PERSPECTIVE

Macular pigment and age related macular degeneration

S Beatty, M Boulton, D Henson, H-H Koh, I J Murray

The yellow coloration of the macula lutea is attributable to the presence of macular pigment in the axons of its photoreceptors.[1] In the 1980s several investigators demonstrated that macular pigment consists of the xanthophyll isomers, lutein and zeaxanthin.[2 3] Although the role of the macular pigment remains uncertain, several functions have been hypothesised and these include reduction of the effects of light scatter and chromatic aberration on visual performance,[4 5] limitation of the damaging photo-oxidative effects of blue light through its absorption,[6–8] and protection against the adverse effects of photochemical reactions because of the antioxidant properties of the carotenoids.[9 10]

Age related macular degeneration (AMD) is the leading cause of visual loss in people over the age of 65 years in the Western world.[11] Although the aetiopathogenesis of AMD remains a matter of debate, there is a growing body of evidence to indicate that oxidative damage plays a role.[12–14] Consequently, the possibility that the absorption characteristics and antioxidant properties of macular pigment confer protection against AMD has been postulated.[10 15] A proved protective effect of macular pigment may be of therapeutic value, as it has recently been reported that human macular pigment can be augmented with dietary modification.[16]

In this article we review the current literature germane to macular pigment and AMD, and examine the evidence that retinal carotenoids are protective against AMD.

Historical background

The absorption of blue light by the macular pigment was first described in 1866 by Max Schultze who concluded: "Therefore, under an otherwise equal organisation, a retina without a yellow spot would see more blue light than one with such a spot".[17] He believed that absorption of the "most refractable violet" reduced chromatic aberration, but also hypothesised that macular pigment might provide some protection against the hazards of short wavelength visible light.[17]

In 1945 Wald demonstrated that macular pigment exhibited a characteristic carotenoid absorption spectrum, and concluded that this pigment belonged to the xanthophyll families found in green leaves.[18 19] It was not until 1985, however, that preliminary identification of the hydroxy carotenoids was published.[2] Using high performance liquid chromatography (HPLC), Bone *et al* suggested that the macular pigment consisted of lutein and zeaxanthin.[2] The presence of lutein and zeaxanthin in the macula was confirmed by Handelman and coworkers in 1988.[3] The identification of macular pigment, and analysis of its stereochemistry, was completed in 1993.[20]

Age related maculopathy

DEFINITION AND GRADING

In 1995 the International ARM Epidemiological Study Group published the international classification and grading system for age related maculopathy and age related macular degeneration.[21] The aim of this system was to achieve consistency of definition and severity scales for epidemiological studies of the future. In that article all age related macular changes are referred to as age related maculopathy (ARM). ARM is characterised by any of the following macular findings: soft drusen; areas of increased pigment or hyperpigmentation associated with drusen; areas of depigmentation or hypopigmentation associated with drusen. Of note, however, hard drusen are not included in the stigmata of ARM. AMD is a term reserved for the late stages of ARM.[21] Dry AMD refers to geographic atrophy, and wet AMD is characterised by choroidal neovascularisation (CNV), detachment of the retinal pigment epithelium (RPE), subretinal haemorrhage, or retinal scarring.

Table 1 Population based prevalence studies of age related maculopathy

Title of study, principal author, and year of publication	*Number of subjects examined*	*Diagnostic criteria*	*Age groups (years)*	*Prevalence of*		
				ARM	*AMD*	*ARM/AMD combined*
Blue Mountains Eye Study (Mitchell, 1995)	3654	Modified WARMGS[29]; no visual criteria; similar to international ARM study group diagnostic and grading criteria	49–54	1.30		
			55–64	2.6	0.2	
			65–74	8.5	0.7	
			75–84	15.5	5.4	
			85+	28	18.5	
Framingham Eye Study (Kahn, 1977)	2477	VA of 6/9 or worse; pigmentary, atrophic or neovascular macular changes, or drusen (hard or soft)	52–64			1.6
			65–74			11
			74–85			27.9
Beaver Dam Eye Study (Klein, 1992)	4775	WARMGS[29] no visual criteria	43–54	8.4	0.1	
			55–64	13.8	0.6	
			65–74	18	1.4	
			75+	29.7	7.1	
NHANES (Klein, 1982)	3056	VA of 6/7.5 or worse; pigmentary, atrophic or neovascular macular changes, or drusen (hard or soft)	45–64			2.3
			65–74			9

WARMGS = The Wisconsin Age Related Maculopathy Grading System. This system grades characteristics of ARM in a semiquantitative fashion using stereoscopic 30 degree colour fundus photographs; VA = visual acuity; ARM = age related maculopathy; AMD = age related macular degeneration; NHANES = National Health and Nutrition Examination Survey.

