The existence of the macula lutea of the human retina has been known for more than 200 years. It is established that the xanthophylls lutein and zeaxanthin are responsible for the yellow color. The effect of macular photopigments on blue-light filtration and color perception is well established. It has been postulated that the pigment might serve to reduce chromatic aberration and to improve visual acuity. The antioxidant capabilities of these xanthophylls combined with their ability to trap short-wavelength light may serve to protect the outer retina, retinal pigment epithelium, and choriocapillaris from oxidative damage. Current ideas on the pathophysiology of age-related macular degeneration may be compatible with the proposed function of lutein and zeaxanthin. This review will summarize our knowledge about macular pigment regarding current efforts in research and the epidemiology of age-related eye disease.

Arch Ophthalmol. 2006;124:1038-1045

The first literature review on the macular yellow spot was published by Home1 in 1798, just 15 years after Buzzi2 published the first description. Soemmering3 independently discovered the foramine centrali limbo luteo in 1795 and observed that the spot is pale in young persons and in older people and is brighter in young adults. There was debate as to whether the yellow spot represented a thinned portion of the retina or a hole; Home1 concluded that it is a postmortem change without significance for vision during life. He also reported that the yellow spot could not be seen in dissections of other animals except in the monkey. The presence of a macula lutea in nonhuman primates has since been well documented.4

Hermann von Helmholtz’s ophthalmoscope of 1851 revolutionized ophthalmology and enabled the yellow spot to be visible in living subjects but did not put to rest the debate (which would simmer for another century) that this appearance was due to the in vivo existence of macular yellow pigment. The classic 1945 article by Wald5 titled “Human Vision and the Spectrum” proved that the yellow pigment absorbed wavelengths between 430 and 490 nm, with maximum absorption at 465 nm. He also documented the first xanthophylls extracted from the human retina and found that they were concentrated in the macula. Further research by other investigators established xanthophylls as the source of macular yellow.6-9 Xanthophylls are a subclass of carotenoids, a large group of plant pigments responsible for the color of bright fruits and vegetables, autumn leaves, salmon, canaries, and flamingos. They act as energy sinks in plants and provide the essential first step toward photosynthesis in association with chlorophyll. Carotenoids are linear hydrocarbons; xanthophylls are the oxygenated form.10 In the 1980s, Bone et al11 identified lutein and its structural iso-mer, zeaxanthin, as the specific xanthophylls in the retina, and Snodderly et al12 located the xanthophyll pigment in the Henle fiber layer in primates.

CAROTENOIDS

Lutein and zeaxanthin are but 2 of the more than 600 plant pigments in the ca-
rothophyll in almost all sources.14 Given and peas. Lutein is the dominant xanthin, and colored vegetables such as leafy green vegetables, many fruits, milk, and poultry. Lutein and zeaxanthin are present in the retina and the periphery. Zeaxanthin, an advantageous mechanism for these compounds.

The retina has the highest concentration of xanthophylls of any tissue, concentrating lutein and zeaxanthin almost exclusively. A regulated active transport mechanism results in a 10 000-fold higher concentration of xanthophyll pigment in the retina compared with the blood. This level of concentration supports the concept of specific xanthophyll-binding proteins; a strong candidate has recently been identified. Retinal tubulin has also been shown to have binding affinities for lutein and zeaxanthin.

ANATOMICAL DISTRIBUTION IN THE RETINA
Snodderly et al12,25,26 showed that the macular pigment density was highest in the monkey in the Henle fiber layer, with a sharp drop-off with increased eccentricity from the macula. High-performance liquid chromatography (HPLC) was used by Bone et al11 and then by Handelman et al27 to identify lutein and zeaxanthin as the carotenoids in the human retina. In the central fovea in humans, the concentration of xanthophyll pigment averages 10^{-3} M; this varies by an order of magnitude across individuals tested.14,17 The xanthophyll pigments can be detected by HPLC throughout the retina, with densities falling by at least 2 orders of magnitude between the central macula and the periphery. Zeaxanthin is dominant only in the macula lutea; its proportion decreases with increasing eccentricity from the fovea. Some of the zeaxanthin in the retina is the chiral isomer meso form, which is not found in serum. There is evidence that this form arises from interconversion of lutein to mesozeaxanthin, an advantageous mechanism to have in a diet dominated by lutein-rich sources.28,29 It is likely that lutein and zeaxanthin occupy different niches: experimentally, zeaxanthin with its linear structure has been shown to orient perpendicularly across a lipid bilayer, while lutein adopts an angle of about 23°.31

