Annu. Rev. Nutr. 2003. 23:171–201
doi: 10.1146/annurev.nutr.23.011702.073307

First published online as a Review in Advance on February 27, 2003

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

Norman I. Krinsky[1], John T. Landrum[2], and Richard A. Bone[3]

[1]*Department of Biochemistry, School of Medicine and the USDA Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts 02111-1837; email: norman.krinsky@tufts.edu*
[2]*Department of Chemistry, Florida International University, Miami, Florida 33199; email: landrumj@fiu.edu*
[3]*Department of Physics, Florida International University, Miami, Florida 33199; email: bone@fiu.edu*

Key Words age-related macular degeneration, carotenoid action, macula, color filter, antioxidant

■ **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

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

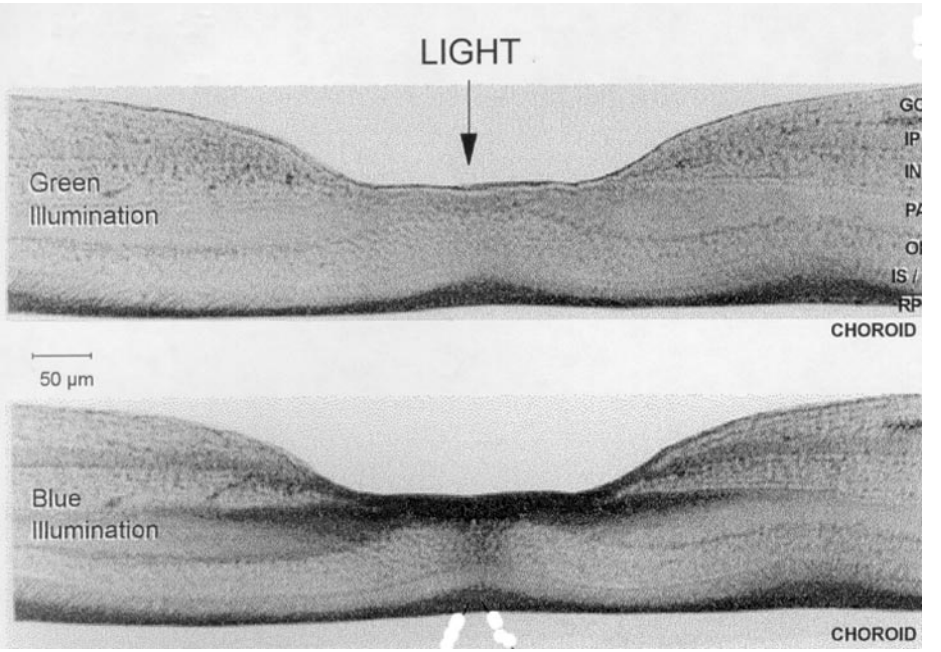

Figure 1 Cross-section of a human macula photographed in either a green or a blue light, indicating the absorption of blue-light by the macular pigment [from (135) and adapted with permission by the *American Journal of Clinical Nutrition.* © *Am. J. Clin. Nutr.*].

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,

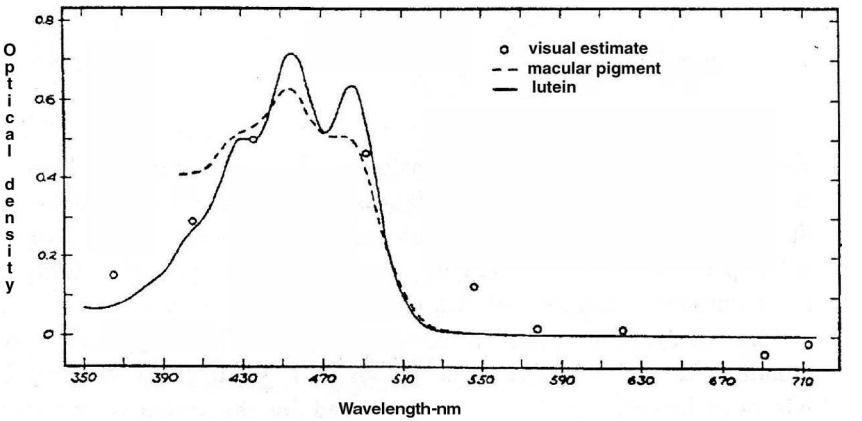

Figure 2 The absorption spectrum of lutein, of the pigment extracted from the macula, and a visual estimate of the macular pigment [adapted from (151) and used with permission of Kluwer Academic Publishers].

