMD than did childless women. Some factors that could be related to women’s hormonal status are discussed later.

**Cardiorespiratory disease and high serum lipid concentrations**

Evidence was found in several studies of an association between cardiorespiratory disease or its risk factors and AMD (Table 2). The Framingham study is especially interesting in this regard, because cardiorespiratory status was assessed 10–30 y before the eye exams for diagnosing AMD. This suggests that long-term cardiorespiratory stress could contribute to the development of AMD, which is consistent with the finding that elevated serum cholesterol or high-density lipoprotein (HDL) are risk factors for AMD, especially the neovascular form. The question arises whether there could be some common effect linked to compromise of the circulation by excess lipids or to insufficient lipid-soluble vitamins. Still, the relation between cardiovascular disease and AMD may not be a strong one. Data from the Beaver Dam study indicate that most cardiovascular risk factors are not related to AMD (57).

**Hyperopia**

Hyperopia (far-sightedness) has been identified as a risk factor for AMD, but without a generally accepted mechanism. It has been suggested that hyperopes, who only wear glasses after middle age, are less protected than are myopes, who wear eyeglasses at all ages and may experience less sunlight exposure (59). However, the apparent influence of hyperopia may be confounded by an age effect. In the Beaver Dam population, hyperopia increased with age from 22\% of the population between 43 and 54 y old to 68.5\% of those aged > 75 y (62).

**Family history of AMD**

Two reports by Hyman et al (32, 35) have concluded that a family history of AMD is one of the risk factors. This could be due to acquired traits such as dietary preferences, inherited ocular characteristics such as pigmentation or refraction, or interactions among such factors. A genetic component is indicated by the close concordance of AMD progression in identical twins (63).

**Factors protecting against AMD**

Antioxidant nutrients are associated with a substantially reduced risk of developing AMD (Table 3). Dietary analyses suggest that carotenoids are the most potent protective factors but blood data indicate that vitamins E and C are also important. No epidemiologic data are available on nutrient concentrations in ocular tissues.

The data from two major studies on the protective effects of blood concentrations of antioxidant nutrients were quantified by dividing the populations into quintiles (EDCC study) or quartiles (Baltimore study). These quintiles and quartiles were collapsed into three groups, low, middle, and high, by forming a single large middle group from either the middle two quartiles or the middle three quintiles. Table 3 includes the cutoffs for the nutrient concentrations in the blood of the high and the low groups of subjects. Because the low group is taken as the reference and the protective effect is judged by comparison with that group, it is revealing that the strongest effect of a given nutrient is seen in the population whose reference group has the lowest blood concentrations of that nutrient.

**Dietary carotenoids**

When the dietary intake of different carotenoids was analyzed, the sum of lutein and zeaxanthin, the retinal carotenoids forming the macular pigment, had the strongest protective effect against neovascular AMD (33). An especially beneficial effect was assigned to intake of spinach, which is rich in lutein but not zeaxanthin (64). Lutein and zeaxanthin intakes were not analyzed separately in this study. Consumption of vitamins E and C, including the use of supplements, did not have a significant effect.

**Blood carotenoids**

Unfortunately, measurements of carotenoids in the blood for AMD epidemiology have been incomplete. No epidemiologic data with separate values for the two retinal carotenoids, lutein and zeaxanthin, have been published. In only one study were data presented for any carotenoids besides \(\beta\)-carotene (29), which is not normally found in the retina (65, 66). The authors reported that the same odds ratio was found whether the sum of all carotenoids, \(\beta\)-carotene alone, or lutein and zeaxanthin alone were considered (29). The other large-scale study to present data on \(\beta\)-carotene (the Baltimore population; 26) found a modest protective effect that did not quite reach the 0.05 level of statistical significance.

**Blood vitamin E**

In the Baltimore study, higher blood concentrations of vitamin E were significantly protective against AMD. This population had a wide range of plasma values, including values that were lower than those in the population in the EDCC study. Only the middle group of the EDCC population had a statistically significant reduction in risk compared with the group with low plasma values. This conclusion should be qualified by the fact that there was lower statistical power to detect an effect in the highest quintile because of the small number of subjects compared with the middle group, in which subjects were lumped from three quintiles.