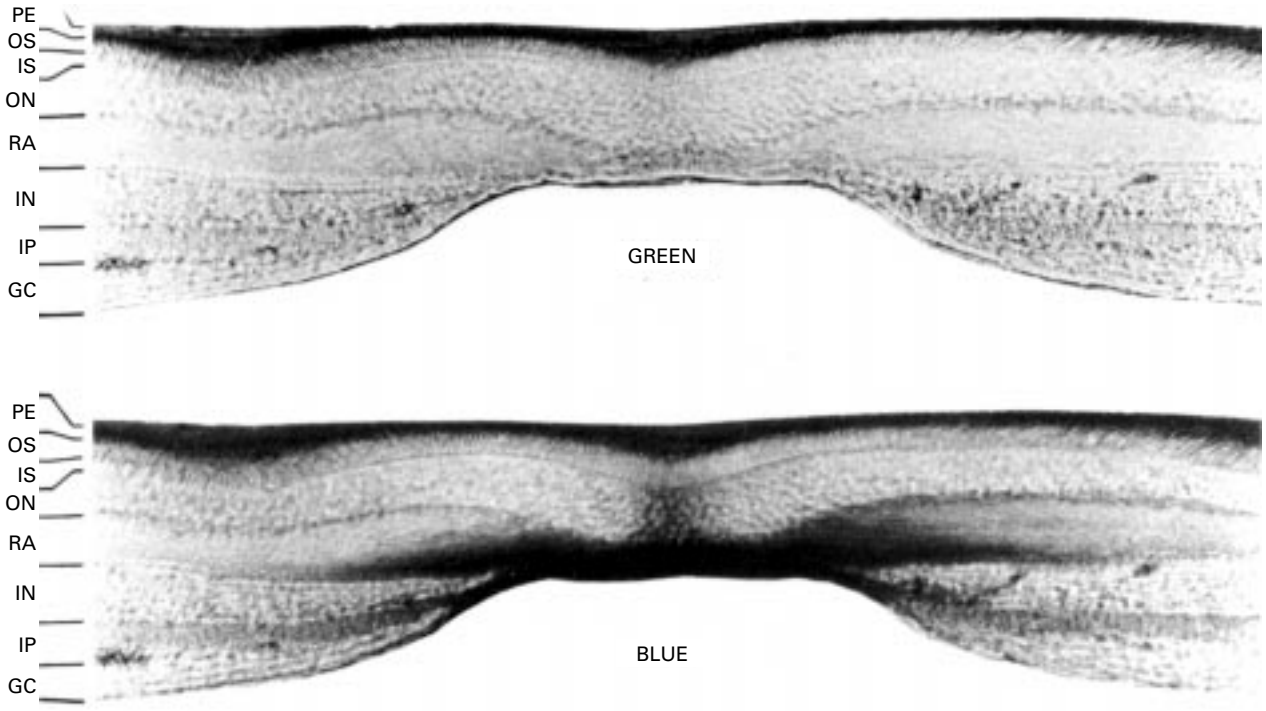

Figure 1 Photographs of a section through the fovea of an adult female Macaca mulatta in green light (top panel) and blue light (lower panel). Dark regions in blue light that are absent in green light represent areas of high macular pigment density. PE= retinal pigment epithelium; OS= outer segment layer; IS= inner segment layer; ON= outer nuclear layer; RA= receptor axon layer; IN= inner nuclear layer; IP= inner plexiform layer; GC= ganglion cell layer. (Reprinted from Snodderly et al,[1] with permission from G J Chader, editor in chief, IOVS, and Dr Max Snodderly.)

INCIDENCE AND PREVALENCE

AMD remains the leading cause of legal blindness in the elderly population of the Western world,[11 22–25] and its prevalence has been determined by several population based studies.[11 26–28] (Table 1). The 5 year incidence, in a population of minimum age 55 years, has been calculated to be 18.9% for ARM and 5.4% for AMD.[30]

Macular pigment

ANATOMICAL CONSIDERATIONS

Human macular pigment consists of the two hydroxy carotenoids, lutein and zeaxanthin.[2] Lutein and zeaxanthin reach their greatest concentrations at the centre of the fovea, and diminish with eccentricity. Snodderly *et al* have measured macular pigment in retinas of macaque and squirrel monkeys using microdensitometry and found the central peak of macular pigment to extend about 100 μm from the foveal centre, and to be flanked by shoulders before declining exponentially to optically undetectable levels at an eccentricity of 1.2–1.5 mm.[31 32] These findings are consistent with those of humans as reported by Bone *et al*.[2] The investigators reported an average mass of the carotenoids per unit retinal area of 1.33 (SD 4.3) ng/mm^2 at the foveal centre compared with 0.81 (0.25) ng/mm^2 at an eccentricity of 1.6–2.5 mm.[33] Although lutein and zeaxanthin both reach their maximum concentrations at the foveola, zeaxanthin is the dominant carotenoid at this location.[31 33] With increasing eccentricity zeaxanthin declines more rapidly than lutein and this results in lutein being the dominant carotenoid in the perifoveal zone in most cases (lutein/zeaxanthin mass ratio at foveola: 0.42 (0.04); lutein/zeaxanthin mass ratio at parafovea: 1.04 (0.24)).[31 33] This observation prompted Snodderly *et al* to suggest that particular ratios of lutein and zeaxanthin are preferentially associated with specific cone types.[31] Although the carotenoids are concentrated in the macula, lutein and zeaxanthin are found throughout the whole retina.[3 33] In five human donor retinas examined by Handelman *et al*, the mean macular pigment mass per unit area of the macula was 70.3 ng (range 35–120.2 ng) and this accounts for 36.4% (31.2%–51.5%) of total carotenoids found in the entire retina.[3] At 7 degrees eccentricity, which corresponds to a linear surface distance of approximately 2 mm, retinal carotenoids become optically undetectable, and the total mass pigment per unit area decreases from the macula to peripheral retina by a factor of almost 300.[33]

The distribution of macular pigment within the layer structure of the monkey retina has also been studied by Snodderly and coworkers, who examined serial sections of primate retinas with two wavelength microdensitometry. Although macular pigment was spectroscopically detectable in all macular layers, regions of dense pigmentation were evident by a vertical band at the centre of the fovea and two horizontal bands in the non-foveolar macula (Fig 1).[1] Difference scans were then calculated for the two wavelength specific density scans for each retinal layer, and this revealed that the greatest concentration of macular pigment was in the photoreceptor axons of the foveola (the foveola was defined as that part of the fovea lacking the inner retinal layers) and relatively high concentrations were also found in the receptor axon and inner plexiform layers outside the foveola (Fig 1).[1] As the macular pigment is an intracellular compound and the axons of the outermost cones pass through the outer nuclear layer, it is believed that the carotenoids within these cone axons contribute to the vertical band at the centre of the fovea and account for the outer band throughout the rest of the macula.[1] The inner band of the inner plexiform layer has been attributed to the presence of carotenoids within the processes of interneurons.[1] The concentration of macular pigment in the inner retina declines more rapidly than that of the outer retina with eccentricity, and at a linear distance of only 400 μm from the foveal centre there is as much lutein and zeaxanthin in most retinal layers as there is in the receptor axon layer.[1] Although the distribution of macular pigment

within the retinal layers is radially symmetric around the foveola, foveal architecture can influence its topography.[1] For example, deep and compact foveas have a higher density of macular pigment at the inner border of the receptor axon layer compared with its outer border, whereas no such pattern is seen in wider and flatter foveas.[1]

There is a consensus among investigators that there is dramatic variability of macular pigment density between individuals.[32 34–36] Pease and Adams found a sixfold variation in the central peak of macular pigment optical density among different subjects,[34] whereas Werner *et al* reported an eightfold variation.[36] The lateral extent of macular pigment distribution, which is also subject to considerable interindividual variability, is significantly and positively related to the central peak density of the pigment.[36]

There is good intraindividual interocular agreement of macular pigment optical density, with mean differences of only 5% for zeaxanthin and 11% for lutein between fellow eyes.[35 37] Macular concentrations of the carotenoids are consistent over long periods of time in individuals on a relatively constant diet,[32] and are unrelated to age.[32 33]

PHYSIOLOGICAL CONSIDERATIONS

De novo synthesis of carotenoids does not occur in animals, and the macular pigment of primates can be traced to its dietary origins.[38 39] Malinow *et al* studied the retinas of macaque monkeys fed a carotenoid-free diet for 3 or more years, and compared the results with those of control primates on a standard laboratory diet which did contain lutein and zeaxanthin.[40] Colour fundus photography and fundus fluorescein angiography indicated a total absence of macular pigment in those animals not receiving carotenoids, whereas a normal foveal appearance was evident in the control monkeys. Also, lutein and zeaxanthin were undetectable in the plasma of primates deprived of dietary carotenoids but were within normal ranges in the monkeys fed unmodified diets.[40]