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-ß, 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

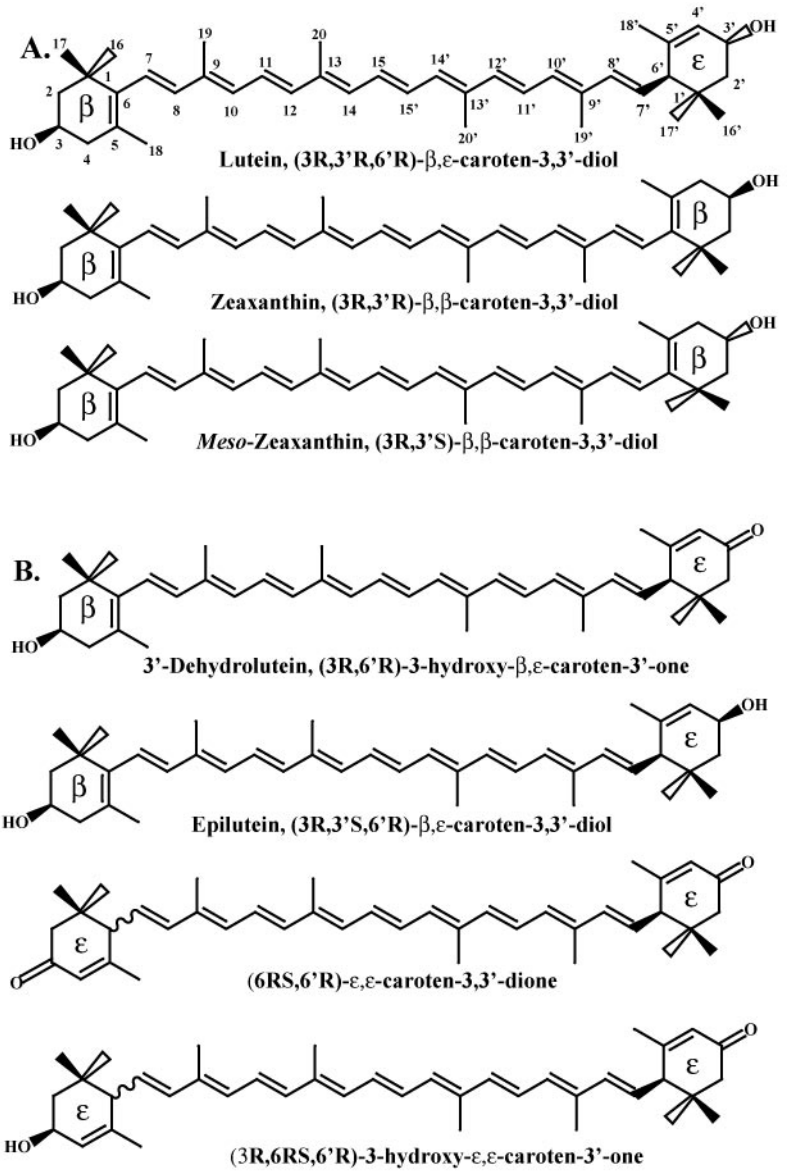

Figure 3 Structures of the major pigments isolated from the human macula (A) as well as minor xanthophylls found in the retina that may be involved in metabolic interconversions in the formation of *meso*-zeaxanthin (B).

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 (*vida 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)-$\beta\varepsilon$-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 peroxyl 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 peroxyl radicals by a hydrogen abstraction mechanism generating a neutral carotenoid radical. In some instances, direct addition of a peroxyl 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 O_2 to generate carotenoid-peroxyl 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 stereosiomers 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 peroxyl 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 peroxyl 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 O_2. This secondary reaction generates a carotenoid peroxyl radical whose formation will depend upon the oxygen partial pressure. At sufficiently high partial pressures of O_2 this carotenoid peroxyl radical can generate additional radicals by cleavage of the resulting peroxyl bond. This O_2-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 peroxyl radicals. However with increasing oxygen concentration, secondary oxygen-generated peroxyl 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 peroxyl 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 $\sim$ 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 that 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 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 that 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).

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

LITERATURE CITED

1. Abadi RV, Cox MJ. 1992. The distribution of macular pigment in human albinos. *Invest. Ophthalmol. Vis. Sci.* 33:494–97
2. Age-Related Eye Disease Study Research Group. 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and

