Review

The rationale and evidence base for a protective role of macular pigment in age-related maculopathy

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ABSTRACT

Age-related maculopathy (ARM) remains the most common cause of blind registration in people aged 50 years or over in the developed world, and its prevalence continues to rise. Although effective new treatments have become available in the recent past, these are expensive and cumbersome to the healthcare provider and to the patient, and many cases remain resistant to such therapy. There is a biologically plausible rationale whereby macular pigment, which is entirely of dietary origin, may prevent or delay the onset, or ameliorate the clinical course, of ARM. In this article, we review this rationale, and critically appraise the current evidence base germane to the use of supplements containing the macular carotenoids in patients with, or at risk of developing, ARM.

Age-related macular degeneration (AMD) is the advanced form of age-related maculopathy (ARM), and is the leading cause of blindness in people over 50 years of age in the developed world.[1 2] The number of adults registered blind as a result of AMD in industrialised countries continues to rise, primarily due to increasing longevity.[3 4] Beyond its inevitable impact on the individual sufferer, AMD poses a growing socio-economic challenge to modern society.[5–7]

Three dietary carotenoids, lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (*meso*-Z), accumulate at the macula, where they are collectively referred to as macular pigment (MP). L and Z are present in many foods, whereas *meso*-Z is not found in a conventional diet, although it is found in certain types of seafood.[8 9] In recent years, the anatomic, biochemical and optical properties of MP have provoked interest in the putative protection that this pigment may confer against ARM.[10]

In this article, we review the literature germane to the rationale and evidence base for the putative protective effect of MP against ARM.

RATIONALE

Aetiopathogenesis of ARM

There is a consensus that genetic background and environmental/lifestyle risk factors, and an interaction between these variables, predispose to ARM. In other words, the risk that an individual's genetic make-up represents for ARM is subject to modification by environmental/lifestyle factors.[11] The three undisputed risk factors for ARM are: increasing age, positive family history of disease and smoking. Tobacco smoking is, therefore, the only proven environmental/lifestyle risk factor for this disease.[12–14] Putative environmental/lifestyle risk factors for ARM include dietary deficiency of antioxidants relevant to retinal health, and chronic and cumulative exposure to ambient short-wavelength (blue) light.[15 16]

Oxidative stress

As ARM is, by definition, an age-related disorder, the free radical and the evolutionary theories of ageing are of particular relevance to its aetiopathogenesis. The free radical theory of ageing proposes that ageing and age-related disorders are the result of cumulative damage resulting from tissue reactions involving reactive oxygen intermediates (ROIs). The evolutionary theory of ageing proposes that the force of natural selection declines with increasing age, such that we may have evolved with genes, which promote morbidity and mortality once we have passed our period of procreation. In other words, genes that have a beneficial effect, or no effect, in early life are not eliminated by natural selection, even though they may have a detrimental effect in later life. Thus, both genetic background and antioxidant defences may be important for ageing and age-related morbidity, and parallels with gene–environment interactions in ARM are inescapable.

Oxidative stress occurs when the level of oxidants (ROIs) in a system exceeds the detoxifying capacity of its antioxidants.[17] ROIs, which include free radicals, hydrogen peroxide and singlet oxygen, are unstable by-products of oxygen metabolism and interact with macromolecules causing damage to cells and tissues. In addition to oxygen metabolism, atmospheric pollution, asbestos exposure, tobacco use, excess consumption of alcohol, inflammation and ageing are also known to promote the production of ROIs. Interestingly, the body has an antioxidant defence system, which consists of exogenous and endogenous antioxidants, acting synergistically to quench ROIs.

The retina is ideally suited for the production of ROIs, due to its high oxygen demand, exposure to light, metabolic activities (such as retinal pigment epithelium (RPE) phagocytosis, known to generate ROIs) and its abundance of photosensitisers. Furthermore, the photoreceptor outer segments are rich in polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), compounds that represent an ideal substrate for oxidative damage. Oxidation of PUFAs initiates a self-perpetuating chain reaction, which culminates in further production of damaging ROIs and further oxidation of neighbouring PUFAs.[10]

Evidence of oxidative stress can be seen in the RPE and in the neurosensory retina with increasing age, and evidence for such injury is most prominent at the macula.[18 19] Many investigators have

proposed that the age-related changes within the RPE, including an accumulation of lipofuscin within the RPE cells, represent the earliest changes that ultimately lead to ARM. Indeed, there is convincing evidence that RPE dysfunction is related, at least in part, to lipofuscin accumulation within this cell layer, which, in turn, may be attributable to incomplete phagocytosis of oxidatively damaged photoreceptor outer segments.[20 21]