These relations have also been studied in humans by Hammond and coworkers. In their first article addressing the subject, the optical density of macular pigment was measured psychophysically in 88 subjects and attempts were made to correlate the results with serum levels of lutein and zeaxanthin and with the dietary intake of carotenoids for males and females.[41] Dietary intake of carotenoids was assessed using the health habits and history questionnaire.[42] It was found that macular pigment optical density for males was 38% higher than for females, and was positively and significantly related to dietary intake of carotenoids for males only.[41] In contrast, plasma lutein and zeaxanthin correlated significantly and positively with the density of macular pigment and with dietary intake of carotenoids for both sexes.[41] These apparently contradictory findings can be explained by the comparatively weaker relation between blood and diet measures of carotenoids among female subjects. The investigators did not find any significant differences in blood or dietary intake of lutein and zeaxanthin between men and women, and therefore postulated that the greater optical density of macular pigment in males was the result of differences in the way the carotenoids are metabolised by the male and female retina.[41]

Hammond *et al* have also conducted a prospective study to investigate the relation between plasma, dietary, and macular carotenoids.[16] Macular pigment and serum lutein and zeaxanthin were measured in 13 subjects (four men, nine women) before dietary supplementation with spinach (60 g per day, containing 10.8 mg of lutein and 0.3 mg of zeaxanthin) and/or corn (150 g per day, containing 0.4 mg of lutein and 0.3 mg of zeaxanthin) for a period of 6–15 weeks. It was calculated that the volunteers were therefore receiving about four times as much lutein, and two to three times as much zeaxanthin, as a typical diet. Macular pigment optical density was determined psychophysically and serum carotenoids were analysed using HPLC, and measurements were repeated at 4, 8, and 12–15 weeks into the trial, as well as 1–6 months following discontinuation of the modified diet. Three types of response to corn and spinach supplements were identified. Firstly, there were eight "retinal responders" in this group of 11 subjects. "Retinal responders" were subjects in whom significant increases in the density of macular pigment (mean +19% (SD 11%)) and serum lutein (mean +33% (22%)), but not zeaxanthin, were observed. Following discontinuation of the nutritional supplements, serum lutein returned to baseline levels but macular pigment remained augmented in all subjects up to the longest period of follow up, which was 9 months. There were two "retinal non-responders" in whom serum lutein (but not zeaxanthin) increased significantly (mean +31%) without a parallel increase in macular pigment optical density. Finally, there was one "retinal and blood non-responder" in whom no significant rise in macular pigment or serum carotenoids was noted.[16] Of the two subjects whose diets were modified by supplementation with corn only, one showed a substantial increase in macular pigment optical density (+25%) and serum zeaxanthin (+70%) but only a small increase in serum lutein (+11%), whereas the other exhibited little change in any of these factors (macular pigment optical density +6%; serum zeaxanthin +7%; serum lutein −6%). Although these data were preliminary, the investigators were able to draw some reasonable conclusions. Firstly, there are individual differences in the response to dietary modification with carotenoid supplements. Secondly, increases in macular pigment optical density, where seen, were not followed by a rapid decline following discontinuation of the modified diet. And, thirdly, the phenomenon of "retinal non-responders" highlights the discrepancy between tissue and serum responses to lutein and zeaxanthin supplements, and suggests that blood levels of carotenoids are insufficient when investigating the possible protective effect of carotenoids against retinal degenerative disorders.

In a preliminary study by Landrum *et al*, significant increases in serum lutein concentration and macular pigment optical density were observed following a 83 day course of oral lutein supplementation (30 mg/day) in two subjects.[15] The rise in macular pigment optical density was observed to be a slow process, as the first significant increase was not noted until day 14 of supplements. Follow up of the same two subjects until the end of the 140 day course of lutein supplements revealed mean macular pigment optical density increases ranging from 21% to 41% (subject A right eye, 37% (7%); left eye, 41% (7%); subject B right eye, 21% (4%); left eye, 21% (3%)).[43] Interestingly, a statistically significant degree of interocular asymmetry of macular pigment optical density at baseline in subject A was maintained throughout the study, indicating that the accumulation of the macular carotenoids is under the influence of transport mechanisms within the individual eye. The longer duration of follow up revealed a levelling off of the rise in macular pigment optical density in subject B between day 90 and day 140 of supplements. Further, the increase in measures of macular pigment continued at approximately the same rate for 50 days following discontinuation of the lutein supplements, despite falling serum levels of lutein.[43] The persistence of raised macular pigment optical density following discontinuation of lutein supplements and return to pre-supplementation serum levels of lutein prompted Landrum *et al* to postulate a low turnover of carotenoids in the retina, and to speculate that

an individual's macular pigment levels therefore reflect long term carotenoid consumption.[43]

There are between 40 and 50 carotenoids present in a typical Western diet,[44 45] but only 14 have been detected in human blood.[46–48] Sommerburg *et al* have recently measured the content of lutein, zeaxanthin, cryptoxanthin, lycopenes, α carotene, β carotene, neoxanthins, and violoxanthins in a variety of vegetable, fruit, fruit juices, and egg yolk.[49] They found lutein to be present in many kinds of fruit and vegetables, whereas zeaxanthin was present in only a few. The highest amount of zeaxanthin was in orange pepper, and the highest mole percentage of lutein and zeaxanthin (89 mol%) was found in egg yolk.

The most prominent plasma carotenoids include lycopene, α carotene, β carotene, lutein, and zeaxanthin.[50] Of these, only lutein and zeaxanthin are found in the retina.[2 48] Although there exists a significant and positive relation between the density of macular pigment and serum concentrations of lutein and zeaxanthin,[15 41] it is worth noting that the lutein/zeaxanthin ratios of blood and macula do not correlate and this is attributable to the stereochemistry of macular carotenoids.[20] Bone *et al* identified the stereoisomers of lutein and zeaxanthin in human retina and blood using mass spectrometry and chiral column HPLC. The lutein component of macular pigment consists of a single stereoisomer, lutein [(3R,3'R,6'R)-β,ε-carotene-3,3'-diol]. The zeaxanthin component of macular pigment consists of all three possible stereoisomers and these include zeaxanthin itself or RRZ [(3R,3'R)-β,β-carotene-3,3'diol], SSZ [(3S,3'S)-β,β-carotene-3,3'-diol], and *meso*-zeaxanthin [(3R,3'S)-β,β-carotene-3,3'-diol].[15 20] Of the macular carotenoids and their stereoisomers, however, only lutein and zeaxanthin (RRZ) are found in human blood.[20] These findings prompted Bone *et al* to speculate that, as RRZ is the only isomer of human macula found in high quantities in a normal diet, *meso*-zeaxanthin is probably the result of chemical processes occurring within the eye. Further, the observation that a base catalysed reaction known to isomerise lutein into zeaxanthin yielded only *meso*-zeaxanthin suggests that *meso*-zeaxanthin is a conversion product derived from retinal lutein.[20] These findings are consistent with an approximate 2 to 1 predominance of zeaxanthin and *meso*-Z over lutein in the retina and a lutein/zeaxanthin ratio close to 3 in human plasma.[20]