vision loss: AREDS report no. 9. *Arch. Ophthalmol.* 119:1439–52
3. Age-Related Eye Disease Study Research Group. 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* 119:1417–36
4. Aleman TS, Duncan JL, Bieber ML, de Castro E, Marks DA, et al. 2001. Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome. *Invest. Ophthalmol. Vis. Sci.* 42:1873–81
5. Andrewes AG, Borch G, Liaaen-Jensen S. 1974. Carotenoids of higher plants. 7. On the absolute configuration of lutein. *Acta Chem. Scand. B* 28:139–40
6. Anonymous. 2000. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Washington, DC:Natl. Acad. Press. 506 pp.
7. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. 1999. Macular pigment and age related macular degeneration. *Br. J. Ophthalmol.* 83:867–77
8. Beatty S, Koh H, Phil M, Henson D, Boulton M. 2000. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv. Ophthalmol.* 45:115–34
9. Beatty S, Murray IJ, Henson DB, Carden D, Koh H–H, Boulton ME. 2001. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest. Ophthalmol. Vis. Sci.* 42:439–46
10. Berendschot TTJM, Goldbohm RA, Klöpping WAA, van de Kraats J, van Norel J, van Norren D. 2000. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest. Ophthalmol. Vis. Sci.* 41: 3322–26
11. Bernstein PS, Balashov NA, Tsong ED, Rando RR. 1997. Retinal tubulin binds macular carotenoids. *Invest. Ophthalmol. Vis. Sci.* 38:167–75
12. Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao D-Y, Katz NB. 2001. Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Exp. Eye Res.* 72:215–23
13. Bernstein PS, Yoshida MD, Katz NB, McClane RW, Gellermann W. 1998. Raman detection of macular carotenoid pigments in intact human retina. *Invest. Ophthalmol. Vis. Sci.* 39:2003–11
14. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. 2002. Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. *Ophthalmology* 109:1780–87
15. Block G, Woods M, Potosky A, Clifford C. 1990. Validation of a self administered diet history questionnaire: multiple diet records. *J. Clin. Epidemiol.* 43:1327–35
16. Bone RA, Landrum JT. 1983. Dichroism of lutein: a possible basis for Haidinger's brushes. *Appl. Opt.* 22:775–76
17. Bone RA, Landrum JT. 1984. Macular pigment in Henle fiber membranes: a model for Haidinger's brushes. *Vision Res.* 24:103–8
18. Bone RA, Landrum JT, Cains A. 1992. Optical density spectra of the macular pigment in vivo and in vitro. *Vision Res.* 32:105–10
19. Bone RA, Landrum JT, Dixon Z, Chen Y, Llerena CM. 2000. Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Exp. Eye Res.* 71:239–45
20. Bone RA, Landrum JT, Fernandez L, Tarsis SL. 1988. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest. Ophthalmol. Vis. Sci.* 29:843–49
21. Bone RA, Landrum JT, Friedes LM, Gomez CM, Kilburn MD, et al. 1997. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp. Eye Res.* 64:211–18
22. Bone RA, Landrum JT, Hime GW, Cains

A, Zamor J. 1993. Stereochemistry of the Human Macular Carotenoids. *Invest. Ophthalmol. Vis. Sci.* 34:2033–40

23. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. 2001. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest. Ophthalmol. Vis. Sci.* 42:235–40
24. Bone RA, Landrum JT, Tarsis SL. 1985. Preliminary identification of the human macular pigment. *Vision Res.* 25:1531–35
25. Bour LJ, Koo L, Delori FC, Apkarian P, Fulton AB. 2002. Fundus photography for measurement of macular pigment density distribution in children. *Invest. Ophthalmol. Vis. Sci.* 43:1450–55
26. Brindley GS, Willmer EN. 1952. The reflexion of light from the macular and peripheral fundus oculi in man. *J. Physiol.* 116:350–56
27. Britton G. 1995. UV/visible spectroscopy. In *Carotenoids*, ed. G Britton, S Liaaen-Jensen, H Pfander, pp. 13–62. Basel, Switzerland: Birkhäuser
28. Broekmans WMR, Berendschot TTJM, Klöpping-Ketelaars IAA, de Vries AJ, Goldbohm RA, et al. 2002. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am. J. Clin. Nutr.* 76:595–603
29. Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan-Taber L, et al. 1999. A prospective study of carotenoid intake and risk of cataract extraction in US men. *Am. J. Clin. Nutr.* 70:517–24
30. Buchecker R, Eugster CH, Weber A. 1978. Absolute konfiguration von α-doradexanthin and von frishiellaxanthin, einem neuen carotinoid aus Fritschiella tuberosa Iyeng. *Helv. Chim. Acta* 61: 1962–68
31. Buchecker R, Noack K. 1995. Circular dichroism. In *Carotenoids*, ed. G Britton, S Liaaen-Jensen, H Pfander, pp. 63–116. Basel, Switzerland: Birkhäuser
32. Burton GW. 1989. Antioxidant action of carotenoids. *J. Nutr.* 119:109–11
33. Burton GW, Ingold KU. 1984. β-Carotene: an unusual type of lipid antioxidant. *Science* 224:569–73
34. Chasan-Taber L, Willett WC, Seddon JM, Stampfer MJ, Rosner B, et al. 1999. A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. *Am. J. Clin. Nutr.* 70:509–16
35. Chylack LT Jr., Brown NP, Bron A, Hurst M, Köpcke W, et al. 2002. The Roche European American Cataract Trial (REACT): a randomized clinical trial to investigate the efficacy of an oral antioxidant micronutrient mixture to slow progression of age-related cataract. *Ophthalmic Epidemiol.* 9:49–80
36. Ciulla TA, Curran-Celentano J, Cooper DA, Hammond BR Jr., Danis RP, et al. 2001. Macular pigment optical density in a midwestern sample. *Ophthalmology* 108:730–37
37. Ciulla TA, Harris A, Chung HS, Danis RP, Kagemann L, et al. 1999. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. *Am. J. Ophthalmol.* 128:75–80
38. Coren S, Girgus JS. 1972. Density of human lens pigmentation: in vivo measures over an extended age range. *Vision Res.* 12:343–46
39. Craft NE. 1992. Carotenoid reversed-phase high-performance liquid chromatography methods: reference compendium. *Methods Enzymol.* 213:185–205
40. Curran-Celentano J, Hammond BR Jr., Ciulla TA, Cooper DA, Pratt LM, Danis RB. 2001. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am. J. Clin. Nutr.* 74:796–802
41. Dagnelie G, Zorge IS, McDonald TM. 2000. Lutein improves visual function in some patients with retinal degeneration: a pilot study via the Internet. *Optometry* 71:147–64