MEASUREMENT IN VIVO

There are several methods for non-invasively measuring lutein and zeaxanthin levels in the macula of the living eye. The most commonly used methods are heterochromatic flicker photometry and Raman spectroscopy. Recent advances and testing of fundus autofluorescence imaging suggest that more widespread application of this technique to measurement of macular pigment optical density is possible as well.32 Heterochromatic flicker photometry, a psychophysical test, is the most widely used method. There is solid evidence that this measurement reflects lutein and zeaxanthin levels specifically.33,34 Some heterochromatic flicker techniques also provide a means of assessing the spatial distribution of macular pigment.29,35 Advantages include the inexpensive instrumentation, simple training, lack of need for mydriasis, relative immunity from most cataractous changes, and high test-retest reliability in subjects who are able to cooperate with the testing. Disadvantages include the required reliable participation by the subject for adjusting the light source and for judging when the flicker disappears, as well as the fact that those with advanced ocular disease and advanced age may have the most difficulty with the test.34 Fundus autofluorescence imaging presents an option for easily and objectively measuring the spatial distribution of macular pigment that requires less patient compliance. Clinically practical methods that result in values that correlate highly with psychophysical methods have been described.35

®2006 American Medical Association. All rights reserved.

Downloaded From: by a Non-Human Traffic (NHT) User on 11/21/2018
The phenomenon of Raman scattering is the basis for another means of measuring macular pigment types and density. In this technique, an argon laser light is used to resonantly excite specific carotenoid pigment properties, and the signal generated is measured by a spectrometer. Advantages include the minimal required cooperation by the subject and the rapidity of the examination. There is evidence ex vivo that the Raman signal correlates with biochemical assessment of carotenoids in human autopsy specimens. Disadvantages include the requirements of more expensive equipment, patient fixation, and adequate dilation, as well as the fact that other substances or opacities in the lens or ocular media could affect measurements.

VARIABILITY AMONG SUBJECTS

Ocular carotenoids measured by various means vary in density among individuals, and it appears that there are many effects of the level of macular pigment. The diet content of carotenoids among most subjects. However, the response to supplementation with foods or pills is variable. Response variability could suggest that there are numerous dietary attributes that affect the bioavailability of carotenoids from foods. The variability in response may also reflect the ability to transport them into tissues. The bioavailability of carotenoids varies considerably depending on the source. Cooking and dietary fat can also increase the bioavailability of lutein and zeaxanthin.

There appear to be many other variables in the ability to accumulate carotenoids in the retina outside of the diet. There is some evidence to suggest that lutein in the diet might accumulate in the retina more readily in men than in women. Macular pigment density was higher in men in several studies but not in others. Higher levels in men may be related to the lower level of body fat in men compared with women. Three published studies and our observation (J.A.M.; T. L. LaRowe, PhD; D. M. Snodderly, PhD; M. Klein, MD; B. R. Wooten, PhD; and M. Gruber, MS; unpublished data, 2005) to date have observed macular pigment to be lower among persons with more body fat.

Genetics may play a role as well. In some studies, macular pigment density was lower in the maculae of individuals with blue to gray eyes than in individuals with dark irises. Smoking is associated with lower macular pigment density in some studies. This could reflect the oxidant burden of smoking, which could increase the turnover of carotenoids in the blood.