**Blood vitamin C**

A similar pattern has been reported for vitamin C. In the EDCC study the middle group had lower risk than did the group with low plasma values, but the high group was no better protected and perhaps even less protected. The Baltimore population showed a protective trend that did not reach the 0.05
TABLE 3
Factors protecting against age-related macular degeneration (AMD)

<table>
<thead>
<tr>
<th>Protective factor and reference</th>
<th>Odds ratio (CI)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary carotenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goldberg et al (58), NHANES I, n = 178&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 (0.37, 0.99)</td>
<td>Foods high in vitamin A (or carotenoids) once a day; result on vitamin C clouded by lack of information about supplements</td>
</tr>
<tr>
<td>Seddon et al (33), n = 356&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 (0.2, 0.7)</td>
<td>Lutein-zeaxanthin intake strongest effect</td>
</tr>
<tr>
<td>Blood carotenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDCC (29), n = 390&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3 (0.2, 0.6)</td>
<td>Lutein-zeaxanthin, β-carotene, sum of all, same odds ratio; high versus low group; low group β-carotene ≤ 0.25, high ≥ 0.74 μmol/L</td>
</tr>
<tr>
<td>West et al (26), Baltimore, n = 226&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.62 (0.36, 1.07)</td>
<td>Only β-carotene; high versus low group; low group β-carotene &lt; 0.34, high &gt; 0.88 μmol/L</td>
</tr>
<tr>
<td>Blood vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDCC (29), n = 390&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.6 (0.4, 0.9)</td>
<td>Middle versus low group (no improvement in high group); low group ≥ 24.5, high ≥ 43.4 μmol/L</td>
</tr>
<tr>
<td>West et al (26), Baltimore, n = 226&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.43 (0.25, 0.73)</td>
<td>High versus low group: low group &lt; 18.6, high &gt; 82.3 μmol/L</td>
</tr>
<tr>
<td>Blood vitamin C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDCC (29), n = 390&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.6 (0.4, 0.9)</td>
<td>Middle versus low group (no improvement in high group); low group ≥ 39.8, high ≥ 90.8 μmol/L</td>
</tr>
<tr>
<td>West et al (26), Baltimore, n = 226&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.55 (0.28, 1.08)</td>
<td>High versus low group: low group &lt; 59.6, high &gt; 82.3 μmol/L</td>
</tr>
<tr>
<td>Blood antioxidant index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDCC (29), n = 390&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3 (0.2, 0.6)</td>
<td>Carotenoids, vitamin E, vitamin C, and selenium</td>
</tr>
<tr>
<td>West et al (26), Baltimore, n = 226&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.43 (0.26, 0.70)</td>
<td>Vitamin E, vitamin C, and β-carotene</td>
</tr>
<tr>
<td>Hyman et al (35), n = 182&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.3</td>
<td>Vitamin E, vitamin C, and β-carotene</td>
</tr>
<tr>
<td>Sex-related (women only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDCC (28), n = 190&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.3 (0.1, 0.8)</td>
<td>Estrogen use, after multiple adjustments; only eight current users</td>
</tr>
</tbody>
</table>

<sup>a</sup> For case-control studies, the number of case subjects included to analyze the specific factor in the most complete analysis (eg, multivariate) is listed. Population studies are identified by the name of the population; the number of people with the relevant stage of AMD is indicated.

<sup>b</sup> AMD defined as loss of macular reflex, pigment dispersion and clumping, and drusen; some loss of acuity attributed to AMD.

<sup>c</sup> Neovascular AMD only.

<sup>d</sup> Eye disease case-control study group. Neovascular AMD only; required some loss of acuity.

<sup>e</sup> Only 11 case subjects had severe AMD defined as either atrophic or neovascular. All others had large drusen > 63 μm, confluent drusen, disturbances of retinal pigment epithelium (RPE) pigmentation or nongeographic atrophy, defined as poorly demarcated, depigmented RPE with overlying thinning of the retina.

level of statistical significance. This is the third example of what appears to be a consistent pattern when comparing the results from the EDCC and the Baltimore populations: for each nutrient, the study population with the reference group having the lowest blood concentration is the population that shows the strongest effect. This suggests that persons with low blood concentrations of antioxidant nutrients are at risk, but that once the nutrients reach moderate concentrations, substantial protection is already conferred and any additional benefits of further increases are more difficult to show. Such a pattern could be obscured by the practice of coarse division into low, middle, and high groups. To identify the underlying pattern with greater clarity, it might be useful to divide the study populations into smaller groups for analysis of the data with higher resolution so that a dose-response relation could be sought.