42. Delori FC, Goger DG, Dorey CK. 2001. Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Invest. Ophthalmol. Vis. Sci.* 42:1855–66
43. Delori FC, Goger DG, Hammond BR, Snodderly DM, Burns SA. 2001. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J. Opt. Soc. Am.* 18:1212–30
44. Delori FC, Pflibsen KP. 1989. Spectral reflectance of the human ocular fundus. *Applied Optics* 28:1061–77
45. DeVries HL, Spoor A, Jielof R. 1953. Properties of the eye with respect to polarized light. *Physica* 19:419–32
46. Dorey CK, Staurenghi G, Delori F, Sarks S, Sarks J. 1993. Lipofuscin fluorescence in donor eyes with AMD. *Invest. Ophthalmol. Vis. Sci.* 34:1161
47. Duncan JL, Aleman TS, Gardner LM, De Castro E, Marks DA, et al. 2002. Macular pigment and lutein supplementation in choroideremia. *Exp. Eye Res.* 74:371–81
48. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, et al. 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation* 103:2922–27
49. Eagle RC Jr. 1984. Mechanisms of maculopathy. *Ophthalmology* 91:613–25
50. Eliel EL. 1962. *Stereochemistry of Carbon Compounds*. New York: McGraw-Hill
51. Erdman JW Jr., Bierer TL, Gugger ET. 1993. Absorption and transport of carotenoids. In *Carotenoids in Human Health*, ed. LM Canfield, NI Krinsky, JA Olson, pp. 76–86. New York: NY Acad. Sci.
52. Eye Disease Case-Control Study Group. 1992. Risk factors for neovascular age-related macular degeneration. *Arch. Ophthalmol.* 110:1701–8
53. Eye Disease Case-Control Study Group. 1993. Antioxidant status and neovascular age-related macular degeneration. *Arch. Ophthalmol.* 111:104–9
54. Foote CS, Chang YC, Denny RW. 1970. Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. *J. Am. Chem. Soc.* 92:5216–18
55. Gale CR, Hall NF, Phillips DI, Martyn CN. 2001. Plasma antioxidant vitamins and carotenoids and age-related cataract. *Ophthalmology* 108:1992–98
56. Gao G, Wei CC, Jeevarajan AS, Kispert LD. 1996. Geometrical isomerization of carotenoids mediated by cation radical/dication formation. *J. Phys. Chem.* 100: 5362–66
57. Gellermann W, Ermakov IV, McClane RW, Bernstein PS. 2002. Raman imaging of human macular pigments. *Optics Lett.* 27:833–35
58. Goodman GE, Valanis B, Meyskens FL Jr., Williams JH Jr., Metch BJ, et al. 1998. Strategies for recruitment to a population-based lung cancer prevention trial: the CARET experience with heavy smokers. Beta-Carotene and Retinol Efficacy Trial. *Cancer Epidemiol. Biomarkers Prev.* 7:405–12
59. Goodwin TW. 1980. *The Biochemistry of the Carotenoids*. London: Chapman & Hall. 377 pp.
60. Goodwin TW. 1984. *The Biochemistry of the Carotenoids*. London: Chapman & Hall. 224 pp.
61. Grant JL, Kramer VJ, Ding R, Kispert LD. 1988. Carotenoid cation radicals: electrochemical, optical, and EPR study. *J. Am. Chem. Soc.* 110:2151–57
62. Gruszecki WI, Sujak A, Strzalka K, Radunz A, Schmid GH. 1999. Organisation of xanthophyll-lipid membranes studied by means of specific pigment antisera, spectrophotometry and monomolecular layer technique lutein versus zeaxanthin. *Z. Naturforsch. [C]* 54:517–25
63. Haegerstrom-Portnoy G. 1988. Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? *J. Opt. Soc. Am. A* 5:2140–44