Cumulative exposure to short-wavelength light

The energy of a photon is inversely proportional to its radiation frequency; thus ultraviolet and short-wavelength (blue) light have more energy than longer wavelengths of light (eg, red and yellow). Light is composed of that part of the electromagnetic spectrum extending from ultraviolet light (with a wavelength of approximately 10–400 nm) to infrared light (with a wavelength of approximately 700–1 000 000 nm). The visible light spectrum extends from a wavelength of approximately 400–700 nm.[22 23] The cornea and lens filter out most short-wavelength ultraviolet light. However, substantial quantities of high-energy visible light irradiate the retina, especially in young people with clear lenses. In this population, transmission of light to the retina is almost 90% at a wavelength of approximately 450 nm. In contrast, nuclear cataracts and the natural yellowing of the lens, associated with ageing, are known to limit the amount of blue light incident on the retina, with only 70–80% of incident light being transmitted below wavelengths of approximately 540 nm.[22]

Since 1965, investigators have demonstrated that damage to the photoreceptors and the RPE of laboratory animals can be induced by ambient levels of visible light.[24] Indeed, premature induction of drusen formation in young mouse ARM models has been demonstrated with exposure to white, fluorescent light (10 000 lux, equal to outdoor sunlight) 24 h per day for 8 weeks.[25] However, it has been found that the blue part of the visible light spectrum is the most injurious.[23]

Lipofuscin appears to be a key mediator of photo-oxidative stress, and has been shown to be a photo-inducible generator of ROIs, with the threshold for generation of these unstable molecules being lowest for light at the blue end of the visible spectrum.[26] Furthermore, the generation of singlet oxygen by lipofuscin increases as local oxygen tension rises, and this is particularly important in the retina, given the high oxygen tension within this tissue.[27] Indeed, cultures of RPE cells, laden with the lipofuscin constituent A2E, demonstrate a predilection for apoptosis when exposed to visible light, particularly the shorter wavelengths.[28 29] In these circumstances, cellular apoptosis is secondary both to DNA damage and to mitochondrial dysfunction, caused either directly by A2E epoxides and/or ROIs or by the release of pro-apoptotic proteins (eg, cytochrome C from mitochondria).

MACULAR PIGMENT

L, Z and *meso*-Z are naturally occurring hydroxycarotenoids that accumulate at the macula (to the exclusion of other circulating carotenoids), where they are collectively known as MP.[30 31] L and Z are not synthesised de novo in humans, and are entirely of dietary origin, whereas *meso*-Z is primarily formed in the retina following conversion from L.[32]

An average western diet contains 1.3–3 mg/day of L and Z combined,[33 34] with significantly more L than Z (represented by an estimated ratio of 7:1). Approximately 78% of dietary L and Z is sourced from vegetables.[8] L is found in highest concentrations in dark green leafy vegetables, such as spinach, kale and collard greens.[8] Z is the major carotenoid found in corn, orange peppers and oranges, with a high mole percentage of both L and Z being found in egg yolk.[8] Possible dietary sources of *meso*-Z include shrimp, certain marine fish and turtles, none of which is found in a typical western diet.[9]

MP represents the most conspicuous accumulation of carotenoids in the human body. Previous investigators have described the anatomic distribution of MP in the primate retina, and have demonstrated that it generally peaks at the centre of the macula, with a concentration of almost 1 mM at this location.[31 35 36] Of note, Z is the predominant carotenoid in the foveal region, whereas L predominates in the parafoveal region.[36 37] The concentration of *meso*-Z peaks centrally (*meso*-Z:Z ratio is 0.82 in the central retina (within 3 mm of the fovea) and 0.25 in the peripheral retina (11–21 mm from the fovea)).[38] The above observations are most probably attributable to the fact that retinal *meso*-Z is produced primarily by isomerisation of retinal L, thus accounting for lower relative levels of L, and higher relative levels of *meso*-Z, in the central macula, and vice versa in the peripheral macula.

Typically, the optical density of MP reaches its half-peak optical density at an average of only 1.03° (0.3 mm) retinal eccentricity.[39] Although MP is optically undetectable at a retinal eccentricity of 7° (2 mm), L and Z are present in the peripheral retina in minute concentrations (at distances greater than 8.7 mm from the fovea, the concentration of L and Z (combined) is about 1/300 of that within 0.25 mm of the fovea).[36] However, the aggregate amount of total peripheral L and Z is substantial, accounting for approximately 50% of the total amount of the carotenoids within the entire retina.[38 40]

L, Z and *meso*-Z are intracellular compounds, separated between the cell cytosol and the cell membrane of the photoreceptor outer segment membranes, where they are possibly bound to the ubiquitous structural protein tubulin, or to specific xanthophyll-binding proteins.[41–43] Demonstrated initially by Snodderly *et al* in primates, and more recently confirmed in human donor eyes by Trieschmann *et al*, MP has been shown to reach its maximum concentration within the photoreceptor axon layer (fibres of Henle) of the foveola, whereas outside the foveola, the highest concentrations are found both within the photoreceptor axons and within the inner and outer plexiform layers.[35 44] It is noteworthy that MP has both a vertical (within the photoreceptor axons) and a horizontal (within the outer plexiform layer) orientation, thereby maximising prereceptoral absorption of incident blue light.[45] Of note, it is believed that Z is oriented perpendicular to the cell membrane, whereas L is arranged both parallel and perpendicular to the cell membrane.[45]