Blood antioxidant index

Because antioxidants may interact and their blood concentrations tend to be correlated, the combined antioxidant effect has been estimated by use of an antioxidant index. Each study has a different definition of the index and none of them is explicitly related to physiology. The benefit of using such an index is to identify subjects who have generally high blood concentrations of antioxidant nutrients and those who have generally low concentrations. The indexes show a protective effect, but in each case the odds ratios for the indexes are about the same as the ratios for the most protective single nutrient. Consequently, it is not clear that using an index adds deeper insight.

Sex-related factors (estrogen use)

Postmenopausal women given unopposed estrogen replacement therapy (no progesterone) have reduced risk of neovascular AMD. Oral estrogen use alters plasma lipid profiles, resulting in increased concentrations of HDL and cholesterol (67, 68). Concentrations of clotting factors in the blood and the activity of several metabolic systems are altered as well (69, 70).

In addition to the fact that estrogens can have antioxidant activity (71), the question arises whether the hormonally induced changes could alter the profiles of antioxidant nutrients in blood. This is plausible for two reasons: 1) The distribution of vitamin E among the plasma lipid fractions is different in women and men (72) and is influenced by HDL cholesterol concentrations (73). 2) Carotenoids are distributed differently among the plasma lipid fractions, so that changes in the relative amounts of the different lipoprotein fractions might alter the blood carotenoid composition. Lutein is distributed relatively uniformly, whereas β-carotene is preferentially associated with the low-density lipoproteins (74, 75). Consequently, alterations...
of blood lipid profiles by hormonal interventions may produce changes in the transport and metabolism of lipid-soluble antioxidants that should be evaluated.

**BIOLOGICAL MECHANISMS**

**Age-related changes in the retina and RPE**

Most of the changes that occur in the visual system with aging are gradual and relatively subtle (76, 77). The alterations that are most easily related to AMD are those involving the photoreceptors and the RPE. The most vulnerable retinal cells are thought to be the rods and the blue-sensitive cones. Over the course of a life span of 90 years, 30% of the rods within 4 mm of the foveal center are lost (18). Moreover, the percentage of rods lost is greatest in an annulus between 0.5 and 3 mm from the foveal center (18). This retinal region is also where degenerative changes of AMD are often first seen (15).

Of the three cone types, the blue-sensitive (B) cones are the least numerous (78) and the most likely to be killed by repeated exposure to light (79, 80). Like the rods, the B cones are absent from the very center of the fovea (78) but are numerous along the slope of the foveal depression. With age, the sensitivity of the neural pathways receiving input from B cones declines, but the B cone pathways in the fovea suffer less sensitivity loss than elsewhere (81). The foveal B cones are thought to be protected from age-related damage by the yellow macular pigment, which acts as a filter and absorbs blue light (81).

In the RPE, changes with age include a loss of cells (82), reduced amounts of melanin per cell (83), and accumulation of complex granules (83) and of lipofuscin (19, 83). The loss of melanin may result in reduced protection against light-induced oxidative (photooxidative) damage to the RPE (83).

**The effect of oxidative processes on the retina and RPE**

**Photoreceptor renewal and lipofuscin formation**

The outer segments of the photoreceptors consist of stacks of membranous disks (Figure 1) that are continually being renewed by synthesis of new disks and by shedding of old disks at the tips. Shedding of disks by the rods occurs preferentially after the onset of light in the normal diurnal cycle. In this way, the rods renew their entire 22-37-μm outer segment every 9–12 d (84, 85). The cones also shed their disks, but in a less synchronized manner and perhaps with a slightly slower rate of renewal (86, 87). In both cases, the RPE phagocytoses the shed disks and digests them. As one of the factors modulating this high flux of membranous material, it has been suggested that oxidation of disk membranes may increase the amount of disk shedding (88).

In the RPE, the digestion of the shed disks is a source of lipofuscin (89), which if accumulated in sufficient amounts may be toxic to the cells (90). RPE lipofuscin is a complex fluorescent mixture of materials containing at least 10 different fluorophores, many of which appear to be vitamin A metabolites, including some oxidation products of vitamin A (91). Vitamin A reacts with ethanolamine that is probably derived from the phospholipids of the phagocytosed photoreceptor membranes to form a particularly distinct fluorophore (91).