64. Halliwell B, Gutteridge JM. 1995. The definition and measurement of antioxidants in biological systems [letter; comment]. *Free Radic. Biol. Med.* 18:125–26
65. Ham WT. 1983. Ocular hazards of light sources: review of current knowledge. *J. Occup. Med.* 25:101–3
66. Ham WTJ, Mueller HA, Ruffolo JJ, Millen JE, Cleary SF, et al. 1984. Basic mechanisms underlying the production of photochemical lesions in the mammalian retina. *Curr. Eye Res.* 3:165–74
67. Hammond BR Jr., Curran-Celentano J, Judd S, Fuld K, Krinsky NI, et al. 1996. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res.* 36:2001–12
68. Hammond BR Jr., Johnson EJ, Russell RM, Krinsky NI, Yeum K-J, et al. 1997. Dietary modification of human macular pigment density. *Invest. Ophthalmol. Vis. Sci.* 38:1795–801
69. Hammond BR Jr., Wooten BR, Snodderly DM. 1997. Individual variations in the spatial profile of human macular pigment. *J. Opt. Soc. Am. A* 14:1187–96
70. Hammond BR Jr., Wooten BR, Snodderly DM. 1998. Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest. Ophthalmol. Vis. Sci.* 39:397–406
71. Handelman GJ, Dratz EA, Reay CC, van Kuijk FJGM. 1988. Carotenoids in the human macula and whole retina. *Invest. Ophthalmol. Vis. Sci.* 29:850–55
72. Handelman GJ, Nightingale ZD, Lichtenstein AH, Schaefer EJ, Blumberg JB. 1999. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. *Am. J. Clin. Nutr.* 70:247–51
73. Hankinson SE, Stampfer MJ, Seddon JM, Colditz GA, Rosner BN, et al. 1992. Nutrient intake and cataract extraction in women: a prospective study. *Brit. Med. J.* 305:335–39
74. Harris A, Chung HS, Ciulla TA, Kagemann L. 1999. Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog. Retin. Eye Res.* 18:669–87
75. Hill TJ, Land EJ, McGarvey DJ, Schalch W, Tinkler JH, Truscott TG. 1995. Interactions between carotenoids and the $CCl_3O_2\bullet$ radical. *J. Am. Chem. Soc.* 117:8322–26
76. Jacques PF. 1999. The potential preventive effects of vitamins for cataract and age-related macular degeneration. *Int. J. Vitam. Nutr. Res.* 69:198–205
77. Jaffe GJ, Wood IS. 1988. Retinal phototoxicity from the operating microscope: a protective effect by the fovea. *Arch. Ophthalmol.* 106:445–46
78. Johnson EJ, Hammond BR, Yeum K-J, Qin J, Wang XD, et al. 2000. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am. J. Clin. Nutr.* 71:1555–62
79. Kaplan LA, Lau JM, Stein EA. 1990. Carotenoid composition, concentrations, and relationships in various human organs. *Clin. Physiol. Biochem.* 8:1–10
80. Khachik F, Beecher GR, Goli MB, Lusby WR, Smith JC Jr. 1992. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. *Anal. Chem.* 64:2111–22
81. Khachik F, Bernstein PS, Garland DL. 1997. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest. Ophthalmol. Vis. Sci.* 38:1802–11
82. Khachik F, de Moura FF, Zhao D-Y, Aebischer C-P, Bernstein PS. 2002. Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. *Invest. Ophthalmol. Vis. Sci.* 43:3383–92
83. Khachik F, Spangler CJ, Smith JC Jr., Canfield LM, Steck A, Pfander H. 1997. Identification, quantification, and relative concentrations of carotenoids and their

