

BIOLOGIC MECHANISMS OF THE PROTECTIVE ROLE OF LUTEIN AND ZEAXANTHIN IN THE EYE

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■ **Abstract** The macular region of the primate retina is yellow in color due to the presence of the macular pigment, composed of two dietary xanthophylls, lutein and zeaxanthin, and another xanthophyll, *meso*-zeaxanthin. The latter is presumably formed from either lutein or zeaxanthin in the retina. By absorbing blue-light, the macular pigment protects the underlying photoreceptor cell layer from light damage, possibly initiated by the formation of reactive oxygen species during a photosensitized reaction. There is ample epidemiological evidence that the amount of macular pigment is inversely associated with the incidence of age-related macular degeneration, an irreversible process that is the major cause of blindness in the elderly. The macular pigment can be increased in primates by either increasing the intake of foods that are rich in lutein and zeaxanthin, such as dark-green leafy vegetables, or by supplementation with lutein or zeaxanthin. Although increasing the intake of lutein or zeaxanthin might prove to be protective against the development of age-related macular degeneration, a causative relationship has yet to be experimentally demonstrated.

CONTENTS

INTRODUCTION AND THE HISTORY OF LUTEIN AND ZEAXANTHIN IN THE EYE	172
Description of the Macular Region of the Retina	172
Identification of the Macular Pigment	172
Potential Role of Lutein and Zeaxanthin in Protecting the Eye	174
CHEMICAL AND PHYSICAL PROPERTIES OF LUTEIN AND ZEAXANTHIN	174
Chemical Properties	174
Physical Properties	178

BIOLOGICAL PROPERTIES OF LUTEIN AND ZEAXANTHIN	179
Light Filter; Chromatic Aberration	179
Membrane Properties	180
Antioxidation and Pro-oxidation	181
SOURCES OF LUTEIN AND ZEAXANTHIN	183
Dietary	183
Supplemental	184
MEASUREMENT OF MACULAR PIGMENT IN VIVO	184
Criteria for Measuring Macular Pigment in Vivo	184
Heterochromatic Flicker Photometry	185
Reflectometry	186
Fluorescence of Lipofuscin	187
Resonance Raman Spectroscopy	187
EPIDEMIOLOGICAL EVIDENCE FOR A ROLE OF LUTEIN AND ZEAXANTHIN IN VISION	188
Observational Epidemiology	188
Observational Epidemiology: Cataracts	189
Interventional Epidemiology: Age-related Macular Degeneration (AMD)	189
Interventional Epidemiology: Cataracts	190
Interventional Epidemiology: Other Eye Diseases	190
PROTECTIVE ACTIONS OF LUTEIN AND ZEAXANTHIN	191
CONCLUSIONS: EYE HEALTH AND CAROTENOIDS	192
Current Recommendations	192
Future Recommendations	193

INTRODUCTION AND THE HISTORY OF LUTEIN AND ZEAXANTHIN IN THE EYE

Description of the Macular Region of the Retina

In the middle of the retina there is a depression called the fovea, an area so rich in cone receptors that it permits us to have our maximal visual acuity. In primates, the fovea is a yellow, pigmented structure, which because of its color is called the macula lutea, or more commonly, the macula. This pigmentation is not due to the cones, but to the accumulation of carotenoids that are some of the major xanthophylls, or dihydroxy-carotenoids, found in plants. In cross-section, the macula can be readily characterized both by the depression in the retinal surface as well as the presence of blue-absorbing carotenoid pigments, as illustrated in Figure 1. The anatomy is described very well in a recent review (135).

Identification of the Macular Pigment

The first report that the yellow spot in the macula of human retinas might be a carotenoid appeared in 1945. George Wald dissected the foveal region of 10 human retinas, extracted them with chloroform, and reported that the spectrum

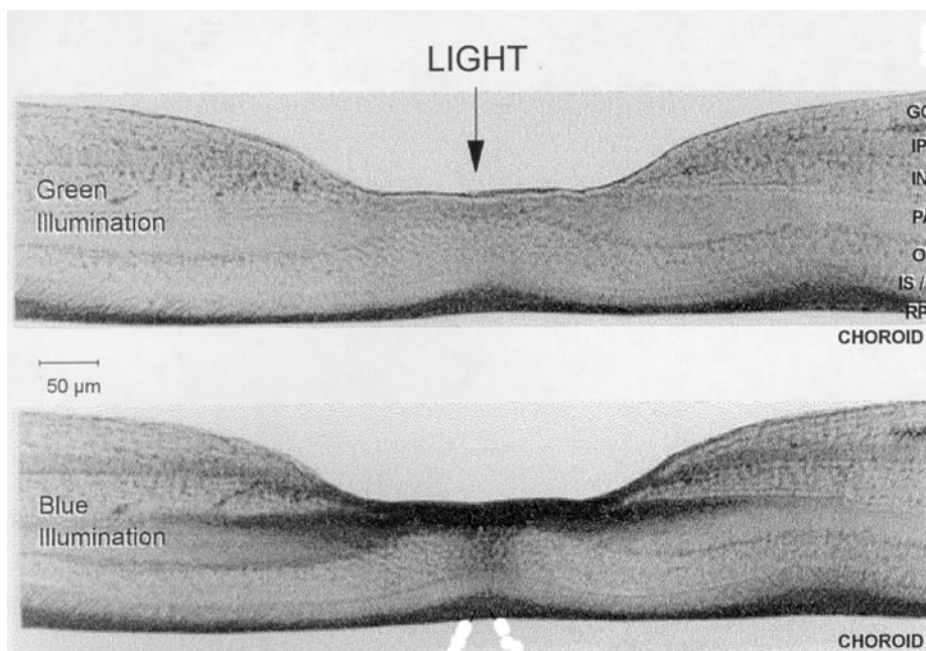


Figure 1 Cross-section of a human macula photographed in either a green or a blue light, indicating the absorption of blue-light by the macular pigment [from (135) and adapted with permission by the *American Journal of Clinical Nutrition*. © *Am. J. Clin. Nutr.*].

of the yellow pigment agreed quite well with the visual estimate of the macular pigment, derived from the differences in the log sensitivity of peripheral and foveal cones (150). Furthermore, the spectrum resembled that of a preparation of leaf xanthophyll, or lutein, and based on this property as well as its solubility, Wald concluded that the macula pigment was the xanthophyll lutein. This work was extended in a subsequent study (151), from which Figure 2 is derived. Fifty years after this observation, carotenoids were also identified in the lens of the human eye (162) and several years later, carotenoids were identified in virtually all of the tissues of the eye (12).