According to this schema, oxidative processes should increase lipofuscin concentrations in the RPE by two different mechanisms. First, they increase the amounts of material shed by the photoreceptors, thus making available more reactants such as ethanolamine for lipofuscin formation. Second, oxidized forms of vitamin A contribute to more lipofuscin production. In humans, lipofuscin concentrations in the RPE dip to a local minimum in the fovea, where macular pigment absorption of blue light may help to retard photooxidative processes contributing to lipofuscin formation (17, 19). Human retinal photoreceptors also form lipofuscin granules that accumulate with age (92, 93), but the factors influencing their formation are apparently unknown.

In rats, the buildup of RPE lipofuscin can be accelerated by antioxidant deficiency (especially vitamin E) (12, 94). However, this lipofuscin has a different chemical composition from the lipofuscin that accumulates in normal aging, and it is not clear whether it has any special relation to the development of pathology (95). The chemistry of the lipofuscin-like products that are formed in the RPE in the antioxidant deficiency states discussed below is not well understood.

**Oxidative damage initiated by light**

An extensive body of literature from experiments on primates, rodents, and other vertebrates shows that the retina is damaged by visible light (12, 96). Many mechanisms may be involved in light damage to the retina, but one of them is thought to be the peroxidation of polyunsaturated membrane lipids (97–99). Lipid peroxides are toxic to the retina (100) and the photoreceptors are rich sources of fatty acids that can be oxidized. Rod outer segments have some of the highest concentrations of polyunsaturated fatty acids of any known membrane systems (101). Although cone cell membrane composition is not known, it has been shown that n-3 fatty acid deficiency causes acuity deficits in monkeys, implying that these polyunsaturates contribute to cone as well as rod function (102).

Of all the spectral regions, primate retinas have the lowest damage thresholds for blue light (103, 104). Evidence has been accumulating that oxygen and its radicals participate in the damage from blue light (105–107). Damage from short exposures of 100-1000 s at high power is greatest in the RPE and there is some involvement of the photoreceptors (107, 108).

The mechanism by which melanin may modulate blue-light damage to the RPE is not securely established (12). Nevertheless, the high damage potential of blue light is probably conferred in part by endogenous photosensitizers in the tissues. Known or suspected photosensitizers that occur as normal constituents of cells include cytochromes, flavins, and hemoproteins, all of which are excited by blue light (109). The flavin enzymes of the mitochondria, as well as free riboflavin, are powerful photosensitizers and are readily damaged by exposure to blue light (110).

The retinal carotenoids are well suited to inhibit blue light damage. First, they absorb some of the blue light before it reaches many of the retinal elements or the RPE (Figure 1). Second, carotenoids can quench the triplet state of photosensitizers, therefore minimizing subsequent photochemical reactions (111). Third, they can also quench the photochemical products such as singlet oxygen (112–114) and possibly oxygen radicals (115), thereby preventing photosensitized damage. Finally, they can act as chain-breaking antioxidants to retard the peroxidation of membrane phospholipids (116). Consistent
with these chemical results, experiments on monkey retinas show that the macular region of the RPE and the retina are less vulnerable to blue light damage than are other regions (108, 117).

A series of studies have shown that in primates, cones are more vulnerable to acute damage than are rods. Exposure of the retina for several hours to powers of illumination below those needed to damage the RPE affects all retinal layers, but the cones are the most sensitive elements (117). In fact, primate cones are damaged by broad-spectrum light at intensities that have been routinely permitted in industrial environments (118).

Intermittent exposure to light for prolonged periods at lower power that is two to three orders of magnitude below acute damage thresholds causes damage specific to particular cone subtypes. After exposure regimes with wavelengths of light near their visual pigment absorption peaks, the B cones are permanently destroyed; the green-sensitive cones are damaged by light that they absorb but they slowly recover (80). This wavelength-specificity suggests that the cone visual pigments may initiate the damage or else may start a physiologic cascade that can have damaging effects at a later stage.

**Oxygen tension and its relation to the laminar distribution of antioxidant nutrients**

Many studies have shown that antioxidant nutrients are important in preventing light damage to ocular tissue (12, 13). The protection of the retina is an intricate process that makes use of multiple agents with different distributions in the retina. Nothing is currently known about the spatial distribution of most of the aqueous antioxidants such as ascorbate. However, some aspects of the distributions of the lipid-soluble antioxidants, the carotenoids and vitamin E, are known. These data establish a striking correspondence between the chemistry of these protective agents and the profile of oxygen tension in the retinal layers.