At this point, it is unclear if macular pigment levels vary throughout life in the absence of disease or gross dietary deficiency. Small observational investigations found stable to moderately decreased xanthophyll levels or decreases of macular pigment levels with advancing age. Consistently, eyes with age-related macular degeneration (AMD) have low levels. It is unclear whether this is a cause or a consequence of the condition. A study of macular pigment levels measured by HPLC in postmortem donor eyes documented no significant differences across subjects aged 3 through 95 years. Samples from very young children documented lutein as the dominant central macular pigment, being superseded by zeaxanthin probably around the age of 3 years. Human breast milk is rich in lutein and zeaxanthin.

KNOWN AND PROPOSED FUNCTIONS

The functional roles of macular carotenoids have not been completely characterized. However, some hypothetical functions in the human eye have been extrapolated from their known biological, optical, and photochemical properties. Blue-light filtration effects, including glare reduction, minimization of chromatic aberration, improved fine detail distinction, contrast enhance-
optical properties. The cone axons radiate in a stellate fashion from the fovea, forming the Henle fiber layer. Because of their linear structure, lutein and zeaxanthin exhibit dichroic properties. If these molecules are arranged perpendicular to the radially oriented axon, they will preferentially absorb plane-polarized light that is polarized in a direction parallel to the linear carotenoid molecule (perpendicular to the axonal direction).31,37

The extent of glare reduction by preferential absorption of polarized light has not been quantified, but several inferences can be made. Macular pigment is certainly reduced in individuals with age-related macular disease; this may be partly responsible for problems with glare in this population. Macular pigment is absent or severely reduced among those with albinism and advanced retinitis pigmentosa; the photophobia of these patients could be partly explained by their inability to dampen glare with macular pigment. Given the substantial interindividual variability of macular pigment levels, it is plausible that sensitivity to glare is increased in those with low pigment levels.60,62

Macular pigment probably increases visual sensitivity. Macular pigment density and scotopic and photopic visual sensitivity were measured in a healthy group of 27 subjects aged 60 to 84 years and were compared with those in a group of 10 younger subjects aged 24 to 36 years.60 With effects of lens density corrected for, subjects with high macular pigment density from the older group did not have visual sensitivity that was substantially different from that in subjects from the younger group. Subjects from the older group with low levels of macular pigment, however, had poorer sensitivity than subjects from the younger group. No causality can be conferred, although better visual sensitivity and youthful levels of macular pigment in older subjects appear to be associated in the study.

Another major proposed function of macular pigment, and the one that is pertinent to age-related disease, is neutralization of reactive oxygen species. The antioxidant system in cells and tissues includes enzymes (catalase, glutathione peroxidase, and superoxide dismutase), primary water-soluble antioxidants (such as glutathione and vitamin C), and lipid-soluble antioxidants (xanthophylls, retinoids, and vitamin E). The potential for the creation of reactive oxygen species in the retina is high. The outer retina, especially membranes of the outer segments of the photoreceptors, has high concentrations of polyunsaturated fatty acids that are susceptible to photo-oxidation.63-66 The outer retina also has a high oxygen tension (70 mm Hg), almost that of arterial blood. A vulnerable substrate, rich oxygen supply, and high-energy blue light creates ideal conditions for oxidative damage. The means of damage is actinic, a subthermal photochemical response caused by selective absorption of light by photoactive molecules. Another potential anatomical site for damage mediated by a photochemical response is the choriocapillaris. A theorized source of reactive oxygen species are hemoglobin precursors (protoporphyrins).67-69

Reactive oxygen species are produced by absorption of UV and blue light by a photosensitizing compound or molecule (lipofuscin, protoporphyrin, or cytochrome). Single state molecules rapidly form and can create triple state molecules via intersystem crossing. These longer-lived molecules can then react with oxygen to produce reactive oxygen species, including superoxide anion, hydroxyl radical, hydrogen peroxide, and singlet oxygen. These in turn can cause lipid peroxidation by attacking polyunsaturated fatty acids, resulting in DNA damage, protein and transmembrane glycoprotein oxidation, and other forms of cellular vandalis.64 Carotenoids are potent scavengers of free radicals (eg, superoxide anion and hydroxyl radical) and are particularly efficient at neutralizing singlet oxygen.70-73 Because their lipid-soluble nature relieves them to membranes, it is likely that they especially protect the polyunsaturated fatty acid–rich membranes of the outer retina.59,60,71