Bone & Landrum carried out the first chromatographic characterization of the macular pigment using a high performance liquid chromatography (HPLC) analysis to demonstrate that there were actually two xanthophylls present in the macula, namely lutein and zeaxanthin (20, 24). Shortly thereafter, they and others reported that there was a different ratio of lutein to zeaxanthin between the central fovea and the more peripheral regions, with zeaxanthin predominating in the central fovea and lutein in the periphery (20, 71). Subsequently, Bone et al. identified *meso*-zeaxanthin as an important component of the macular pigment (22). The structures of the major macular pigments are shown in Figure 3. More recently,

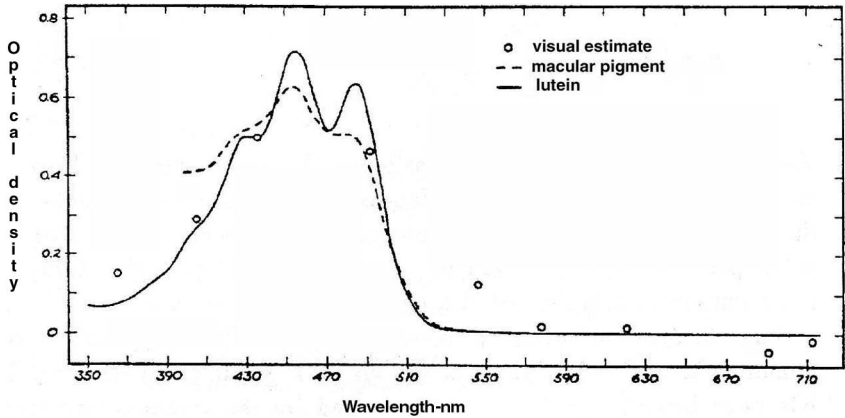


Figure 2 The absorption spectrum of lutein, of the pigment extracted from the macula, and a visual estimate of the macular pigment [adapted from (151) and used with permission of Kluwer Academic Publishers].

Khachik et al. have reported that some of the minor peaks observed in the HPLC analysis of the macular pigment consist of oxidation products of both lutein and zeaxanthin, such as 3'-epilutein and 3-hydroxy- β , ϵ -caroten-3'-one, as well as geometric isomers of the major pigments (81). The presence of *cis*-isomers in the retina is not surprising, since the macula is exposed to bright light, which is known to isomerize carotenoids. However, the presence of oxidative metabolites suggests that the pigments are susceptible to oxidation in the tissue, or that an active metabolic process takes place, with some potential for interconversions from among the reported intermediates.

Potential Role of Lutein and Zeaxanthin in Protecting the Eye

Although there is ample evidence that there is an epidemiological association between the ingestion of, or the blood levels of, lutein and zeaxanthin and the risk of age-related macular degeneration (AMD), the exact mechanism of protection is still unresolved.

CHEMICAL AND PHYSICAL PROPERTIES OF LUTEIN AND ZEAXANTHIN

Chemical Properties

Lutein and zeaxanthin are isomeric carotenoids that differ chemically from one another in subtle ways (95). Thus, it might be surprising to a novice in the field that these two carotenoids are not interchangeable wherever they have a functional

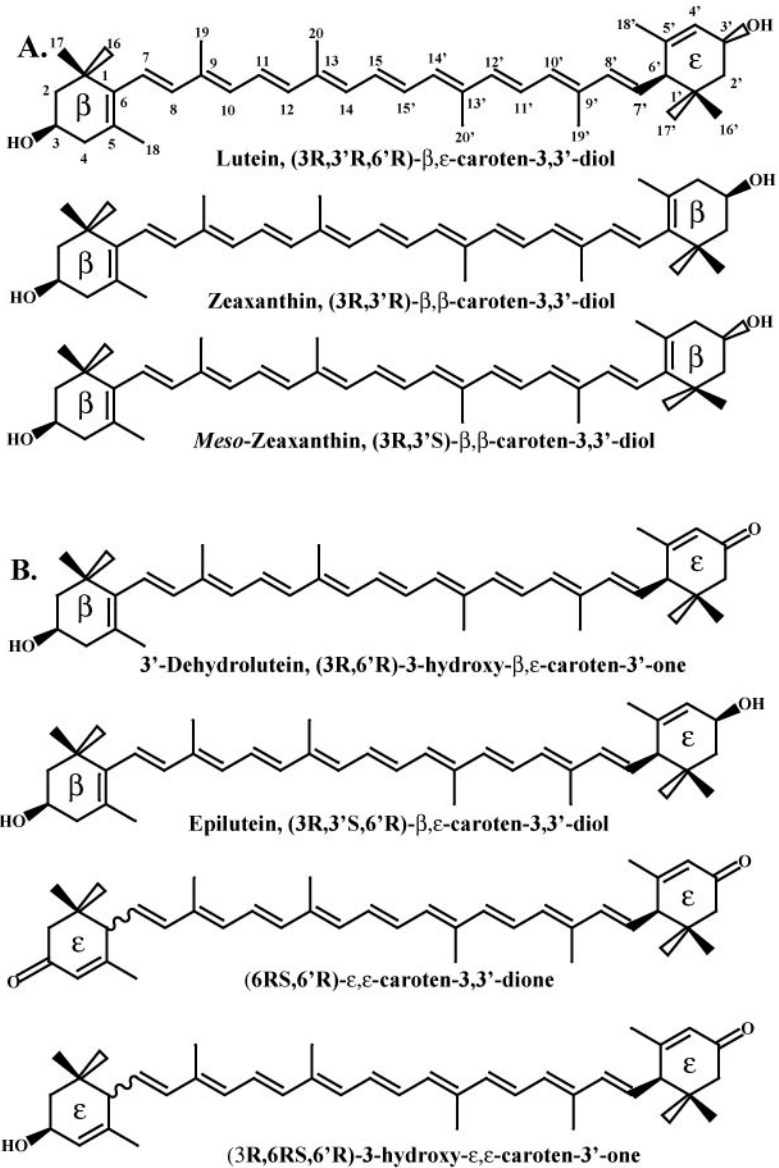


Figure 3 Structures of the major pigments isolated from the human macula (A) as well as minor xanthophylls found in the retina that may be involved in metabolic interconversions in the formation of *meso*-zeaxanthin (B).

role. Experience has shown us that we must look carefully at the nature of the subtle differences and their consequences (59, 60).

CHARACTERIZATION Any description of the characteristics of these two molecules must originate with a review of their chemical structures (Figure 3A). Lutein and zeaxanthin are both dihydroxy-carotenoids with the ionone ring systems being substituted at both the 3 and 3' carbon.

In zeaxanthin, the less abundant of these two isomers in most plant sources (60), the ionone rings are both β types. The β -ionone ring double bond is found between the C5 and C6 carbons, placing it in a position to interact, albeit weakly, with the conjugated polyene chain. A strong steric interaction between the C18 methyl found on C5 of the ionone ring and the hydrogen on C8 constrains the ring double bond to an angle of about 40° to the plane of the conjugated polyene chain (27, 31). The β -ionone ring double bond is therefore functionally isolated and behaves largely independently of the conjugated system. This has spectroscopic as well as chemical implications for zeaxanthin (*vide infra*). The carbons bearing the two hydroxyl groups share an identical R stereochemical configuration in the most common form of zeaxanthin, 3R, 3'R-zeaxanthin, that is found in most higher plants (153). The other stereoisomers of zeaxanthin (3S, 3'S-zeaxanthin and 3R, 3'S-*meso*-zeaxanthin), while less common, have been identified in a number of animals (126–128), including humans where a significant amount of 3R, 3'S-*meso*-zeaxanthin is present, concentrated in the central retina (*vide infra*). It is thought that these isomers, when found in animal tissues, are the result of biochemical transformations and are not dietary in origin.