The retina gets its oxygen supply from two spatially separated sources (Figure 1). A dense capillary bed underneath the retina in the choroid supplies the photoreceptors, whereas capillary networks in the inner retina nourish the other cellular components (3). In the foveal center, oxygen tension is high near the choroid (4) and drops rapidly with distance (Figure 1, right column, second row) because the center of the fovea is avascular and there is no retinal oxygen supply. Readers not familiar with visual physiology may be surprised to realize that the photoreceptors are metabolically more active in the dark, consuming more energy and more oxygen. Because so much oxygen is consumed in the dark, the oxygen tension in the retina is lower in the dark than in the light. In the parafovea, oxygen tension in the dark (Figure 1, right column, first row) reaches a local minimum of ≈7 mm Hg in the vicinity of the photoreceptor axons and the outer nuclear layer (4).

The enzyme systems of the retina that generate energy are distributed so that they match the oxygen profile. Enzymes associated with anaerobic glycolysis are most active near the oxygen minimum, whereas enzymes associated with oxidative metabolism show a complementary profile, peaking near the oxygen maximum (5). The profile of oxidative metabolism presumably reflects the distribution of mitochondrial activity in the retina.

The anatomical distribution of the lipid-soluble antioxidants is also intimately related to the retinal oxygen profile. Studies of many species (119–121) have shown that vitamin E is more concentrated in rod outer segments than in the inner retina (12). The macular carotenoids exhibit a complementary pattern, being denser in the rod-sparse area of the foveal avascular zone and in the fiber layers of the inner retina (1, 2). The overall laminar pattern places the highest densities of retinal carotenoids in the layers with lowest oxygen tension and the highest concentration of vitamin E in the layer with the highest oxygen tension (Figure 1).

Model chemical studies on β-carotene and vitamin E provide a mechanistic rationale for the complementary arrangement of carotenoids and vitamin E in the retinal layers. Relative to α-tocopherol, β-carotene (and presumably the retinal carotenoids) becomes progressively more effective as an antioxidant as the oxygen tension decreases (122). At low oxygen tensions near 4 mm Hg, β-carotene (and presumably the retinal carotenoids) can inhibit lipid peroxidation more effectively than can α-tocopherol (123). Therefore, their high densities in regions of low oxygen tension places them where they should be able to be effective. Because carotenoids can be destroyed relatively rapidly at high oxygen tensions (122), the low oxygen tension in the inner retina, coupled with protection (124) by the substantial amounts of vitamin E in the foveal center (20), may facilitate the accumulation of high carotenoid densities.

**Special properties of the retinal carotenoids**

From experiments on monkeys, Ham and his colleagues (106) reported that the threshold for retinal light damage at ambient oxygen concentrations increased by a modest factor of 10% after massive dietary supplements of β-carotene. More recently, Tso (125) and colleagues substantially elevated retinal concentrations of β-carotene in rats (126) and monkeys (personal communication, 1990) by massive supplementation and reported striking protection against retinal light damage. Although these are intriguing results, they do not necessarily mean that β-carotene plays a leading role in protecting the retina, because it is normally present only in trace amounts (65, 66).

Why does the retina accumulate zeaxanthin and lutein to the exclusion of other carotenoids such as β-carotene, which are also abundant in the blood (64)? The two hydroxyl groups, at either end of the retinal carotenoids (Figure 2) may have special importance. This has been emphasized by studies of zeaxanthin in phospholipid bilayers. In model systems, zeaxanthin spans the lipid membrane like a rivet or a tie-bar (127).
brane systems (eg, dimyristoyl phosphatidylcholine). These results are consistent with the orientation of the retinal membranes appears to be another key factor.

Although much of the biochemical literature has focused on β-carotene, the available evidence indicates that the retinal carotenoids may be more effective antioxidants in biological systems. Lutein is no more effective in solution than β-carotene at quenching singlet oxygen (130, 131), but in cultured cells it is more effective at inhibiting autoxidation of cellular lipids (10). Similarly, zeaxanthin is only as effective as β-carotene in inhibiting autoxidation of lipids in solution (132), but about 50% more effective in retarding hydroperoxide formation in phosphatidylcholine liposomes (116). This is true whether the radicals that initiate the oxidation are generated in the lipid phase or the aqueous phase. Some of the greater antioxidant effectiveness of zeaxanthin may be due to its relative resistance to destruction by oxidative processes (133), but its ability to integrate into mem-

branes appears to be another key factor.