metabolites in human milk and serum. *Anal. Chem.* 69:1873–81
84. Khaled M, Hadjipetrou A, Kispert LD. 1990. Electrochemical and electron paramagnetic resonance studies of carotenoid cation radicals and dications: effect of deuteration. *J. Phys. Chem.* 94:5164–69
85. Kilbride PE, Alexander KR, Fishman M, Fishman GA. 1989. Human macular pigment assessed by imaging fundus reflectometry. *Vision Res.* 29:663–74
86. Kirschfeld K. 1982. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Proc. R. Soc. Lond. B* 216:71–85
87. Kohler BE. 1995. Electronic structure of carotenoids. In *Carotenoids*, ed. G Britton, S Liaaen-Jensen, H Pfander, pp. 1–12. Basel, Switzerland: Birkhäuser
88. Krinsky NI. 1989. Antioxidant functions of carotenoids. *Free Radic. Biol. Med.* 7:617–35
89. Krinsky NI. 2002. Possible biologic mechanisms for a protective role of xanthophylls. *J. Nutr.* 132:540S–42S
90. Landrum JT, Bone RA. 2001. Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* 385:28–40
91. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. 1997. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp. Eye Res.* 65:57–62
92. Landrum JT, Bone RA, Kilbum MD. 1997. The macular pigment: a possible role in protection from age-related macular degeneration. *Adv. Pharmacol.* 38:537–56
93. Landrum JT, Bone RA, Moore LL, Gomez CM. 1999. Analysis of zeaxanthin distribution within individual human retinas. *Methods Enzymol.* 299:457–67
94. Le Marchand L, Hankin JH, Bach F, Kolonel LN, Wilkens LR, et al. 1995. An ecological study of diet and lung cancer in the South Pacific. *Int. J. Cancer* 63:18–23
95. Liaaen-Jensen S. 1971. Isolation, reactions. In *Carotenoids*, ed. O Isler, pp. 61–188. Basel/Stuttgart: Birkhäuser
96. Mangels AR, Holden JM, Beecher GR, Forman ME, Lanza E. 1993. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J. Am. Diet. Assoc.* 93:284–96
97. Maoka T, Arai A, Shimizu M, Matsuno T. 1986. The first isolation of enantiomeric and *meso*-zeaxanthin in nature. *Comp. Biochem. Physiol.* 83B:121–24
98. Mares-Perlman JA. 1999. Too soon for lutein supplements [editorial]. *Am. J. Clin. Nutr.* 70:431–32
99. Mares-Perlman JA, Klein R. 1999. Diet and age-related macular degeneration. In *Nutritional and Environmental Influences on the Eye*, ed. A Taylor, pp. 181–214. Boca Raton: CRC
100. Mares-Perlman JA, Klein R, Klein BEK, Greger JL, Brady WE, et al. 1996. Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch. Ophthalmol.* 114:991–97
101. Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. *J. Nutr.* 132:518S–24S
102. Martin H-D, Ruck C, Schmidt M, Sell S, Beutner S, et al. 1999. Chemistry of carotenoid oxidation and free radical reactions. *Pure Appl. Chem.* 71:2253–62
103. McLellan JS, Marcos S, Prieto PM, Burns SA. 2002. Imperfect optics may be the eye's defence against chromatic blur. *Nature* 417:174–76
104. Moeller SM, Jacques PF, Blumberg JB. 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *J. Am. Coll. Nutr.* 19:522S–27S
105. Moreland JD, Robson AG, Soto-Leon N, Kulikowski JJ. 1998. Macular pigment and the colour-specificity of visual evoked potentials. *Vision Res.* 38:3241–45
106. Mortensen A, Skibsted LH, Truscott TG. 2001. The interaction of dietary