Lutein has both a β -ionone ring and an ϵ -ionone ring. The presence of the hydroxyl groups at both the 3 and 3' carbons suggests that a close similarity in physical properties exists between lutein and zeaxanthin. The ϵ -ionone ring has a C4-C5 double bond and an allylic 3'-hydroxyl group. Interestingly, in the predominant form of lutein, (3R,6'R, 3'R)- $\beta\epsilon$ -carotene-diol (5), the designation of the stereochemical configuration of the ϵ -ring hydroxyl (R) is identical to that of the β -ring because of the Cahn-Ingold-Prelog rules (50). However, the ϵ -ring hydroxyl group is oppositely directed with respect to the hydroxyl group in the β -ionone ring. As shown in Figure 3A, the 3'-hydroxyl of the zeaxanthin ring projects forward from the surface of the page whereas the 3'-hydroxyl of lutein is folded back away from the plane of the page. This is a major stereochemical distinction between the dominant forms of lutein and zeaxanthin. The relative orientation of the hydroxyls may be a factor of some importance for specific recognition of these two isomers by proteins (142), and it may also influence the preference in site selection exhibited by these carotenoids when they are incorporated into membranes (*vide infra*) (62). Another significant feature resulting from the presence of the ϵ -ionone ring in lutein is that C6', which is attached to the polyene chain, is a stereocenter. The consequence of a tetrahedral sp^3 hybridized carbon at C6' in the ϵ -ionone ring is that a slight rotation about the C6'-C5' bond can relieve strain caused by the C18' methyl group. This is not possible for the β -ionone ring where the double bond constrains the geometry between C6-C5. The consequence of the β -ionone

versus ϵ -ionone ring substitution is that the β hydroxyl groups in zeaxanthin and lutein are directed in an axial direction whereas that of the lutein ϵ group is directed equatorially with respect to the ring plane.

REACTIVITY, SOLUBILITY, POLARITY AND AGGREGATION The presence of the hydroxyl groups makes lutein and zeaxanthin distinctly more polar than their respective carotene analogs, α - and β -carotene. This is demonstrated dramatically by their relative retention times on both normal and reversed-phase chromatographic columns where the difference in retention times is due primarily to polarity (39). The ratio of the retention times of the carotenes to those of the xanthophylls is approximately 4:1 on C-18 (octadecylsilane) derivatized reversed-phase HPLC columns. As would be expected, the polarities of lutein and zeaxanthin are very similar, and baseline chromatographic separation is not easily achieved. This has resulted in a tendency of many researchers to report combined lutein/zeaxanthin values (129). Lutein and zeaxanthin are most soluble in nonpolar or dipolar solvents such as hexane, benzene, ethers, methylene chloride, and chloroform. They are also soluble in alcohols generally. The solubility of lutein and zeaxanthin in methanol is less than in alcohols having long alkyl chains.

In natural systems, lutein and zeaxanthin are found in many different chemical environments (59, 60). Much of the lutein and zeaxanthin in the leaves of plants is protein-bound. In fruits and flower petals, the xanthophylls are esterified and are concentrated into chromoplasts where they are found to be solubilized in the membranes (59). In humans and higher animals, lutein and zeaxanthin are accumulated in lipophilic tissues (79, 130) such as adipose tissue and are carried in the blood by the lipoproteins, probably in a nonspecific manner similar to cholesterol. Lutein and zeaxanthin are distributed equally between LDL and HDL fractions in human blood, in contrast to the hydrocarbon carotenoids that are preferentially found in LDL fractions, up to 75% (51). The approximate concentration of lutein in human tissues is: serum, 0.1–1.23 μM ; liver, 0.1–3.0 μM ; kidney, 0.037–2.1 μM ; and lung, 0.1–2.3 μM (6). In the human retina, the concentration of the pigments reaches its highest levels, between 0.1 and 1 mM (93), providing solid evidence for active uptake or storage (20). It is unclear whether the carotenoids are protein-bound or are incorporated into the membranes of nerve fibers (11, 17, 160).

OXIDATION AND OXIDATION/REDUCTION PRODUCTS Oxidation of alcohols to produce carbonyl functional groups readily occurs both *in vitro* and *in vivo*. Secondary alcohols such as those in lutein and zeaxanthin produce ketones upon oxidation. In lutein, the alcohol of the ϵ -ionone ring is allylic to the double bond of the ring and consequently is more readily oxidized than the β -ring hydroxyl groups of either lutein or zeaxanthin. *In vitro* oxidation of lutein with MnO_2 produces 3-hydroxy- β,ϵ -carotene-3'-one (3'-dehydrolutein) in 80% yield (30); this compound has also been identified in human retinas (81). Zeaxanthin also reacts with MnO_2 *in vitro* but forms a multitude of products, including apocarotenals and epoxides, in addition to oxidation of the alcohol functional group.

The carotenoid polyene chain can also be oxidized by reaction with a peroxy radical or similar species (61). Loss of a single electron from the conjugated chain will result in the formation of a cation radical (155). In vitro, a carotenoid cation radical can react further and act as a potent oxidant itself. Thus, one reaction of the cation radical is reduction to regenerate the carotenoid (106). Carotenoids can also react with peroxy radicals by a hydrogen abstraction mechanism generating a neutral carotenoid radical. In some instances, direct addition of a peroxy radical to the polyene chain may occur (164). Carotenoid radicals are not as reactive as many carbon-centered radicals due to the conjugation present in the polyene system. Carotenoid radicals can react, abstracting a hydrogen atom from a suitable donor, again regenerating the carotenoid and producing a secondary radical, or they can react with O₂ to generate carotenoid-peroxy radicals (164).

The in vivo oxidation of the hydroxyl groups present in lutein and zeaxanthin to produce corresponding keto carotenoids has been reported to occur in several biological systems, including that of humans. (3R, 3'S, 6'R)-Lutein (3'-epilutein), 3-hydroxy- β,ϵ -caroten-3'-one (3'-dehydrolutein), 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -caroten-3,3'-dione, and ϵ,ϵ -caroten-3,3'-diol have been identified in human serum and milk (80, 83). It is likely that, as in other animals, these carotenoids probably do not originate from the diet but are the result of metabolism or degradative oxidation, possibly occurring in the liver; however, experiments have not established this.

3'-Epilutein and ϵ,ϵ -caroten-3,3'-diol are thought to be formed by reduction of 3-hydroxy- β,ϵ -caroten-3'-one and 3'-hydroxy- ϵ,ϵ -caroten-3-one or ϵ,ϵ -caroten-3,3'-dione, respectively. Such an oxidation/reduction pathway has also been suggested to explain the occurrence of *meso*-zeaxanthin, which is found in many animals (97). *Meso*-zeaxanthin comprises a very significant proportion of the macular pigment and, like epilutein, may originate via an oxidation/reduction pathway involving formation of 3-hydroxy- β,ϵ -carotene-3'-one (Figure 3B) followed by reduction. 3-Hydroxy- β,ϵ -carotene-3'-one has been identified in human retinal tissue and so the presence of this intermediate compound supports this proposal (81, 82). An alternate hypothesis suggests that a double bond isomerization may occur, converting lutein directly to *meso*-zeaxanthin (82).

Physical Properties

LIGHT ABSORPTION The ability of carotenoids to absorb light arises from the presence of a conjugated polyene chain. The wavelength maximum of the absorption band is related to the extent of the conjugation in the polyene chain (27, 87). Both lutein and zeaxanthin have nine conjugated double bonds in the polyene chain. Lutein has an absorption maximum of 445 nm in ethanol whereas that of zeaxanthin is 451 nm. In addition to the length of the polyene chain, the nature of the end-group attached to the polyene chain has significance for the spectral characteristics of carotenoids. The small difference in the wavelength of maximum absorption for lutein and zeaxanthin is due to the interaction of the double bonds

in the β -ionone ring(s) with the polyene chain. The β -ring double bond, which might seem to be conjugated with the polyene chain, interacts with it only weakly. Nevertheless, the presence of β -ring double bonds induces a modest red shift in the carotenoid absorption spectrum.