**BIOCYCLICAL PLASIBILITY FOR INTERVENTION IN AGE-RELATED DISEASE**

Visual performance in humans decreases slowly until the fifth or sixth decade of life, after which time the decline rate steepens.62,63,74 Decreased lens clarity, loss of rods, and slowed retinal pigment epithelium (RPE) functions contribute to decreased visual capabilities.70,75-77 Still, there is a tremendous interindividual range of ocular function in the aging population; it is this disparity that drives an ever-expanding research effort that seeks to elucidate the etiologies of age-related visual loss with the objective of someday enabling more people to see better longer. Oxidation-mediated damage has emerged as a major mechanism that is proposed to contribute to cancers, cataract, AMD, vascular and heart disease, and some neurodegenerative diseases.

The youthful lens allows far more blue light to reach the macula than it does in senescence. By the age of 60 years, the blue light–filtering capability of the yellowing lens may be similar to that of macular pigment.79 An important implication is that the potential oxidative stress load on the macula has been in place for years before clinical or functional damage is evident. This in turn makes long-term epidemiological studies examining risk factors and potential protective factors crucial in understanding how age-related diseases may be prevented. For instance, it might be assumed that diets over many years that maximize the availability of the ocular antioxidants lutein and zeaxanthin lead to fewer age-related macular changes, but there is little evidence for this.60,78-80

The outer segments of the photoreceptors consist of stacks of membranous discs, which are continually shed and phagocytosed by the RPE. With age, the RPE gradually accumulates lipofuscin, a heterogeneous fluorescent mixture rich in lipid-protein complexes. It is likely composed of by-products of vitamin A metabolism, as well as products of lipid peroxidation.79,81 It is also a photosensitizing source of reactive oxygen species.82-84 There is
solid experimental evidence that a component of lipofuscin, N-retinyl-N-retinylidene ethanolamine (A2E), can damage the RPE, is toxic to mitochondria, and, when exposed to blue light, induces apoptosis of cultured RPE cells.83,85,86 When RPE cells are treated with lutein, this phototoxic effect is reduced greatly.82 The presence of lutein and zeaxanthin has further been shown to reduce the amount of lipofuscin formed in cultured RPE cells.73 Therefore, macular carotenoids appear to have a role in reducing the amount of lipofuscin formed, decreasing the formation of A2E, and, when some A2E is inevitably formed, attenuating its phototoxic effects on RPE cells.

It has also been documented experimentally that lipofuscin exposed to light in the blue part of the spectrum induces higher levels of superoxide anion than in full-spectrum white light or red light (660–730 nm), with increasing amounts generated as the intensity increases.80,81 Lipofuscin accumulates in the RPE with age: cells may contain up to 19% lipofuscin by cytoplasmic volume by the age of 80 years.70,81 Retinal pigment epithelium cells also produce lipofuscin-rich deposits that may accumulate as drusen beneath the RPE or within the Bruch membrane. As they enlarge, drusen become the earliest lesions in AMD. Despite vigorous efforts to understand the multifactorial pathogenesis, it is unknown at this point if AMD is a disease set into play by defects in the chorioid, RPE, or photoreceptors.64,88

An analysis quantified lutein and zeaxanthin levels by HPLC in 112 donor eyes with AMD and in 112 donor eyes without AMD. The type of AMD (wet or dry) was specified in only about a quarter of the eyes. Eyes from donors with diabetes mellitus or those known to have eye diseases other than AMD were excluded. Samples from 3 zones, corresponding to visual angles of 5°, 5° to 19°, and 19° to 38°, were analyzed. Eyes with AMD had 62% of the lutein and zeaxanthin levels compared with eyes of control subjects in the central 5°.80 It is unclear from the study if the reduced amount of xanthophyll in eyes with AMD represents a contributory cause or an effect of the disease. Other studies48,49,90 documented lower levels of macular pigment in patients with AMD.