The incorporation of zeaxanthin into phospholipid mem-
branes systems has at least three known effects, which are not restricted to antioxidant activity: 1) zeaxanthin increases the ordering of the hydrocarbon chains, effectively modifying the structure of the membrane (128, 134), 2) zeaxanthin decreases the oxygen diffusion-concentration product, which controls the rate of chemical reactions with oxygen and should help to protect the fatty acids from oxidation (128), and 3) zeaxanthin (but not β-carotene) mechanically stabilizes model membranes and slows the flow of water across them (134). Analogous effects have been observed for zeaxanthin incorporated into natural biological membranes (135).

In plants, zeaxanthin has a well-accepted photoprotective role. When plants are exposed to excess light, zeaxanthin is synthesized and it helps to dissipate energy that cannot be used for photosynthesis so that the plant is protected from light damage. When the plant returns to moderate light levels, zeaxanthin must be removed or the energy continues to be dissipated and the normal efficiency of photosynthesis is diminished. In fact, zeaxanthin concentrations go through a daily cycle, low at night and high at midday, whereas β-carotene concentrations fluctuate less and are lower when the light is more intense. These and other observations have led to the conclusion that zeaxanthin is the carotenoid that protects the photosynthetic apparatus when photon flux density is high (136, 137). The accumulation of zeaxanthin to protect the primate fovea appears to be exploiting an ancient and ubiquitous evolutionary adaptation from the plant kingdom.

Although it is difficult to transfer the understanding of zeaxanthin’s photobiology directly from plants to primates, the data suggest that a very refined discrimination is being made by the retina. Zeaxanthin and lutein are structural isomers, differing only by the placement of one double bond (Figure 2). At the center of the fovea of humans (7) and Old World primates (8), zeaxanthin dominates over lutein, even though there is more than twice as much lutein in the blood (64, 138). Finally, note that much of the retinal zeaxanthin is a chiral isomer, meso-zeaxanthin, that is not present in the blood (139). In fact, it has been suggested that lutein may be converted into meso-zeaxanthin by the retina (139). Because lutein is more widely available in foods than zeaxanthin (140, 141), such a conversion would have the advantage of giving the retina a broader array of sources for its zeaxanthin.

Effects of deficiencies of antioxidant nutrients on the retina and RPE

Carotenoids

The retinas of monkeys fed carotenoid-deficient diets for ≥ 3 y are devoid of macular pigment (142). Microspectro-
metric measurements of the retinas revealed no detectable absorption of macular pigment carotenoids (PK Brown and DM Snodderly, unpublished observations, 1985). The retinas also developed abnormalities that were consistent with defects in the cones (143). First, the retinas were very fragile and prone to split in the region of the photoreceptor axons (layer PA in upper photo of Figure 1, also called Henle fiber layer), where lutein and zeaxanthin were depleted. The authors noted that this may indicate a deterioration of membrane integrity. This deterioration is consistent with results from studies of model membranes cited above, which show that zeaxanthin helps to stabilize membranes and protect the lipids from oxidation. Second, the cone pedicles were swollen, again suggesting stress of the cone photoreceptors.

The RPE was also affected. There were reduced numbers of RPE cells in the fovea and the remaining cells were flattened and cuboidal instead of normally columnar. The RPE cells were engorged with large amounts of lipofuscin. In summary, the cones were abnormal and the RPE was stressed and dying at an accelerated pace in the carotenoid-deficient animals.

These conclusions have to be considered to be tentative, because low carotenoid concentrations were not the only unusual aspects of the diets. The monkeys were fed high-fat diets for studies of the cardiovascular system. The diets contained no taurine. These additional factors could have contributed to some of the abnormalities observed.

Vitamin E

Monkeys fed diets deficient in vitamin E for 2 y developed degenerative changes in the macular region of the retina. The degeneration primarily involved the photoreceptor outer segments and cones were at least as severely affected as rods (144). There was extensive accumulation of lipofuscin in the RPE. Plasma vitamin E concentrations associated with these changes were generally < 2.3 μmol/L; unfortunately, retinal vitamin E was not measured. In this case, the deficiency state included feeding the animals an unusual, semipurified diet that contained no carotenoids.

Vitamin C

The effects of vitamin C deficiency on the retina have been studied in guinea pigs. The retinas of scurvy caused animals showed no pathologic changes in normal lighting but were more severely damaged by continuous bright light than were the retinas of normal animals (145). Other experiments with rats have shown that retinal ascorbate is oxidized during exposure to damaging light regimes and that supplemental ascorbate protects against.
light damage (146). These results are consistent with the idea that oxidation is involved in light damage of the retina and that ascorbate can provide some protection against the injury.