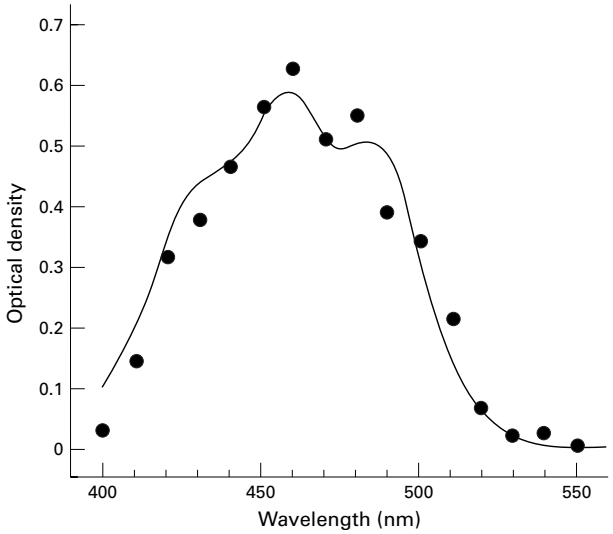

Figure 2 Absorption spectrum of macular pigment as plotted by Wyszecki and Stiles (line) and Werner et al (points). (Reprinted from Werner et al,[36] with permission from Elsevier Science.)

Carotenoids in the blood are known to bind to several proteins, including low density lipoprotein, transthyretin, β lactoglobulin, and albumin.[51–54] However, little is known of the biochemical mechanisms occupied with transport, uptake, and stabilisation of the macular pigment. Bernstein *et al* investigated protein-macular pigment interactions by incubating soluble bovine retinal extracts with radioactive carotenoids, and identified tubulin as the major carotenoid binding protein.[55] Further study performed on human macular tissue confirmed that macular pigment binds to retinal tubulin.[55] These findings are consistent with the spatial distribution of macular pigment in humans as it has been shown that microtubules are oriented axially along the cone myoid and axon, and are virtually absent in the outer segments.[56] Also, the macular pigment binding properties of an abundant structural protein such as tubulin would facilitate the selective accumulation and stabilisation of lutein and zeaxanthin in the human fovea. It appears, therefore, that the macular carotenoids are not primarily bound to the axonal membranes, as suggested by previous investigators,[57 58] if the spatial distribution of macular pigment is to be satisfactorily explained. However, our current understanding of macular pigment accumulation remains limited, and the protein(s) occupied with the specific uptake of the carotenoids has yet to be identified.

Although there is a consensus that macular pigment is of alimentary origin, some investigators have hypothesised that individual differences in the density of macular pigment may be explained in part by heredity.[58] The ability of the retina to accumulate lutein and zeaxanthin to the exclusion of other plasma carotenoids, coupled with the excellent interocular agreement of macular pigment concentrations, prompted Handelman *et al* to suggest that this striking degree of biological control might be passed from one generation to the next.[58] Hammond and coworkers have measured macular pigment, serum carotenoid concentrations, and general dietary patterns in monozygotic twins and found statistically significant differences in the optical density of macular pigment in five of the 10 twin pairs studied.[59] Further studies revealed that the twin with greater concentration of macular pigment also had higher mean levels of dietary lutein and zeaxanthin.[58 59] Although the experimental design did not allow an assessment of the heritability of macular pigment, because dizogytic twins were not included in the study, the authors were able to conclude that deposition of lutein and zeaxanthin is not completely genetically determined.

FUNCTIONAL CONSIDERATIONS

Although the function of macular pigment remains uncertain, several possibilities have been proposed. The filtering effect of macular pigment is thought to reduce chromatic aberration and protect the retina from the damaging effects of incoming short wavelength light, and active antioxidant activity has also been attributed to the macular carotenoids.

Macular pigment as an optical filter

The absorbance spectrum of macular pigment in situ peaks at 460 nm, and therefore reduces the sensitivity of the macular region to short wavelength light by acting as a broad band filter (Fig 2).[34 60] The fovea has the greatest visual acuity of all retinal regions because of its close receptor spacing, and this results in its vulnerability to image degradation. In 1866 Schultze postulated that the presence of macular pigment might result in improved visual acuity through compensation for chromatic aberration in the eye's refractive media by absorbing short wavelength light before it reaches the photoreceptors.[17] In 1974 Reading and Weale presented a theoretical quantification

of the absorption of short wavelength light by macular pigment, and concluded that its filtration of the aberrant part of the spectrum was appropriate to reduce chromatic aberration to below threshold.[5] Hence, it appears that the peak concentration of macular pigment at the centre of the fovea is consistent with its role in minimising chromatic aberration.

Light induced retinal damage may result from its thermal, mechanical, or photochemical effects. The type of light induced retinal injury depends primarily upon wavelength, power level, and exposure time, and only the photochemical reactions are seen at irradiation levels that are well tolerated if experienced transiently.[61 62] In 1976 Ham *et al* analysed light induced retinal damage as a function of wavelength by exposing rhesus monkey retinas to laser illumination, and found that sensitivity to threshold damage rose exponentially with decreasing wavelength.[63] Indeed, the investigators calculated that 100 times less energy is required to produce retinal injury with blue light (440 nm) than with orange light (590 nm). Ruffolo *et al* have investigated the influence of arterial oxygenation on photochemical damage of the retina in macaque monkeys, and found that elevated blood oxygen is associated with a reduced threshold for injury and more severe damage.[64] The oxygen enhancement of blue light damage suggests that the basic mechanism of the photochemical injury is the photodynamic production of free radicals from the toxic combination of light and oxygen.[64]

The cumulative blue light photochemical damage of the retina is reflected in the age related morphological and functional changes that occur in the macula, including a reduction in cone density[65] and loss of sensitivity of the short wavelength cone (*s* cone) pathways.[66] Haegerstrom-Portnoy measured the spectral sensitivity of short wavelength sensitive cones (450 nm) and medium and long wavelength sensitive cones (*m* and *l* cones respectively, 578 nm) at varying degrees of eccentricity for young and aged subjects. The results showed that *S* cone sensitivity attenuates with increasing age and that this attenuation varies as a function of eccentricity, with less loss occurring foveally than extrafoveally.[67] The observed differential loss of *s* cone sensitivity across the retina cannot be accounted for by lens changes alone, and indicates that these cones may be protected centrally by the screening effect of macular pigment.[67]