carotenoids with radical species. *Arch. Biochem. Biophys.* 385:13–19
107. N'soukpoé-Kossi CN, Sielewiesiuk J, Leblanc RM, Bone RA, Landrum JT. 1988. Linear dichroism and orientational studies of carotenoid Langmuir-Blodgett films. *Biochim. Biophys. Acta* 940:255–65
108. Naylor EJ, Stanworth A. 1954. Retinal pigment and the Haidinger effect. *J. Physiol.* 124:543–52
109. Nebeling LC, Forman MR, Graubard BI, Snyder RA. 1997. Changes in carotenoid intake in the United States: the 1987 and 1992 National Health Interview Surveys. *J. Am. Diet. Assoc.* 97:991–96
110. Norren DV, Vos JJ. 1974. Spectral transmission of the human ocular media. *Vision Res.* 14:1237–44
111. Nussbaum JJ, Pruett RC, Delori FC. 1981. Historic perspectives. Macular yellow pigment. The first 200 years. *Retina* 1:296–310
112. Okulski W, Sujak A, Gruszecki WI. 2000. Dipalmitoylphosphatidylcholine membranes modified with zeaxanthin: numeric study of membrane organisation. *Biochim. Biophys. Acta* 1509:216–28
113. Palozza P, Krinsky NI. 1992. Antioxidant effects of carotenoids *in vitro* and *in vivo*: an overview. *Methods Enzymol.* 213:403–20
114. Pauleikhoff D, van Kuijk FJGM, Bird AC. 2001. Macular pigment and age-related macular degeneration. *Ophthalmologe* 98:511–19
115. Pease PL, Adams AJ. 1983. Macular pigment difference spectrum from sensitivity measures of a single cone mechanism. *Am. J. Optom. Physiol. Opt.* 60:667–72
116. Pryor WA, Stahl W, Rock CL. 2000. Beta carotene: from biochemistry to clinical trials. *Nutr. Rev.* 58:39–53
117. Rapp LM, Maple SS, Choi JH. 2000. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest. Ophthalmol. Vis. Sci.* 41:1200–9
118. Rautalahti M, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Heinonen OP. 1997. Beta-carotene did not work: aftermath of the ATBC study. *Cancer Lett.* 114:235–36
119. Reading VM, Weale RA. 1974. Macular pigment and chromatic aberration. *J. Opt. Soc. Amer.* 64:231–34
120. Ritenbaugh C, Peng YM, Aickin M, Graver E, Branch M, Alberts DS. 1996. New carotenoid values for foods improve relationship of food frequency questionnaire intake estimates to plasma values. *Cancer Epidemiol. Biomarkers Prev.* 5:907–12
121. Ruddock KH. 1963. Evidence for macular pigmentation from colour matching data. *Vision Res.* 3:417–29
122. Ruttimann A, Schiedt K, Vecchi M. 1983. Separation of (3R,3′R)-, (3R,3′S)-, (3S,3′S)-zeaxanthin, (3R,3′R,6′R)-, (3R,3′S,6′S)-, and (3S,3′S,6′S)-lutein via the dicarbamates of (S)-(+)-a-[1-naphthyl]ethylisocyanate. *J. High Res. Chrom. Commun.* 6:612–16
123. Sample PA, Esterson FD, Weinreb RN, Boynton RM. 1988. The aging lens: in vivo assessment of light absorption in 84 human eyes. *Invest. Ophthalmol. Vis. Sci.* 29:1306–11
124. Sample PA, Quirante JS, Weinreb RN. 1991. Age-related changes in the human lens. Clinical assessment of age-related changes in the human lens. *Acta Ophthalmol. (Copenh.)* 69:310–14
125. Schalch W, Dayhew-Baker P, Barker FM II. 1999. The carotenoids of the human retina. In *Nutritional and Environmental Influences on the Eye*, ed. A Taylor, pp. 215–50. Boca Raton: CRC
126. Schiedt K. 1989. New aspects of carotenoid metabolism in animals. In *Carotenoids: Chemistry and Biology*, ed. NI Krinsky, MM Mathews-Roth, RF Taylor, pp. 247–68. New York/London: Plenum
127. Schiedt K, Bischof S, Glinz E. 1991. Recent progress on carotenoid metabolism in animals. *Pure Appl. Chem.* 63:89–100
128. Schiedt K, Leuenberger FJ, Vecchi M,

Glinz E. 1985. Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. *Pure Appl. Chem.* 57:685–92