ISOMERIZATION Lutein and zeaxanthin are constitutional isomers and differ in the position of a double bond in one of the ionone rings. Each has a variety of different stereoisomers. These include the geometrical Z- and E- isomers (often referred to as *cis* and *trans* isomers). Many are noted to occur in human serum. The presence of a Z-bond in an otherwise all-E polyene chain of the carotenoid causes the molecule to have a pronounced V-shape and alters the visible spectrum. Because of the methyl substitution of the carotenoids, only 9-, 13-, and 15-Z isomers (and 9'- and 13'-) are encountered (165). Isomers containing multiple Z-bonds are also possible. Minor quantities of lutein Z isomers are detectable in human serum and the human retina (80, 81). Both 9-Z- and 13-Z isomers have been reported.

In addition to the geometrical isomers due to the presence of Z-bonds, there exist stereoisomers that are the result of the absolute configuration around the stereocenters present in these two carotenoids (139). Lutein has three stereocenters whereas zeaxanthin has two stereocenters. There is only a single lutein stereoisomer, (3R, 3'R, 6'R)- β , ϵ -carotene-3,3'-diol, present in significant quantities within the retina, although minor amounts of so-called epilutein, (3R, 3'S, 6'R)- β , ϵ -carotene-3,3'-diol, have been reported. Zeaxanthin exists in three stereoisomeric forms that result from the configurations at its two stereocenters. They are 3R, 3'R- β , β -carotene-3,3'-diol; 3S, 3'S- β , β -carotene-3,3'-diol; and 3R,3'S- β , β -carotene-3,3'-diol, respectively (122). All three zeaxanthin stereoisomers are known in nature, but of these, the 3R, 3'R is the dominant form and is found as the single isomeric zeaxanthin in higher plants that are common human food sources (97). In the human retina, 3R, 3'R- and 3R, 3'S-zeaxanthin are present in nearly equal abundance (81). The 3S, 3'S-isomer has also been reported to be present, but at very low levels. The analysis of the distribution of the zeaxanthin isomers across the retina shows that the highest levels of 3R, 3'S-zeaxanthin are found in the central macula and diminish to very low levels in the peripheral retina (21). These observations support the proposal that this *meso*-isomer is the result of metabolic action occurring within the retina.

BIOLOGICAL PROPERTIES OF LUTEIN AND ZEAXANTHIN

Light Filter; Chromatic Aberration

Because of their very high absorptivity, lutein and zeaxanthin in the inner retina form a very efficient filter for blue-light that reaches the back of the eye (Figure 1). The macular pigment is chiefly accumulated in the Henle fiber layer composed

of the photoreceptor axons that overlay the photoreceptors themselves (135). The macular carotenoids attenuate blue-light prior to its reaching the delicate functional structures including the photoreceptors, the retinal pigment epithelium, and the underlying choriocapillaris. It is deemed highly probable that this reduction in blue-light intensity, which can be as great as 90% and is normally about 40%, could significantly reduce the oxidative stress on the retina and may be sufficient to account for the reduction in risk of AMD that has been observed in some epidemiological studies (92). Studies have now demonstrated that the macular carotenoids protect the retina from damage due to acute exposure to blue-light (125). The extrapolation of this protective role to chronic low dose exposure to blue-light is reasonable, but unequivocal proof remains elusive.

Chromatic aberration arises in optical systems when refraction of different wavelengths occurs to different extents, producing multiple overlapping images most often characterized by the presence of colored fringes and a loss of image sharpness. The reduction of blue fringes as a result of the absorption by the macular pigment has been suggested as a possible advantage resulting from the presence of these yellow pigments (119, 156). The extent to which chromatic aberration is a significant factor limiting the acuity of the human eye is not established, and the role of the macula pigment in improving retinal image has been questioned (103).

Membrane Properties

Haidinger's brushes The precise location of the macular pigment molecules that are present in the Henle fiber layer of photoreceptor axons is not known. Observations using polarized light reveal that the molecules are highly organized (136). The retinal structure is clearly capable of providing them with a preferential, rather than random, alignment. The main evidence for this comes from the entoptical phenomenon known as Haidinger's brushes (45, 108). The brushes can be seen by most subjects if they gaze through a plane-polarizing filter at a surface uniformly illuminated by blue-light. The brushes appear as a slightly darker, hourglass-shaped figure at the fixation point. The orientation of the figure is perpendicular to the electric field vector of the light. With white light, the brushes appear faintly yellow. The explanation of the brushes is to be found in the linear nature of the conjugated polyene chain of carotenoid molecules that renders them dichroic: They absorb blue-light maximally when the electric field vector is parallel to the chain and minimally when it is perpendicular (16). In addition, a preferential alignment of each carotenoid molecule perpendicular to a line connecting it to the center of the fovea is required. Such an alignment places the molecules perpendicular to the Henle fibers that run in radial directions outward from the center of the fovea (17). Incorporation of the carotenoids transversely in the cylindrically shaped membranes of these fibers is one possible arrangement that is consistent with Haidinger's brushes (18).

An alternative hypothesis is that the carotenoids are protein-bound within the photoreceptor nerve axon (11). To account for Haidinger's brushes, the proteins would have to be correctly oriented in relation to the axis of the nerve axon so that the carotenoids were held at, or close to, 90° to the axis. One potential candidate protein, tubulin, is found in abundance in the Henle fiber layer. It has been suggested that the protein-bound carotenoids are associated with the microtubules that run axially along the cone axon (11). However, our current knowledge of lutein or zeaxanthin binding proteins is extremely limited. Only one, occurring in the silkworm larva, has been isolated and characterized (142). A report of a specific lutein binding protein isolated from the human retina is quite exciting, but its characterization has not been completed (160). It is not yet known whether the amount of protein would be sufficient to bind all of the macular carotenoids, nor is anything known about its distribution within the retina or its ability to orient carotenoids within the nerve fibers. Indeed the fundamental question of whether it is a transport protein or a binding protein remains to be answered. Interestingly, upon binding with this protein, lutein exhibits a shift in its absorption maximum to 460 nm, the same wavelength maximum that is determined psychophysically for the macular pigment.

SOLUBILITY AND ORIENTATION IN MEMBRANES The possibility of lutein and zeaxanthin being solubilized in the membranes of Henle's fibers, with the required orientation to account for Haidinger's brushes, is supported by several studies (16, 17). Bone & Landrum (18) incorporated these two carotenoids in phosphatidylcholine liposomes in proportions similar to those found in the center of the fovea. The absorbance spectra of these membrane-bound carotenoids were in remarkably good agreement with macular pigment spectra determined psychophysically both by heterochromatic flicker photometry and by a method based on the dichroic properties of the macular pigment.

The orientation of lutein and zeaxanthin relative to the plane of the membrane appears to be different for lutein and zeaxanthin (62, 112). Zeaxanthin becomes incorporated in the membrane, spanning the lipid bilayer with the hydroxyl group at each end apparently hydrogen-bonded in the polar head group regions. This is precisely the orientation that would account for Haidinger's brushes. Lutein, on the other hand, appears to adopt a less completely oriented configuration. Theoretical calculations indicate that the average angle that the carotenoid molecules assume normal to the membrane surface should be less than $\sim 55^\circ$ (18). This value is completely consistent with an experimentally determined average value of about 42° for carotenoids incorporated in Langmuir-Blodgett films (107).

Antioxidation and Pro-oxidation

The term antioxidant is applied to many different biomolecules and has no single exclusive definition. A good working definition was proposed in the recently

published National Academy of Sciences report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (6):

“A dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on the normal physiological function in humans.”