The macular pigment is well suited to act as a filter of incoming blue light for several reasons. Firstly, the absorbance spectrum of macular pigment peaks as 460 nm. Secondly, macular carotenoids reach their highest concentrations in the prereceptoral receptor axon layer of the foveola and the extrafoveolar macula. Thirdly, the macular pigment is distributed throughout the photoreceptor cell and therefore each photoreceptor screens other photoreceptors as well as itself because of the lateral course of the axons.[68] And fourthly, comparisons of biochemical and densitometric studies of the macular pigment have shown that its orientation enhances light absorption.[37]

Snodderly *et al* studied the filtering effect of macular pigment in primate retinas. Firstly, the investigators compared the foveolar filtering densities from light entering axially and from the side, and found that the results did not differ by more than 10%.[1] Then, by converting the optical density to extinction per unit length, they calculated the integrated density of macular pigment from the vitreal edge to the RPE ("total retina") and from vitreal edge to the outer segment/inner segment border ("total screening"). The results indicated that most of the absorption by macular pigment occurs before light reaches the photoreceptors. The contribution that each retinal layer made to the screening effect of macular pigment was also analysed, and it was observed that carotenoids within the photoreceptor axons accounted for most of the filtering effect between the centre of the fovea and an eccentricity of about 200–250 μm, whereas carotenoids in the interneurons were largely responsible for the absorption of short wavelength light more peripherally.[1] It was calculated that the fraction of blue light that was absorbed before reaching the rods and short wavelength cones, the photoreceptors spectrally closest to macular pigment, was approximately 0.4 at the foveola.[1] However, it is worth noting that the crystalline lens may also protect against the damaging effects of blue light. Under normal circumstances, wavelengths between 400 nm and 1400 nm can penetrate to the retina,[69] but nuclear cataracts are known to filter out visible blue light.[70] Further, AMD is negatively associated with nuclear lens opacities,[71] whereas cataract extraction is positively associated with progression of ARM and development of AMD.[72]

Antioxidant properties of macular pigment

In the retina, the generation of reactive oxygen species (ROS) can occur as the byproducts of cellular metabolism or as the result of photochemical reactions. These ROS include free radicals, which are partially reduced oxygen species containing one or more unpaired electrons (for example, superoxide anion, hydroxyl radical), and species with their full complement of electrons in an unstable or reactive state (for example, singlet oxygen, hydrogen peroxide). These molecules are highly reactive and will readily react with lipid, protein, and nucleic acids, thereby resulting in impaired cell function or cell death.[73] The retina is particularly susceptible to damage by these ROS for two reasons. Firstly, it is exposed to light and high levels of oxygen which provide an ideal environment for the generation of ROS. And, secondly, it contains high levels of polyunsaturated fatty acids which are readily oxidised by the ROS. Further, it has been shown that photochemical injury at the level of the RPE is related to wavelength, the threshold for damage being lowest for the blue light region of the visible spectrum,[63] and continuing to decrease for wavelengths below 400 nm.[74] It is likely that macular pigment acts to protect the retina from photochemical damage both directly by acting as a free radical scavenger and indirectly by filtering out the potentially damaging blue light.

The antioxidant properties of the retinal carotenoids have been investigated and they include the ability to quench the triplet state of photosensitisers[75] and singlet oxygen,[76] reactivity with free radicals,[77] and chain breaking antioxidant properties to retard the peroxidation of membrane phospholipids (Table 2 and Fig 3).[78] Firm evidence that the retinal carotenoids play an antioxidative role in the retina was provided by Khachik *et al* in 1997, who identified all major and minor carotenoids, and their metabolites, within the monkey retina.[50] Three major and 11 minor carotenoids were detected. The major carotenoids were lutein, zeaxanthin, and a direct oxidation product of lutein known as 3-hydroxy-β,e-caroten-3'-one. The minor carotenoids included several oxidation products of lutein and zeaxanthin, and one of lycopene. Although the carotenoid metabolites are not of dietary origin, they have been previously detected in human plasma, albeit at lower

Table 2 The carotenoids can quench reactive oxygen species (equation 1) and free radicals (equations 2 and 3)

$^1O_2^* + CAR \rightarrow {}^3O_2 + {}^3CAR^*$	(eqn 1)
$R^* + CAR(H) \leftrightarrows RH + CAR^*$	(eqn 2)
$R^* + CAR \leftrightarrows R^- + CAR^{*+}$	(eqn 3)

$^1O_2^*$= singlet oxygen; R*= free radical; CAR= carotenoid.

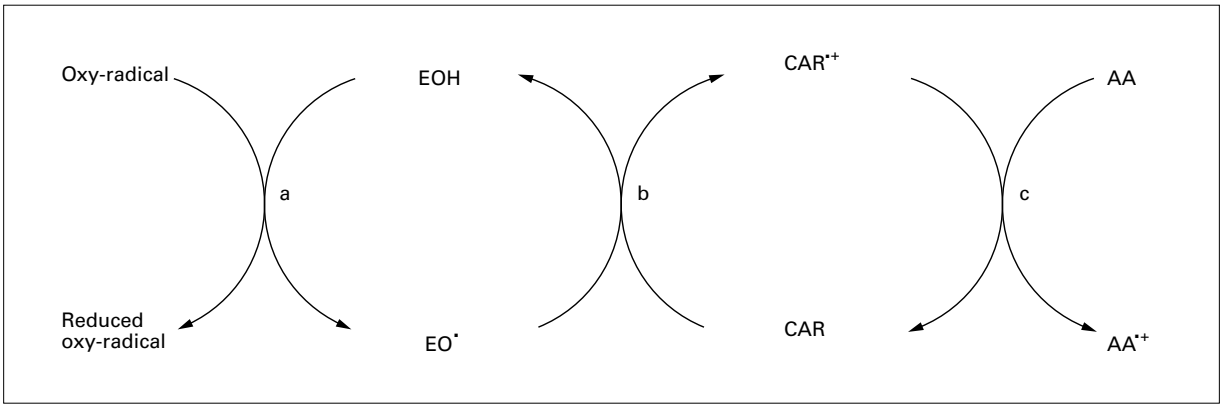

Figure 3 A schematic representation of the cooperative antioxidant interactions of vitamin E, vitamin C, and the carotenoids. EOH+= radical cation of α tocopherol; AA*+= radical cation of ascorbic acid; CAR*+= radical cation of carotenoid.*

concentrations.[46 47] The investigators were uncertain, therefore, whether their findings represented an accumulation of blood borne carotenoid oxidation products or oxidative reductive processes involving retinal lutein and zeaxanthin. However, the relatively high concentrations of 3-hydroxy-β,e-caroten-3'-one in the retina does suggest in vivo metabolic oxidation of retinal lutein. The proposed metabolic pathways for conversion of the carotenoids to their oxidation products involves a series of oxidation and double bond isomerisation reactions.[50 79] In brief, the presence of direct oxidation products of lutein and zeaxanthin in human retina confirms the active antioxidant activity of macular carotenoids, and supports the hypothesis that macular pigment protects against photo-oxidative damage.