**Issues regarding the use of nutritional supplements: possible harmful effects**

The hope of improving their health has led many multivitamin supplement users in the United States to consume excessive amounts of some nutrients. A survey conducted in 1986 showed that the intake of riboflavin for the 95th percentile of adult supplement users was an astonishing 36 times the recommended dietary allowance (RDA) (147, 148). Another nutrient, iron, which is known to have strong oxidizing effects on the eye (12), was consumed by those at the 95th percentile in amounts five times the RDA. However, when attempts were made to evaluate the effect of supplement use on the risk for AMD, no evidence for protection was found (26, 33).

The concern is that excess amounts of some nutrients, such as flavins, could sensitize the retina and RPE and promote light damage and oxidation. Rats fed supplemental riboflavin in amounts two to four times the recommended dietary amount suffered loss of rod photoreceptors (149). It is thus important to consider the possibility that excessive nutritional supplementation may constitute a risk factor for AMD.

Unfortunately, current AMD epidemiology provides very limited estimates of the quantities of supplemental nutrients that are consumed by free-living study populations. The situation is complicated by the practice of fortifying prepared foods so that total vitamin and mineral intake is difficult to estimate. It is hoped that realization of the existence of both prooxidant and antioxidant effects of nutrients (150) will focus increased attention on these issues in future studies.

**Unintended interactions: areas for further study**

When humans consume supplemental α-tocopherol, plasma γ-tocopherol decreases (73). Because γ-tocopherol is generally considered to be the most potent form of vitamin E, the loss of γ-tocopherol would normally cause little concern. For people who smoke cigarettes, however, γ-tocopherol may have a special role. One of the principal oxidants in cigarette smoke is thought to be nitrogen dioxide (40), which is more effectively detoxified by γ-tocopherol than by α-tocopherol (151). Hence, one must ask whether smokers with their high risk of AMD would need to balance α- and γ-tocopherol for maximum benefit if they are considering using tocopherol supplements.

A similar concern for a balanced intake arises for carotenoid supplementation. A large clinical trial (the AREDS study) in the United States will be testing the effects of β-carotene on people who may be at risk for AMD. Although long-term studies have not yet shown evidence of interactions, Miccozzi et al (152) reported that short-term supplementation of the diet with 12–30 mg β-carotene/d reduces serum lutein concentrations in humans. This type of interaction needs to be studied more thoroughly because it implies that supplemental β-carotene, which is not normally found in the retina (65, 66), may unintentionally reduce the amounts of the macular carotenoids lutein or zeaxanthin that are available to the retina.

Animal studies have also documented interactions among dietary carotenoids. In chicks, supplementation of the diet with a relatively massive β-carotene dose reduced the sum of zeaxanthin and lutein in serum to ~41% of baseline values (116). The relative quantities of the interacting carotenoids may also be important. For rats, the utilization of β-carotene to form tissue vitamin A was increased by small amounts of dietary lutein but decreased by large amounts of lutein (153). The results were attributed to interactions within the gastrointestinal tract. These results illustrate the need for better understanding of the interactions among different carotenoids and the specific roles that each may play. However, to be most applicable to human concerns, animal studies will need to use more realistic dosages when evaluating these effects.

**Toward the prevention of age-related macular degeneration**

The information summarized above implies that many cases of AMD should be preventable. Smoking cessation, adequate intake of fruit and vegetables, and avoidance of excessive sunlight exposure are lifestyle factors that can reduce the risk of disease. An important step would be to make available an assessment procedure to determine each person’s biological status. Such an assessment should include measurement of blood and tissue concentrations of carotenoids and antioxidant vitamins at an affordable cost. Knowing their personal status would help motivate individuals to control their risk factors and perhaps modify their lifestyles. Nevertheless, some people, such as women with low ocular melanin, may remain at risk for reasons beyond their control. For their sake, future research should help to identify what contribution carotenoids and antioxidant vitamins can make to maintaining their vision and their quality of life.

I thank K Dorey, P Jacques, and R Hammond for helpful discussion of the scientific issues and M Mullan-Sandstrom and N Krinsky for contributions to the figures.

**REFERENCES**


10. Zhang L-X, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/
CAROTENOIDs, ANTIOXIDANT VITAMINs, AND AMD