IN VITRO EVIDENCE Several highly oxidizing species are generated in biological systems, including singlet oxygen, hydroxyl radical, superoxide, hydrogen peroxide, organic hydroperoxides, and peroxy radicals (64). These species can react with carotenoids by three distinctly different pathways: electron transfer, hydrogen abstraction, and radical addition. Carotenoids, including lutein and zeaxanthin, have long been described as natural antioxidants (32, 54, 75, 88, 113). With the exception of their ability to quench singlet oxygen, there is a paucity of evidence demonstrating an *in vivo* antioxidant function of carotenoids (164).

Carotenoids are easily oxidized, losing an electron from the polyene chain to form a radical cation (56, 84). “Preferential” oxidation of a carotenoid to form a radical cation, which in turn reacts with ascorbate regenerating the unaltered carotenoid, is a hypothesis that may explain how carotenoids prevent irreversible oxidation of polyunsaturated fatty acids, nucleic acids, and proteins (106). On the basis of *in vitro* reaction chemistry, Truscott (146) has proposed that the hydrophobic radical cation of tocopherol, formed within membranes by reaction with radical oxygen species, could be regenerated by membrane-bound carotenoids, such as zeaxanthin, forming a carotenoid radical cation that in turn would be reduced by ascorbate external to the membrane system. This *in vitro* reactivity is consistent with the antioxidant hypothesis and provides a solid theoretical basis to encourage further investigation of potential antioxidant function of carotenoids.

The argument for a radical addition pathway in which a peroxy radical adds directly to the carotenoid was put forward by Burton & Ingold (33). They showed that this reaction mechanism results in a carbon-centered carotenoid radical that can react directly with O₂. This secondary reaction generates a carotenoid peroxy radical whose formation will depend upon the oxygen partial pressure. At sufficiently high partial pressures of O₂ this carotenoid peroxy radical can generate additional radicals by cleavage of the resulting peroxy bond. This O₂-dependent step is often referred to as a pro-oxidant effect since it generates more radicals than it consumes. Martin and coworkers (102) have elegantly demonstrated that at low partial pressures many carotenoids are dramatically antioxidant, interrupting substrate oxidation by peroxy radicals. However with increasing oxygen concentration, secondary oxygen-generated peroxy radical formation becomes important and can, at sufficiently high (e.g., 1 atm) pressures, result in loss of antioxidant behavior. At physiological partial pressures of oxygen and carotenoid concentrations, it appears that the pro-oxidant step is a sufficiently small effect that carotenoids will have only net antioxidant capability.

IN VIVO EVIDENCE In the retina, the possibility that carotenoids act as antioxidants remains an issue of great interest. Careful analysis of rod outer segments isolated from the perifoveal and peripheral regions of the retina by both Rapp et al. (117) and Sommerburg et al. (138) shows that lutein and zeaxanthin are present in these cellular structures. This is an essential requirement for the antioxidant function because it is in the outer segments and the retinal pigment epithelium where the effects of oxidation appear to produce the greatest damage. Prior to this discovery, the largest concentration of lutein and zeaxanthin was thought to reside in the Henle fiber layer, approximately 100 μm removed from the outer segments, where it was visibly discernible. It has proven infeasible to isolate the cone photoreceptor outer segments, which are the site of greatest morphological change in the development of AMD, to determine whether they too contain lutein and zeaxanthin. Nevertheless, the hypothesis that lutein and zeaxanthin function as antioxidants is consistent with the observation that the retina is a highly aerobic tissue with an exceptionally high rate of metabolism, and there is significant evidence that AMD results from oxidative degradation and radical processes occurring in the outer segments and retinal pigment epithelium (RPE) (8).

The light-filtering capability of the lutein and zeaxanthin found in the inner Henle fiber layers is a passive antioxidant function. It reduces the rate of radical generation by blue-light and therefore the chances of peroxy radical-induced oxidative chain reactions. This function for the macular pigment was first put forward by Kirschfeld (86). Although not a chemical antioxidant mechanism in the usual sense, this role may be extremely important. The oxygen partial pressure in most tissues is relatively low, ca. 30 mm Hg or lower. In the outer segments of the retina, the oxygen partial pressure is very high and may result in a very high rate of singlet oxygen formation through a blue-light-initiated photosensitization step (66) and lead to irreversible damage to various cell structures.

In addition to the principal retinal components of the macular pigment, lutein, zeaxanthin, and *meso*-zeaxanthin, several minor carotenoids are present in the retina. The presence of oxidative metabolites in the retina, although not proof of carotenoid antioxidant activity, is at least consistent with such a hypothesis. Likewise, the presence of *meso*-zeaxanthin, 3S, 3'S-zeaxanthin, and epilutein, all of which could be formed via an oxidation/reduction pathway, are consistent with an active participation in oxidative metabolism within the retina.

SOURCES OF LUTEIN AND ZEAXANTHIN

Dietary

The two foods that have the highest amount of lutein are spinach and kale (15, 96, 120). Other major dietary sources include broccoli, peas, brussels sprouts, and egg yolk. Although the values found in eggs are relatively low, recent data suggest that

lutein and zeaxanthin from this food source are highly bioavailable (72, 140). In fact, the best sources of zeaxanthin are egg yolks, corn, orange peppers, orange juice, oranges, and honeydew (137). Data on the lutein content of foods frequently include zeaxanthin and are reported as lutein + zeaxanthin, making examination of specific effects of dietary lutein difficult. However, in terms of food sources, human metabolism, and tissue storage, lutein and zeaxanthin are similar. The intake of lutein and zeaxanthin in the United States is generally lower than that of β -carotene or lycopene, but levels of about 3 mg/d can be easily achieved with a high fruit and vegetable diet (161). Although lutein and zeaxanthin are considered to be major carotenoids in the U.S. diet, data from the 1987 and 1992 National Health Interview Surveys suggest that there was a decline in lutein intake, particularly from dark-green leafy vegetables (109).

Supplemental

Currently, health food stores offer a variety of supplement products that contain lutein, or lutein diester, in amounts of 6–25 mg/capsule. In addition, many manufacturers of multivitamin supplements are adding lutein to their products, although at levels of only 0.25 mg/capsule. But an editorial saying that it is still too early for recommending lutein supplements has been published (98).

MEASUREMENT OF MACULAR PIGMENT IN VIVO

Criteria for Measuring Macular Pigment in Vivo

The growing body of evidence linking low levels of macular pigment with an increased risk of AMD (9, 23, 53, 131) underscores the need for reliable methods of measuring the amount and distribution of macular pigment in the retina. Several such methods exist; those that are psychophysical are subjective in nature (18, 91, 105, 115, 121), whereas other methods are objective (13, 43, 85). Methods are to be found in both categories that provide the density distribution of macular pigment over a reasonably wide area, while others provide the density only within a limited region, for example the central 1° of the retina. The former reveal distributions that vary widely from subject to subject. For some, the distribution is sharply peaked at the center of the fovea and for others it is shallower and broader (69). In some cases a “volcano-like” distribution emerges, with a pronounced dip in the center of the fovea (57). These differences are indistinguishable by methods that measure the macular pigment density only within a small central area. Furthermore, these methods typically measure the density at the center of the retina relative to some eccentric location, e.g., at 7°, where the density is assumed to be negligibly small. If the subject’s macular pigment density distribution is broad, the assumption may not be strictly justified.