Observational evidence for the protective effects of macular pigment comes from topographic localization of geographic atrophy in AMD and pigment levels. Often in the evolution of atrophy, the perifoveal zone is affected first, with relative preservation of the central fovea until later in the disease course. The perifovea is still subject to high light levels and metabolic activity but corresponds to significantly less pigmentary concentration than that enjoyed by the fovea. The perifovea also experiences a dramatic loss of rods and blue cones in aged eyes without AMD (about 30% of rods within 4 mm of the foveal center are lost by the age of 90 years, with an annulus between 0.5 and 3 mm experiencing the most rod loss).57 Blue cone loss is least in the central zone of densest pigment.87 Although the aging process decreases scotopic and short-wave sensitivity, higher levels of macular pigment seem to preserve shortwave and scotopic function to an extent.62

**EFFECTS OF DEPRIVATION AND SUPPLEMENTATION OF CAROTENOIDS**

The source of retinal carotenoids is dietary. Monkeys that were fed diets deficient in carotenoids for an average of 3 years had no detectable macular pigment.91 In a long-term study,92 18 monkeys were fed xanthophyll-free diets for 7 to 16 years. They had no detectable macular pigment measured noninvasively. Moreover, monkeys that were fed xanthophyll-free diets had a lower RPE cell density compared with normally fed monkeys.93 Initiation of lutein-supplemented, zeaxanthin-supplemented, or regular chow diets demonstrated rapid increases in serum xanthophyll levels, much higher in the lutein- or zeaxanthin-supplemented diets than in the regular chow diet. Macular carotenoids also increased steadily, achieving steady states by 24 to 32 weeks.

Another animal model that may be used to study the effects of retinal pigment levels is the quail. Like primates, quail retinas have a high percentage of cones and a similar propensity to concentrate lutein and zeaxanthin. Recent studies showed that (1) dietary supplementation of zeaxanthin rapidly and significantly raised the retina levels of lutein or zeaxanthin as measured by HPLC94 and (2) quail that were exposed to bright light and received zeaxanthin-supplemented diets had significantly less light-induced photoreceptor apoptosis than quail fed carotenoid-deficient diets.95,96

Human supplementation studies have been small; however, convincing evidence emerges that short-term supplementation can increase lutein and zeaxanthin levels in the macula. Already it is clear that there is considerable interindividual variability in native levels of macular carotenoids and in response to supplementation. In most cases, supplementation with lutein esters causes serum and macular levels of pigment to rise significantly. Ten milligrams of lutein taken by 8 male subjects caused plasma levels to rise 5-fold and macular levels to increase 4% to 5%.37 In another study, 30 mg of lutein taken daily by 2 male subjects caused plasma levels to rise 10-fold, and macular pigment increased about 40% in one subject and 20% in the other. Serum levels rose promptly, while macular levels began to rise after 20 days, continued to rise for 50 days after lutein supplements were stopped, and remained elevated for more than a year.90 In a study involving 38 healthy subjects given doses of lutein (36 subjects, 2.4–30 mg/d) and zeaxanthin (2 subjects, 30 mg/d), all subjects had increased variable serum concentrations. Most subjects also showed increased macular concentrations; those who did not were given lower doses. In a study in which 4 men and 9 women consumed prescribed amounts of daily spinach (10.8 g of lutein and 0.3 mg of zeaxanthin) or sweet corn (0.4 mg of lutein and 0.3 mg of zeaxanthin) for up to 15 weeks, 3 subjects (2 men and 1 woman) were nonresponders. In the others, serum levels of these nutrients rose 33% and macular pigment levels rose 19%, on average.
Epidemiological studies are helpful in providing a perspective about whether the effect of carotenoids on eye diseases suggested by short-term animal experiments or human clinical studies can be generalized to longer periods in persons with differing environmental, cultural, and genetic circumstances. Such studies so far have not revealed consistent findings. The Eye Disease Case-Control Study Group analyzed the serum and dietary levels of micronutrients in 421 patients with the neovascular form of AMD vs those in 615 control subjects (clinic patients with other diagnoses; patients with other diseases predisposed to choroidal neovascularization were excluded).