129. Schmitz HH, Poor CL, Gugger ET, Erdman JW Jr. 1993. Analysis of carotenoids in human and animal tissues. *Methods Enzymol.* 214:102–16
130. Schmitz HH, Poor CL, Wellman RB, Erdman JW Jr. 1991. Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. *J. Nutr.* 121:1613–21
131. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, et al. 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration (correction in JAMA 273:622, 1995). *JAMA* 272:1413–20
132. Shaban H, Richter C. 2002. A2E and blue light in the retina: the paradigm of age-related macular degeneration. *Biol. Chem.* 383:537–45
133. Smith W, Mitchell P, Rochester C. 1997. Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. *Am. J. Ophthalmol.* 124:838–40
134. Smith W, Mitchell P, Webb K, Leeder SR. 1999. Dietary antioxidants and age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology* 106:761–67
135. Snodderly DM. 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.* 62 (Suppl.):1448S–61S
136. Snodderly DM, Brown PK, Delori FC, Auran JD. 1984. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest. Ophthalmol. Vis. Sci.* 25:660–73
137. Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM. 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Brit. J. Ophthalmol.* 82:907–10
138. Sommerburg OG, Siems WG, Hurst JS, Lewis JW, Kliger DS, van Kuijk FJ. 1999. Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Curr. Eye Res.* 19:491–95
139. Straub O. 1987. *Key to Carotenoids*. Basel: Birkhäuser. 296 pp.
140. Surai PF, MacPherson A, Speake BK, Sparks NH. 2000. Designer egg evaluation in a controlled trial. *Eur. J. Clin. Nutr.* 54:298–305
141. Suter M, Remé C, Grimm C, Wenzel A, Jäättela M, et al. 2000. Age-related macular degeneration. The lipofuscin component of *N*-retinyl-*N*-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. *J. Biol. Chem.* 275:39625–30
142. Tabunoki H, Sugiyama H, Tanaka Y, Fujii H, Banno Y, et al. 2002. Isolation, characterization, and cDNA sequence of a carotenoid binding protein from the silk gland of *Bombyx mori* larvae. *J. Biol. Chem.* 277:32133–40
143. Teikari JM, Laatikainen L, Virtamo J, Haukka J, Rautalahti M, et al. 1998. Six-year supplementation with alpha-tocopherol and beta-carotene and age-related maculopathy. *Acta Ophthalmol. Scand.* 76:224–29
144. Thomson LR, Toyoda Y, Langner A, Delori FC, Garnett KM, et al. 2002. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Invest. Ophthalmol. Vis. Sci.* 43:3538–49
145. Thomson LR, Toyoda Y, Langner A, Delori FC, Garnett KM, et al. 2002. Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. *Exp. Eye Res.* 75:529–46
146. Truscott TG. 1996. β-Carotene and disease: a suggested pro-oxidant and antioxidant mechanism and speculations concerning its role in cigarette smoking. *J.*

Photochem. Photobiol. B: Biol. 35:233–35

147. van de Kraats J, Berendschot TT, van Norren D. 1996. The pathways of light measured in funds reflectometry. *Vision Res.* 36:2229–47
148. Van Norren D, Tiemeijer LF. 1986. Spectral reflectance of the human eye. *Vision Res.* 26:313–20
149. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BEK, Brady WE, Palta M. 1998. Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am. J. Epidemiol.* 148:204–14
150. Wald G. 1945. Human vision and the spectrum. *Science* 101:653–58
151. Wald G. 1949. The photochemistry of vision. *Doc. Ophthalmol.* 3:94–137
152. Weale RA. 1953. Spectral sensitivity curves and the absorption of light by the ocular media. *Brit. J. Ophthalmol.* 37:148–57
153. Weedon BCL. 1971. Stereochemistry. In *Carotenoids*, ed. O Isler, pp. 267–323. Basel/Stuttgart: Birkhäuser
154. Werner JS, Donnelly SK, Kliegl R. 1987. Aging and human macular pigment density. *Vision Res.* 27:257–68
155. Woodall AA, Lee SW-M, Weesie RJ, Jackson MJ, Britton G. 1997. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim. Biophys. Acta* 1336:33–42
156. Wooten BR, Hammond BR. 2002. Macular pigment: influences on visual acuity and visibility. *Prog. Retin. Eye Res.* 21:225–40
157. Wooten BR, Hammond BR Jr., Land RI, Snodderly DM. 1999. A practical method for measuring macular pigment optical density. *Invest. Ophthalmol. Vis. Sci.* 40:2481–89
158. Wooten BR, Wald G. 1973. Color-vision mechanisms in the peripheral retinas of normal and dichromatic observers. *J. Gen. Physiol.* 61:125–45
159. Wustemeyer H, Jahn C, Nestler A, Barth T, Wolf S. 2002. A new instrument for the quantification of macular pigment density: first results in patients with AMD and healthy subjects. *Graefes Arch. Clin. Exp. Ophthalmol.* 240:666–71
160. Yemelyanov AY, Katz NB, Bernstein PS. 2001. Ligand-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retina. *Exp. Eye Res.* 72:381–92
161. Yeum K-J, Booth S, Sadowski J, Lin C, Tang G, et al. 1996. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr.* 64:594–602
162. Yeum K-J, Taylor A, Tang G, Russell RM. 1995. Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Invest. Ophthalmol. Vis. Sci.* 36:2756–61
163. Yeum KJ, Shang FM, Schalch WM, Russell RM, Taylor A. 1999. Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr. Eye Res.* 19:502–5
164. Young AJ, Lowe GM. 2001. Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys.* 385:20–27
165. Zechmeister L. 1962. *Cis-Trans Isomeric Carotenoids, Vitamins A, and Arylpolyenes*. New York: Academic. 251 pp.

Annual Review of Nutrition
Volume 23, 2003

CONTENTS