Heterochromatic Flicker Photometry

By far the most commonly employed psychophysical method of measuring macular pigment density is heterochromatic flicker photometry (HFP). The method is based upon the altered spectral sensitivity of that part of the retina that is overlain with the blue-light-absorbing macular pigment layer. The spectral sensitivity at each point is reduced in the wavelength range ~ 400 to 520 nm by an amount that depends on the corresponding macular pigment optical density. Thus the reduction in sensitivity shows a maximum at 460 nm, the peak wavelength in the macular pigment optical density spectrum (18).

Flicker photometry was originally developed as a method for comparing the luminosities of two similar light sources, e.g., a standard and a substandard. The lights were presented in counter-phase with each other in a visual field, producing a sensation of flicker. When the luminosities were matched, the field appeared steady. In HFP, as adapted for measuring the macular pigment, the stimulus is a visual field, typically 1° to 2° in diameter, alternating between 460 nm (blue) and a reference wavelength, typically 540 nm (green), where the macular pigment optical density is essentially zero (157). The intensity of the blue-light is controlled by the subject. The frequency of alternation is critical. At low frequencies, the individual colors are discernible. As the frequency is increased, color fusion occurs, resulting in a flickering, turquoise-colored stimulus. At a higher, critical frequency, the sensation of flicker can be eliminated, or minimized, by adjusting the blue-light intensity to a particular value. (Above this critical frequency, flicker is eliminated over a range of blue-light intensities that increase with frequency.) It is assumed that the sensation of flicker is minimized or eliminated when the blue and green lights are of equal luminosity at the level of the photoreceptors. Thus a subject with a higher macular pigment density will require a higher blue-light intensity to compensate for the attenuation of blue-light by the macular pigment.

Other factors, beside macular pigment, influence the amount of blue-light needed to minimize flicker. Older subjects tend to need a higher intensity to compensate for the age-dependent absorption of blue-light by the lens (38, 110, 123, 124). In addition, the relative sensitivity of the cones to blue- and green-light may vary among subjects (152). In order to eliminate these potential sources of error, the subject makes two series of measurements: one while gazing directly at the center of the stimulus, and another while gazing at a fixation mark that is located at, say, 8° from the center of the stimulus. The stimulus is then imaged on the retina 8° from the center of the fovea, an area assumed to be virtually free of macular pigment. The blue-light intensity setting that the subject makes will, however, be affected by the other factors mentioned above. An implicit assumption in the method, backed by supporting evidence (158), is that the relative sensitivity of the cones to blue- and green-light is the same in the two retinal locations where the stimulus is imaged. Accepting this provision, the macular pigment optical density (at 460 nm) is given by the log ratio of intensity settings for the two viewing conditions. Certainly when the experiment is repeated with different wavelengths in

place of the 460 nm light, a macular pigment absorption spectrum can be generated that is remarkably similar to that obtained spectrophotometrically from lutein and zeaxanthin mixtures (18).

A remaining question is whether the macular pigment optical density value obtained by this method represents an average over the area of the retina where the stimulus is imaged. There is evidence that the sensation of flicker, or lack thereof, is determined by receptors at the edge of the stimulus image (154). If this is the case, a 1° stimulus will determine the macular pigment optical density at 0.5° eccentricity from the center of the fovea. By employing stimuli of different diameters, a pigment density profile across the central retina may be generated. This may also be achieved using narrow annuli of varying diameters as stimuli.

There is a technique similar to HFP in which the stimulus consists of alternating blue and green bars that move across the visual field, and whose relative luminances can be adjusted (105). When the luminances are matched, the perception of motion is minimized. As with HFP, the test is performed with the stimulus viewed centrally and peripherally in order to determine the optical density of the macular pigment. The spatial distribution of the macular pigment can be obtained by viewing stimuli at various eccentricities.

Reflectometry

The earliest methods of measuring the macular pigment objectively were based on the observation that the spectra of light reflected from the central and peripheral parts of the retina were different (26). Prior to measuring the reflectance spectra, the retina was exposed to light of sufficient intensity to bleach the visual pigments in the photoreceptors. Bleaching was necessary because the distribution of visual pigments is not uniform across the retina. The original investigators, Brindley & Willmer (26), attributed the remaining differences between the central and peripheral reflectance spectra mainly to the macular pigment. They assumed that light incident on the retina was reflected from layers posterior to the macular pigment—the RPE and choroid—and therefore had passed twice through the macular pigment before exiting the eye. Indeed their resulting spectra were consistent with the macular pigment optical density spectrum. In a later study, Van Norren & Tiemeijer, making the same assumption, obtained excellent agreement between the macular pigment optical density spectra obtained by the reflectance method and psychophysically (148).

Delori & Pflibsen (44) and van de Kraats et al. (147) introduced more sophisticated reflectance models that included the effects of absorption by blood, melanin, macular pigment, and ocular media, as well as tissue scattering. Using curve-fitting routines, both groups were able to determine the contribution of each absorbing component to the reflectance spectra.

In the studies described above, reflectance measurements were made only at discrete and widely separated retinal locations and therefore could not provide information on the spatial distribution of macular pigment. However, this can be

achieved using imaging reflectometry. Kilbride et al. (85) used a retinal camera to obtain digital images of the bleached fundus using two wavelengths, 462 and 559 nm, at which macular pigment optical density is close to the maximum and zero, respectively. After alignment of the two images, a density map of the macular pigment was obtained by taking the difference between the logarithms of corresponding pixel values in the two images. In some studies, the use of a reference wavelength was eliminated. Abadi & Cox (1) obtained single images of the retina at 460 nm and attributed the lower reflectance in the center of the retina entirely to the macular pigment. The method of reflectometry can be adapted for use with a retinal camera that uses film, and this modification was used by Bour et al. (25) to examine the distribution of macular pigment in children. An added complication is that the developed film must be scanned and digitized.

The scanning laser ophthalmoscope is an instrument capable of providing the highest quality retinal images, and it too has been adapted for the purpose of generating spatial density distributions of the macular pigment. Wüstemeyer et al. (159) obtained high resolution images at the 488 and 514 nm wavelengths available from an argon laser, and demonstrated that subjects with dry AMD had lower levels of macular pigment than normal subjects without ocular pathology. When using such wavelengths, corrections must be made to the calculated macular pigment optical densities to account for the relative extinction coefficients of the macular pigment at these two wavelengths.

Fluorescence of Lipofuscin

The so-called aging pigment, lipofuscin, tends to accumulate with age in the RPE, posterior to the macular pigment layer. Its autofluorescent properties have been exploited in a novel method that provides a single-pass measurement of the optical density of the macular pigment (43). The bleached retina is illuminated in turn by two different exciting wavelengths, e.g., 470 and 550 nm, that are differentially absorbed by the macular pigment. Each wavelength causes the lipofuscin to fluoresce, and the intensity of the emitted light is measured at ~ 710 nm, a wavelength outside the absorption limits of the macular pigment. The differential absorbance of the two exciting wavelengths by the macular pigment can be obtained from these intensity measurements, leading to a single-pass measure of its optical density. It is, of course, necessary to take into account the difference in the quantum efficiency of fluorescence of lipofuscin at 470 and 550 nm. The method may be of limited use in the case of young subjects and those with AMD because both groups tend to have low amounts of lipofuscin in the RPE (42, 46).