It should be noted, however, that the antioxidative potential of macular pigment is dependent on the local oxygen environment. Jorgensen and Skibsted demonstrated that the antioxidant effects of various carotenoids, including zeaxanthin, decrease with increasing oxygen tensions.[77] Also, at very high oxygen partial pressure the carotenoids may even act as pro-oxidants, but they remain effective antioxidants at oxygen concentrations which do not exceed biologically relevant levels.[77]

If macular pigment does play a direct antioxidant role in the retina, its location is vital because it must be either close to the site of production of the reactive oxygen species or near the vulnerable tissue components, and its distribution should be such that its efficacy at quenching singlet oxygen and free radicals is not adversely affected by the profile of oxygen tension in the retinal layers. We have mentioned that macular pigment reaches its highest concentration in the receptor axon layer of the foveola, and is also abundant in the inner plexiform layer. We have also noted that the concentration of the carotenoids within each retinal layer, including the outer segment layer, peaks at the foveola. The chromophores, which act as photosensitisers involved in triggering the photo-oxidative processes, are found within the photoreceptor outer segments. Although the alterations in ARM involve several layers, including the choriocapillaris, the RPE, Bruch's membrane, and the outer retina, the site of the primary defect has yet to be established. Of note, however, age related degeneration of cells in the outer nuclear layer and their photoreceptors has been described in the absence of significant changes in the adjacent layers.[80] Also, there is strong evidence that the destruction of photoreceptors is the result of light induced oxidative stress in the retina.[81] Further, the age related photoreceptor loss correlates directly with lipofuscin concentration within the adjacent RPE, thus supporting the hypothesis that ARM results at least in part from an excessive phagocytic and metabolic load on the RPE cell.[82] Therefore, a maximum concentration of macular pigment at the foveola, where the density of cone receptors peaks, is appropriate if macular pigment is to actively quench reactive oxygen species.

However, the question remains as to why the density of macular pigment is greatest in the receptor axon layer and the inner plexiform layer. Macular pigment may play an active antioxidant role with unidentified chromophores of the receptor axon and inner plexiform layers that are involved in the production of ROS. This is supported by its spatial profile, as the receptor axon layer is characterised by low oxygen tension relative to other retinal layers,[83] thereby allowing the carotenoids to be effective quenchers of the ROS.[77]

As discussed in the previous section, macular pigment acts as an optical filter, with its peak absorption at 460 nm, and this is thought to protect the retina against the actinic effects of blue light which include damage to the RPE and the overlying photoreceptors. Although numerous photosensitisers exist in the retina, which can result in auto-oxidation of photoreceptor outer segments and impede their lysosomal degradation, the major chromophores involved in the blue light damage are thought to reside in the RPE.[84] Ham *et al* believed melanin, a broad band absorber, to be the major chromophore but evidence is now accumulating that lipofuscin is likely to be more important.[85 86] Recent studies have shown that lipofuscin is a potent photoinducible generator of ROS, with production peaking in response to the blue light region of the visible spectrum.[86] Also, preliminary work indicates that lipofuscin is phototoxic to RPE cells and is associated with a reduction in lysosomal stability and cell viability.[87] Further, excessive intracellular lipofuscin associated with RPE cell vacuolation and blebbing, processes known to contribute to drusen formation.[88 89] Thus, by screening reactive blue light, macular pigment is thought to reduce the potential for auto-oxidation in the central retina.

MEASURING MACULAR PIGMENT

MP can be measured in live subjects or in donor eyes.

Ex vivo techniques

Methods used to quantify macular pigment in postmortem retinas include HPLC[2] and microdensitometry.[31] HPLC is a biochemical analysis which does not depend on the absorptive characteristics of the pigment, whereas microdensitometry calculates the optical density of macular pigment by deriving the difference in its absorption of blue light (460 nm) and green light (say 560 nm). The main limitations of macular pigment measurements in donor eyes include the need for expensive specialist equipment, and the labourious preparation and fixation of the tissues that is required if potential postmortem alterations in the

spatial profile of the pigment are to be avoided.[1] Also, post-mortem measurements do not allow the investigator prospectively to study macular pigment and the factors that influence it, such as diet.

In vivo techniques

Methods of measuring macular pigment in live subjects can be classed as either psychophysical or imaging techniques.

The most commonly used psychophysical technique utilises heterochromatic flicker photometry (HFP) to estimate the optical density of the pigment at the foveal centre, which is proportional to its concentration. A detailed description of this procedure may be found elsewhere.[32] Briefly, a stimulus of blue light close to the peak absorbance of the macular pigment (say 460 nm) alternates with a green light which is not absorbed by macular pigment (say 560 nm). This flickering stimulus is presented to the foveal centre where macular pigment reaches its maximum concentration, and then to the parafovea where macular pigment is optically undetectable. The luminance of one light source (usually the blue light) can be adjusted by the subject, and the flicker can therefore be eliminated if the two wavelength components are matched in luminance. For example, when viewing the flickering stimulus centrally, the intensity of the blue light must be increased to compensate for its attenuation by macular pigment if the end point of no flicker is to be reached, whereas less adjustment is required when viewing the stimulus peripherally. This difference between the foveal and parafoveal sensitivities to blue light is used as a measure of macular pigment optical density. HFP used for taking measurements of macular pigment is reproducible,[32] exhibits good test-retest reliability,[32] and shows good agreement with absorbance spectra generated from in vitro preparations of liposome bound zeaxanthin and lutein.[90] Some subjects, however, find the task of HFP difficult to perform.

To our knowledge, two imaging techniques have been developed to assess the spatial distribution of macular pigment in live subjects. The first of these, known as fundus reflectometry, measures the reflectance of short wavelength light (462 nm) which has passed twice through the pigment containing layers of the retina.[91] In order to correct for the absorptive effects of melanin and oxyhaemoglobin, a digitised image obtained at an illuminating wavelength of 559 nm is subtracted from that taken at 462 nm, thus yielding the spatial variation of the double density of macular pigment. Reflectometry using a modified version of the research scanning laser ophthalmoscope (SLO) and image acquisition system can also be used to measure macular pigment in live subjects.[92] After bleaching of the rod and cone photopigments, images of the macular region are obtained with the SLO under conditions of 488 nm and 514 nm illumination, and the macular pigment density difference is then derived.[92] Macular pigment mapping using SLO based reflectometry is said to be more resistant to light scatter than conventional fundus reflectometry.[92]

Each method of mapping and measuring the macular pigment has its own merits and limitations, and all are laboratory based and require expensive specialist equipment. The need for a reliable and objective technique that can be used in the clinical setting is self evident.

Evidence that macular pigment protects against ARM and AMD

The evidence to support the hypothesis that macular pigment protects against ARM may be classed as circumstantial, epidemiological, experimental, or clinical.