Patients with serum carotenoid levels in the top one third had half the risk of developing neovascular AMD compared with those with levels in the lowest third, while those in the middle third had one third the risk. Having intakes of lutein and zeaxanthin in the highest, compared with the lowest, quintiles lowered the estimated risk for AMD by 60%.

Since the original findings in 1994, the relationship between lutein and zeaxanthin in the diet or blood and AMD has been investigated in several populations. Higher intakes or blood levels of lutein or zeaxanthin have been associated with lower rates of some types of AMD in some studies but not in others. Generally, the relationships between lutein and zeaxanthin and AMD have been more consistent among studies of later stages of AMD and among populations that have consumed higher levels of lutein and zeaxanthin. These inconsistent results might reflect the variability among individuals in the ability to increase macular pigment with dietary or supplemental xanthophylls, as discussed earlier. As we learn and adjust for the dietary, medical, and lifestyle attributes that affect macular pigment, independent of diet, these relationships may become clearer. We may also gain insights about relationships between lutein and zeaxanthin intake and progression of later stages of AMD from clinical trials in which subjects diets’ are supplemented with foods or pills rich in lutein and zeaxanthin. To learn about the long-term effects of lutein and zeaxanthin on preventing earlier stages of AMD, we will look to long-term prospective studies after we better understand the other determinants of macular pigment levels. The Age-Related Eye Disease Study II, which is scheduled to begin recruitment in late 2006, will study the potential efficacy of lutein, zeaxanthin, and omega-3 long-chain polyunsaturated fatty acid supplementation on the progression of AMD. It is designed as a 2 × 2 factorial, placebo-controlled study, with one quarter of the cohort receiving placebo, one quarter lutein or zeaxanthin, one quarter omega-3 fatty acids, and one quarter a combination of the latter.

The Age-Related Eye Disease Study I vitamin supplement, as the standard-of-care supplement formula, will be offered to all participants.

SUMMARY OF EVIDENCE TO GUIDE FURTHER RESEARCH

Our understanding of the macular yellow spot has accelerated in the past few decades. Advances in measuring xanthophyll density in vivo, combined with an expanded understanding of the biochemical and molecular nature of lutein and zeaxanthin, are unveiling several potential roles for these pigments in the human eye. Their antioxidative properties provide a solid basis for investigating their potential in slowing the development of cataract and AMD, in which oxidative damage is believed to play a role in the pathophysiology. The results of studies thus far indicate that carotenoids concentrated in the outer retina can prevent or minimize the damage from photosensitizing reactions in the choriocapillaris are unanswerable questions. Soon, emerging knowledge of the pathophysiology of AMD can be merged with functional knowledge about the ocular pigments, to better understand their potential role in minimizing the effect of these common and costly diseases.

Submitted for Publication: June 10, 2005; final revision received September 14, 2005; accepted September 13, 2005.

Correspondence: Ronald P. Danis, MD, Department of Ophthalmology, University of Wisconsin-Madison, Fundus Photo Reading Center, Park West One, Suite 400, 406 Science Dr, Madison, WI 53711-1068 (danis@rc.ophth.wisc.edu).

Financial Disclosure: None reported.

REFERENCES

1. Home E. An account of the orifice in the retina of the human eye, discovered by Professor Soemmering: to which are added proofs of this appearance being extended to the eyes of other animals. Philos Trans R Soc Lond. 1790; (pt 2):332.
12. Snodderly DM, Auran JD, Delori FC. The macular pigment, II: spatial distribution in primate