Resonance Raman Spectroscopy

Strong, resonance-enhanced Raman signals are emitted by lutein and zeaxanthin when these carotenoids are excited by light in the wavelength range 450 to 550 nm. Originally the 488 or 514.5 nm lines of an argon laser were used as the excitation source for the macular pigment (13). The resulting Raman-scattered light, of

slightly longer wavelength than the excitation light, was imaged onto the entrance slit of a Raman spectrometer. With postmortem retinas, the Raman intensities were found to be highly correlated with the carotenoid content evaluated by HPLC. In another study, levels of macular pigment were found to be 32% lower in subjects with AMD compared with normal elderly control subjects (14). More recently, the laser light source has been replaced with a filtered mercury arc lamp, and the Raman-scattered light from the retina has been imaged with a charge-coupled detector camera (57), thereby creating a density map of the macular pigment. So far this technique has been applied only to human donor eyecups, but will no doubt be further refined for use with living subjects.

EPIDEMIOLOGICAL EVIDENCE FOR A ROLE OF LUTEIN AND ZEAXANTHIN IN VISION

Observational Epidemiology

The evidence supporting a relationship between lutein and zeaxanthin and AMD, which has been reviewed numerous times in the past (7, 92, 99, 114), was based on the presence of lutein and zeaxanthin in the macula, and the relative decrease in these carotenoids in the macula of AMD patients (23). In 1992, the Eye Disease Case-Control Study Group reported that an increased risk of neovascular AMD was associated with decreased levels of serum carotenoids (52), and in the following year, they reported that lutein and zeaxanthin, as well as α - and β -carotene and cryptoxanthin, were responsible for the reduced risk, with patients in the group with the highest level of plasma lutein/zeaxanthin having the largest decrease in risk for AMD (53). In a subsequent study, Seddon et al. reported that intake of lutein and zeaxanthin from dark green, leafy vegetables was associated with a very significant decrease in the relative risk of developing AMD (131). In this case-control study, subjects who were in the highest quintile for their intake of lutein/zeaxanthin had a 57% lower risk of advanced AMD compared to those in the lowest quintile, and subjects in the highest quintile for consumption of spinach had an 86% lower odds ratio of advanced AMD. It has now been observed that there is a positive relationship between dietary intake and serum levels of lutein and zeaxanthin, as well as between serum concentrations of lutein and zeaxanthin and macular pigment density (19, 36).

The relationship between serum carotenoids and macular pigment density may not hold for women. Broekmans et al. (28) found that women had higher serum and adipose fat concentrations of lutein than men, but had significantly lower levels of the macular pigment. This finding may be related to the observations of Hammond et al. (67) that women have a higher incidence of AMD, although Curran-Celentano et al. did not find a difference between men and women in either serum lutein concentration or in macular pigment density (40).

Not all of the epidemiological studies have supported a role for lutein and zeaxanthin in AMD. Mares-Perlman et al. (100), while reporting on the Beaver Dam Eye Study, found a significant relationship between zinc ingestion and early

AMD, but no relationship between carotenoid ingestion and either early or late ARM. However, a later study from this group reported a significant relationship between the intake of pro-vitamin A carotenoids (α -carotene, β -carotene and β -cryptoxanthin) and the incidence of large drusen, frequently used as a marker of early AMD (149). Also, Smith et al. (133), using the Blue Mountains Eye Study cohort, found no protective relationship between serum α -carotene or β -carotene and AMD, from either the diet or supplements (134).

However, the evidence has continued to grow stronger that there is a relationship between the ingestion of lutein and zeaxanthin, primarily from dark green, leafy vegetables, serum levels of these two carotenoids, and the amount of macular pigment in the retina (36, 40). It has been known for many years that short wavelength light, i.e., the kind absorbed by lutein and zeaxanthin, can be damaging to the retina [reviewed by Ham (65)]. Thus the finding that donor eyes from individuals having the highest levels of lutein and zeaxanthin in the peripheral region of the macula had an 82% lower risk for AMD when compared to donor eyes from individuals with the lowest levels of these 2 carotenoids (23) is significant. And when large national surveys were tabulated with respect to carotenoids in the diet and serum, higher levels of lutein and zeaxanthin in the diet were inversely related to signs of early AMD (65).

Observational Epidemiology: Cataracts

An early study linking nutrient intake and cataract extraction was carried out in women, where an inverse correlation was observed for total vitamin A intake, including β -carotene, but it was spinach, and not carrots, that was most frequently associated with a lower risk (73). This observation was followed up by a study involving 77,466 female nurses, where it was reported that lutein and zeaxanthin intake were associated with a decreased risk of cataract extraction (34). The observation that human lenses contain lutein and zeaxanthin as the only carotenoids (162) and that these pigments are localized in the more metabolically active epithelial and cortical layers of the lens (163) has stimulated interest in the possibility that these compounds may play a protective role in the development of cataracts. Several epidemiological studies indicate that there is a modest decrease in cataract extraction in men in the highest quintile of lutein and zeaxanthin intake (29) and that dietary carotenoids protect against cataract development (55). Many of these studies have appeared in reviews evaluating cataract risk and dietary nutrients (76, 104).

Interventional Epidemiology: Age-related Macular Degeneration (AMD)

Dietary lutein and zeaxanthin, either in the form of green, leafy vegetables or corn (68, 78), or lutein supplements (10, 91), can increase the amount of macular pigment. Under these circumstances, there is no question that this would lead to a decrease in chromatic aberration. However, we still do not have the direct evidence that there would be more antioxidant protection in the macula, aside

from blue-light absorption (65, 66, 77). Therefore, several authors have advocated a “go slow” approach with respect to recommending lutein supplements either to inhibit the progression of age-related macular degeneration (7) or to prevent cataract formation (98).

A major intervention trial from the Age-Related Eye Disease Study Research Group (AREDS) was published in 2001, in which 3640 subjects (55–80 years) were supplemented daily with either a cocktail of antioxidants (vitamin C, 500 mg; vitamin E, 400 IU; and β -carotene, 15 mg), zinc (80 mg), or a combination of both (3). When compared to a placebo, the combination led to a significantly decreased odds reduction for the development of advanced AMD, whereas the individual supplements decreased the risk, but not significantly. In the highest risk groups, as determined by the number and size of drusen, both zinc and zinc plus antioxidants significantly reduced the odds for progression to advanced AMD. It is unfortunate that at the time AREDS was initiated, neither lutein or zeaxanthin was available in a commercial formulation, and β -carotene was selected as the carotenoid of interest, even though it is not present in either the human retina or lens, and a long-term study observed no benefit from β -carotene supplementation on the occurrence of AMD in males who smoke (143). Interestingly, this AREDS study reported a nonsignificant decrease in the serum levels of lutein and zeaxanthin (3). In hindsight, the measurements of macular pigment density as affected by these treatments would have been of great interest.

Interventional Epidemiology: Cataracts

Another large AREDS intervention trial involved 4629 subjects, 55–80 years old, who were supplemented daily with an antioxidant cocktail (vitamin C, 500 mg; vitamin E, 400 IU; and β -carotene, 15 mg) for an average of 6.3 years (2). The results indicated that in a relatively well-nourished older adult group, there was no apparent effect on the seven-year risk of development or progression of age-related lens opacities or visual acuity loss.

Another trial, the Roche European American Cataract Trial (REACT), used both U.S. and U.K. patients with early age-related cataract (ARC) who were randomized to receive either a placebo or a daily antioxidant mixture (vitamin C, 750 mg; vitamin E, 600 mg; β -carotene, 18 mg) (35). A significant benefit on inhibiting progression of ARC was observed in the U.S. group after three years, but not in the U.K. group, and no explanation was available for the difference in the response in these two populations.