CIRCUMSTANTIAL EVIDENCE

In this context, the term circumstantial evidence refers to parallels between the risk of developing age related maculopathy and factors associated with low macular pigment density. These parallels include light iris colour,[93] cigarette smoking,[94] female sex,[41] and increasing lens density.[95]

Light iris colour

In 1996 Hammond *et al* reported a significant and positive relation between macular pigment density and iris pigmentation.[93] The authors put forward two possible explanations to account for their findings. Firstly, a shared tendency to accumulate melanin and retinal carotenoids might exist as both mechanisms may have coevolved in response to environmental pressures such as light and oxygen. And, secondly, macular pigment depletion may occur as a result of oxidative stress in those eyes with light coloured irides because of increased light transmission.[96]

However, it is worth noting that although several investigators have demonstrated an inverse relation between iris pigmentation and the risk for ARM[97–101] and the severity of AMD,[102] the reports are not unanimous and some studies have failed to detect any relation.[103–105] Several mechanisms have been put forward to explain the putative protective effect of heavy iris pigmentation and these include its positive correlation with choroidal melanin[9 106] and macular pigment,[93] its association with ethnic origin, and its effect of reducing the amount of light entering the eye.[96]

Cigarette smoking

Although the literature is still divided on whether or not tobacco use is a risk factor for ARM,[11 97 98 107–109] an increased incidence of neovascular and atrophic AMD has been consistently demonstrated among smokers.[98 107–110] In 1996 Hammond *et al* reported their measurements of macular pigment optical density in 34 cigarette smokers and compared the results with those of 34 non-smokers matched for age, sex, dietary patterns, and overall pigmentation.[94] It was found that tobacco users had significantly less macular pigment (mean optical density 0.16) than control subjects (mean optical density 0.34; $p<0.001$). Further, smoking frequency (cigarettes per day) was inversely related to macular pigment density ($r=-0.448$). The authors suggested that reduced antioxidant protection[111 112] and increased oxidative stress[113 114] may result in macular pigment depletion in tobacco users. Therefore, as oxidative damage has been causally linked to choroidal neovascularisation,[115] it is possible that a lack of macular carotenoids among smokers may shift the oxidant/antioxidant balance in favour of neovascular AMD.[94]

Female sex

Despite a lack of consensus on whether or not female sex is a risk factor for ARM,[26 27 30 116–118] there is agreement that women are at greater risk of neovascular AMD than men.[27 98 116 119] In 1996 Hammond *et al* investigated the sex differences in macular pigment optical density, adjusted for age and caloric intake, and found that males had an average of 38% more macular pigment than females ($p<0.001$).[41] Moreover, although there was a positive correlation between serum carotenoids and the density of macular pigment for both sexes, the relation was stronger for men (males $r=0.62$; females $r=0.3$). These poor relations between retinal, diet, and blood carotenoids among females prompted the authors to suspect the presence of moderating variables, possibly hormonal interactions.[41]

Lens density

Hammond *et al* have demonstrated an age related inverse relation between macular pigment density and lens density (r=−0.47; p<0.001),[95] and this supports the concept that ARM and age related cataracts share a common pathogenesis.[120] Further, these findings are consistent with reports of increased cataract risk associated with tobacco use,[121] light exposure,[122 123] and inadequate intake of dietary antioxidants,[124 125] all of which implicate oxidative damage in cataractogenesis. Although the cause of the inverse relation remains uncertain, it has been postulated that individuals with higher macular pigment density may also accumulate greater quantities of lutein and zeaxanthin in the lens, and the lenticular carotenoids may prevent or retard cataract progression through their antioxidant properties.[126] Although there is no direct evidence for a shared mechanism of uptake, the concept is supported by the finding that the lens and the macula both accumulate lutein and zeaxanthin to the exclusion of other carotenoids in the blood.[126]

EPIDEMIOLOGICAL EVIDENCE

The Eye Disease Case-Control Study Group (EDCC) obtained personal, medical, physiological, biochemical, and ocular data on 421 subjects with AMD and 615 without the disease.[98] Of the 21 biochemical variables analysed, only serum carotenoid and serum cholesterol were found to be significantly associated with risk of neovascular AMD. Multivariate analysis identified a markedly decreased risk of neovascular AMD in those subjects with higher levels of serum carotenoids, and a markedly increased risk in those with high levels of serum cholesterol. It is worth noting, however, that blood levels of carotenoids included lutein, zeaxanthin, β carotene, α carotene, cryptoxanthin, and lycopene, of which only lutein and zeaxanthin are found in the retina.[3] Nevertheless, the positive correlation between serum lutein and zeaxanthin and macular pigment density that we have mentioned, coupled with the findings of the EDCC, support the view that macular carotenoids are protective for neovascular AMD.[98]

The National Health and Nutritional Examination Survey (NHANES), designed to measure the health and nutritional status of a cross sectional sample of the US population, used interview based questionnaires to assess dietary intake of vitamins A and C for 178 subjects with ARM (see Table 1 for diagnostic criteria) and compared the results with those of 2904 controls with healthy maculas.[127] After stratified adjustment for demographic and medical factors, it was found that consumption of fruits and vegetables rich in vitamin A was negatively associated with ARM. A diet rich in fruit and vegetables also contains high quantities of lutein and zeaxanthin.[49] The EDCC also evaluated dietary intake of vitamins A, C, E, and the carotenoids in 356 subjects with AMD using a food frequency questionnaire and compared the results with a control group which was statistically similar in terms of age and sex.[128] No protective effect was found for consumption of vitamin C, E, or preformed vitamin A (retinol). However, a higher dietary intake of carotenoids was associated with reduced risk of AMD. After correcting for known risk factors for ARM and AMD, it was found that those in the highest quintile of carotenoid intake had a 43% lower risk for AMD than those in the lowest quintile (odds ratio 0.57; p=0.02). Of the dietary carotenoids, lutein and zeaxanthin were found to be the most protective (p for trend=0.001). The findings of NHANES and the multicentre EDCC provide strong evidence that antioxidant status is related to the risk for ARM/AMD, and that macular pigment may play a protective role.

EXPERIMENTAL EVIDENCE

Landrum *et al* have recently reported their preliminary results of macular pigment measurements using HPLC in 22 ARM and15 control human donor eyes.[15] It was found that eye with ARM had significantly less carotenoids in the macula and whole retina than healthy eyes. Further, 17 of the 22 diseased eyes had less macular pigment than the mean of the control group. The investigators concluded that, as the differences in carotenoid concentrations were consistent across the retina, lower macular pigment levels are probably causally linked to ARM and not simply the result of the degenerative process at the macula.

Hammond *et al* have recently measured macular pigment and visual sensitivity using psychophysical methods in 27 older (aged 60–84 years) and 10 younger subjects (aged 24–36 years), and compared the results.[129] As expected, photopic sensitivity for blue and green light declined with age. For older subjects, however, photopic sensitivity was positively and significantly related to macular pigment density (blue: p <0.001; green: p <0.01). Further, the visual sensitivity of older subjects with high density of macular pigment was not significantly reduced compared with younger subjects. Loss of short wavelength cone sensitivity is a well recognised feature of early ARM.[130–132] Hammond *et al*'s findings suggest that macular pigment protects the retina from these age related sensitivity losses, and may even delay or prevent the disease process of ARM.

CLINICAL EVIDENCE

The central sparing of annular macular degeneration also suggests that macular pigment plays a protective role against certain disease processes. Annular macular degeneration, also known as bull's eye maculopathy, refers to an annular pattern of atrophy in the perifoveal region with sparing of the fovea and is seen in many conditions including cone dystrophies,[133] retinitis pigmentosa,[134] Stargardt's disease, ceroid lipofuscinosis,[135] benign concentric annular macular dystrophy,[136] and AMD.[137–139] Weiter and coworkers measured the diameter of the centrally spared area and the lateral extent of macular pigment, using fundus fluorescein angiography and monochromatic photography respectively, in 45 cases of annular maculopathy.[140] There was no statistically significant difference between the mean diameter of the area of foveal sparing (0.34 (SD 0.15) disc diameters) and the mean diameter of macular pigment (0.31 (0.12) disc diameters). Further, the pattern of macular pigment distribution corresponded exactly to the area of central sparing. The investigators, noting that annular macular degeneration is associated with the use of photosensitising drugs (for example, choloroquine)[141] or disease processes characterised by an accumulation of lipofuscin in the RPE (for example, ARM, cone dystrophies),[142] proposed that annular maculopathies are the result of photo-oxidative damage.[140] With respect to ARM, topographic studies of atrophic AMD have shown that the region most vulnerable to damage lies between 2 and 4 degrees of eccentricity where the density of macular pigment is low,[137 143] and that there is a focal reduction in RPE lipofuscin concentration at the centre of the fovea where the macular carotenoids reach their peak concentrations.[144 145] In brief, Weiter *et al*'s observations support the hypothesis that macular pigment protects against ARM.

Although the cumulative evidence supporting the putative protective role of macular pigment against ARM and/or AMD appears formidable, it should be interpreted in the context of our current and incomplete understanding of the disease, and with full appreciation of the limitations of the studies involved. For example, many of the

cited studies are observational in nature, and report reduced macular pigment among subjects with characteristics that are deemed to be positively associated with ARM or AMD. However, other than age, AMD in the fellow eye is the only risk factor for AMD upon which all investigators agree.[146] Ultimately, longitudinal studies involving serial measurements of macular pigment will be required to ascertain whether or not low macular pigment represents a risk factor for ARM and/or AMD.

Role of nutritional supplements in ARM

The benefits of carotenoid supplements in patients with ARM, or those at risk of developing the disease, remain unproved. Our knowledge of the subject is largely based on retrospective nutritional data of "broad spectrum antioxidant protection" using food frequency questionnaires which do not take account of digestive and absorptive properties, or tissue availability, of the substance under investigation.

West *et al* studied the relation of fasting plasma levels of retinol, ascorbate, α tocopherol, β carotene, and the use of vitamin supplements, with ARM in 976 participants of the Baltimore Longitudinal Study of Aging.[147] It was found that α tocopherol, and an antioxidant index which included α tocopherol, β carotene, and ascorbate, were protective for ARM. However, there was no evidence of a protective effect for vitamin supplements.

The Alpha Tocopherol (AT), Beta Carotene (BC) Cancer Prevention Study Group has conducted a randomised, double blind, placebo controlled trial to determine whether oral supplements of α tocopherol (50 mg daily) and β carotene (20 mg daily) are protective against pulmonary carcinoma.[148] After 6 years of dietary supplementation with these antioxidant vitamins an end of trial eye examination was undertaken, and neither AT nor BC was found to be protective against ARM.[149] However, smoking at least five cigarettes per day was an inclusion criterion, and tobacco use is known to be associated with significantly lower optical density of macular pigment.[94] Furthermore, the definition of a ARM in the ATBC study included the presence of hard drusen, and the prevalence of the disease may have therefore been overestimated.[149] And finally, β carotene is not present in the human retina.[2 48]

Stur *et al* conducted a 2 year double masked, randomised, placebo controlled trial of dietary supplementation with oral zinc, an antioxidant nutrient, in 112 subjects with unilateral neovascular AMD.[150] Serum levels of zinc were significantly greater in the treatment group compared with controls, but the clinical course of the disease in the unaffected eye was similar for the two groups.[150]

Possible harmful effects associated with carotenoid supplements also need to be investigated before ophthalmologists can recommend their use to prevent progression of ARM. Although β carotene is considered safe because its conversion to vitamin A is limited,[151] the ATBC study actually reported a significantly higher mortality among treated than non-treated subjects.[152] To our knowledge, there are no published data concerning toxicity of lutein and zeaxanthin.

Summary

The identification of macular pigment, which consists of lutein and zeaxanthin, was not completed until 1993. Its concentration peaks at the foveola but is optically undetectable at an eccentricity of of 1.2–1.5 mm, and its density is greatest in the receptor axon layer. The absorption spectrum of macular pigment peaks at 460 nm, and the distribution and orientation of macular pigment indicate that it acts as optical filter.

Macular pigment is entirely of alimentary origin, and although its absorptive and transport characterisitics have yet to be fully elucidated it has been shown that macular pigment density can be augmented through dietary modification. In addition to restricting photochemical retinal injury by screening blue light, macular pigment is also suspected of limiting oxidative damage by quenching reactive oxygen species. AMD remains the leading cause of blindness in the developed world, and its prevalence is likely to rise because of increasing longevity. This disease, in addition to causing severe visual disability, will have profound socioeconomic implications in the future as it affects the fastest growing section of the Western world population. We have presented the mounting circumstantial, epidemiological, experimental, and clinical evidence that supports the hypothesis that macular pigment protects against ARM and AMD. The possibility that macular pigment is protective for AMD cannot be ignored, and further research is indicated. In particular, well designed, prospective and randomised clinical trials are needed to evaluate the effects of dietary carotenoid supplementation on the risk for AMD.

Until such time as the beneficial effects of dietary lutein and zeaxanthin supplements have been substantiated, and their long term safety established, routinely prescribing micronutrient preparations containing these compounds to prevent progression of ARM cannot be justified. However, patients with ARM, or at risk of developing the disease, should be encouraged to eat a balanced diet rich in fruit and vegetables.

S BEATTY
M BOULTON
D HENSON

University Department of Ophthalmology, Manchester Royal Eye Hospital

H-H KOH
I J MURRAY

Department of Optometry and Vision Science, University of Manchester Institute of Science and Technology

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