Interventional Epidemiology: Other Eye Diseases

There are several diseases where, unlike AMD, peripheral vision is lost first before total blindness occurs. These are diseases such as retinitis pigmentosa (RP) and choroideremia. Several small studies were initiated using supplementation with helenien, the diester of lutein (also known as adaptinol), and these studies were reviewed by Delori and his associates (111). Adaptinol was administered to both normal subjects and individuals with decreased dark adaptation, retinitis pigmentosa,

progressive myopia, or chorioretinal changes. These studies were small in size, and thus not capable of yielding significant effects.

A small trial using a 26-week program of lutein supplementation (40 mg/d) in 13 patients with RP, and monitored via a self-test utilizing a computer screen to evaluate visual acuity and a wall chart to evaluate central visual-field extent, reported improvement in visual acuity and visual-field area by 6–14 weeks (41). Although this trial was uncontrolled, the results were sufficient to initiate other studies by Jacobson and associates. In a small study using 47 RP patients supplemented with 20 mg/d lutein for six months, all of the patients showed an increase in serum lutein, but not all of them showed an increase in macular pigment density (4). They were not able to differentiate between the macular pigment responders and the nonresponders with respect to baseline serum lutein, mean serum lutein increase, baseline macular pigment densities, age, gender, smoking status, or baseline foveal absolute sensitivity. Furthermore, there was no change in central vision after this six-month supplementation period. A group of seven patients with choroideremia were also supplemented with 20 mg/d lutein for six months, resulting in an increase in both serum lutein and in macular pigment. However, absolute foveal sensitivity did not change (47). It should be noted that both of these studies are relatively short-term, and it would be of interest to see if long-term supplementation might prevent the loss of central vision in these two irreversible conditions leading to blindness.

PROTECTIVE ACTIONS OF LUTEIN AND ZEAXANTHIN

Based on the studies described above there are several mechanisms that could explain why lutein and zeaxanthin might be protective molecules in the eye (89, 90). The first is the ability of the macular pigment to absorb blue-light, particularly before the light impinges on the photoreceptor cells, and the second is an antioxidant action. Absorption of blue-light would not only decrease any chromatic aberration, but it could potentially prevent blue-light from generating reactive oxygen species that could damage photoreceptor cells. In this sense, blue-light absorption can be considered a passive or indirect antioxidant action. Observation of injury resulting from acute photic exposure and the associated protection afforded by the macular pigment has been documented in the literature (77). Recently, Dorey and her associates have demonstrated that Japanese quail supplemented with zeaxanthin accumulate zeaxanthin in retinal oil droplets and are protected from light-induced photoreceptor death (144, 145). We eagerly await data from primates treated with either lutein or zeaxanthin and then exposed to blue-light to see if a similar protective effect can be demonstrated in primates. Additional supporting observations are those of Richter and his associates that a lipofuscin component, *N*-retinyl-*N*-retinylidene ethanolamine (A2E), induces apoptosis in RPE cells, thus inducing AMD (141). A2E is a blue-light-absorbing phototoxic compound, and this offers an example of how the attenuation of blue-light entering the photoreceptor cells and the RPE by the macular pigment might deter the onset of AMD (132).

Although not disease-related, the decline in visual sensitivity with age apparently does not occur in older subjects with high macular pigment densities (70). This suggests that there is some protection associated with the macular pigment, but no specific mechanism has been conclusively demonstrated.

There are other potential mechanisms by which carotenoid antioxidant functions could protect against AMD. AMD may be the result of many different underlying causes. Among these is the idea that AMD is actually an RPE disease, and may be related to a breakdown in the blood-retinal barrier, resulting in macular edema (49). Ciulla et al. (37) have proposed that there is reduced blood flow in the nasal and temporal posterior ciliary arteries in nonexudative AMD, suggesting that choroidal perfusion is abnormal in this form of AMD, and vascular defects have been identified in both nonexudative and exudative AMD patients (74). Plasma lutein has been reported to be directly associated with a decline in the progression of intima-mediated thickness in the common carotid artery in a group of 480 40–60-year-old subjects (48). Lutein might potentially reduce risk for AMD through a similar protection mechanism against thickening of the ciliary arteries. These possibilities require considerable additional investigation.

CONCLUSIONS: EYE HEALTH AND CAROTENOIDS

Current Recommendations

It is clear that the macular carotenoids can absorb blue-light entering the retina and that this can effectively protect the retina at least during acute exposure to high light levels from photic-induced oxidative damage (63). It will be difficult to demonstrate unambiguously that chronic exposure to blue-light at ambient levels is a significant contributory factor in the development of AMD or that the macular pigment is truly protective. This is partly due to the multifactorial nature of the disease, but also because of the challenge of measuring light exposure in individuals as well as their macular pigment optical density. The circumstantial evidence accumulated to date is, however, compelling. It is worth noting that, for nonsmokers, there appears to be no known risk of increasing consumption of lutein- and zeaxanthin-rich food sources or taking supplements containing these carotenoids. South Pacific Islanders consume as much as 27 mg/day (94). Consumption of β -carotene by smokers has been linked to higher rates of lung cancer. Although this has not been demonstrated for lutein or zeaxanthin, it would be imprudent to assume that the same risk does not exist until evidence demonstrates the contrary (58, 116, 118). The Eye Disease Case-Control Study Group (53) found that the upper quintile in their study population consumed greater than 5.6 mg of lutein and zeaxanthin per day and had a lowered odds ratio for the occurrence of late-stage AMD. Given the current state of our understanding, this appears to be a reasonable dietary target level for these two carotenoids. It has been argued that the choice of purified supplements that are now available, rather than a diet rich in these components,

lacks the broader value associated with the consumption of foods having a large variety of potentially beneficial phytonutrients (98).

Future Recommendations

It is essential to develop a complete understanding of the etiology of AMD, including additional studies on the role of genetics and environmental conditions that might be involved with the development of AMD. In addition, the potential role of A2E (N-retinyl-N-retinylidene ethanolamine) in the development of AMD [reviewed by Shaban & Richter (132)] should also be investigated and clarified. Only then will it be possible to incorporate studies on the metabolic role and function of the macular carotenoids in understanding the etiology of AMD, and possibly other eye diseases. It is also important to know more about the site(s) of accumulation of the macular carotenoids. We need to understand microscopically where in the axons these carotenoids are sequestered and how they are transported to these sites. The presence of *meso*-zeaxanthin and other minor carotenoids within the retina has led to the suggestion that several complex metabolic events occur that involve these carotenoids. It is necessary to know if the current hypotheses involving the oxidation of lutein and zeaxanthin in the retina are correct. It is almost impossible to explain the carotenoid distribution pattern and the exceptionally high concentrations of carotenoids found in the retina without imagining a protein that specifically binds and transports these carotenoids into the retina. The nature of this transport mechanism must be understood in order to establish what factors regulate the movement of carotenoids into the retina. The identity and nature of the xanthophyll binding protein and determining whether its absence in some individuals could be a source of increased risk for AMD are important topics for further study.

At a clinical level it will be important to develop a broad base of knowledge about the level of the macular pigment in various populations. It is not yet established if macular pigment levels are completely, or only partially, the result of diet. The question of how predictive low macular pigment levels may be for risk of AMD should be investigated, even though some researchers are advocating supplemental lutein and zeaxanthin for delaying or averting the onset of this disease (9). Until more information is available, particularly from controlled intervention trials, it is still too early to recommend supplemental lutein or zeaxanthin as a means of reducing the risk of eye diseases (101).

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