Functions of MP

Antioxidant

L, Z and *meso*-Z are structural isomers of one another, and their most noteworthy feature, from a biochemical perspective, rests on their high number of double bonds (and, therefore, readily available electrons).[30] The macular carotenoids are capable of quenching singlet oxygen, free radicals and triplet-state photosensitisers, thus limiting membrane phosopholipid peroxidation.[45–49] Kirschfeld was the first to propose the concept that carotenoids protect the macula against oxidative stress.[50] However, firm evidence that carotenoids act as antioxidants in the human retina was provided in 1997 by Khachik *et al*, who demonstrated the presence of direct oxidation products of L and Z in this tissue.[51]

In vitro studies of cultured human RPE cells have demonstrated enhanced survival of these cells when they are subjected to oxidative stress in the presence of Z and other antioxidant compounds, as compared with cells subjected to the same conditions in the absence of such antioxidants.[49] Of note, under in vitro conditions, L and Z are more resistant to degradation than other carotenoids when subjected to oxidative stress, an attribute which may facilitate their selective accumulation and slow biological turnover at the macula.[52] Of the macular carotenoids, it appears that Z is a more potent antioxidant than L.[48 53] Studies have shown that, in conjunction with a Z-binding protein, *meso*-Z is a better antioxidant than Z; however, without the binding protein, this situation is reversed.[46]

Thomson *et al* demonstrated that light-induced photoreceptor apoptosis can be limited by supplemental Z in a dose-dependent fashion in quail (the retina of which, like primates, selectively accumulates L and Z).[54] Consistent with this, Chucair *et al* have recently shown that supplemental L and Z, along with DHA, protect photoreceptors from oxidative stress-induced apoptosis, and that L and Z enhance photoreceptor differentiation.[55] In this study, in vitro cultures of retinal neurons were exposed to paraquat and hydrogen peroxide-induced oxidative stress in rats supplemented with L, Z or β-carotene (with or without DHA) and unsupplemented (control) rats. Cultures of supplemented animals exhibited less oxidative stress-induced apoptosis, and greater preservation of mitochondrial function, when compared with controls. This study provided the first evidence of direct neuroprotection of photoreceptors by the macular carotenoids. However, another recently published study by Kalariya *et al* suggests that carotenoid-derived aldehydes may actually promote oxidative stress in RPE cells.[56]

Optical filter

The absorption spectrum of the macular carotenoids peaks at 460 nm, and thus MP is a filter of blue light and may limit photo-oxidative damage to retinal cells.[57] As mentioned previously, MP levels are maximal within the photoreceptor axons of the foveola and the plexiform layers of the macula.[35 44] Importantly, both the absorptive characteristics of MP and its location in the anterior portion of individual photoreceptors enable the pigment to attenuate the amount of blue light incident upon the photoreceptor.

It has been estimated that the quantity of visible blue light (460 nm) incident upon the photoreceptors of the macula is substantially reduced as a result of the filtering properties of MP; this reduction is estimated at approximately 40%, but varies from 3 to 100% between individuals.[35 58] The orientation of L (which lies both parallel and perpendicular to the cell membrane) confers greater blue-light filtering properties upon this carotenoid when compared with Z (which only lies parallel to the cell membrane), because L absorbs blue light incident from all directions.[45 57] However, it should be borne in mind that L, Z and *meso*-Z have slightly different absorption spectra, and thus the combination of these pigments at the macula results in the prereceptoral absorption of a wider range of short-wavelengths of light than if any were present in isolation.

EVIDENCE

Scientific evidence yielded from clinical and epidemiological research may be categorised and ranked according to the perceived strength of that evidence and its freedom from bias. It is widely accepted that the strongest evidence is derived from well-performed meta-analyses, or systematic reviews, of well-designed prospective cohort studies, and that the impact of treatment on a disease is best assessed by a comprehensive systematic review of carefully conducted randomised controlled trials (RCTs). The evidence supporting a role for MP in the prevention of ARM, or the retardation or arrestation of progression of this disease, is primarily available from observational studies and interventional (supplementation) studies.

Observational studies

Of the 10 observational studies that have examined the relationship between dietary intake of antioxidants relevant to retinal health and risk for ARM, seven have found a protective effect in association with a high intake of such antioxidants (table 1).[16 59–68] 2

Interestingly, the most recent report from the Age-Related Eye Disease Study (AREDS) found that a higher dietary intake of L and Z was independently associated with a decreased likelihood of having neovascular AMD, geographic atrophy, and large or extensive intermediate drusen.[16] Furthermore, a prospective arm of the Blue Mountains Eye Study demonstrated that a high dietary intake of L and Z was associated with a reduced risk of incident neovascular AMD.[68]

Recently, Chong *et al* published a meta-analysis designed to investigate the role of dietary antioxidants in the primary prevention of ARM.[69] They conducted a systematic review of seven databases, covering the years 1800–2007, and specifically directed their search to only include studies evaluating dietary intake of antioxidants relevant to retinal health in individuals without any signs of ARM at baseline. A minimum follow-up of 1 year was required for inclusion, with ARM as the primary outcome, and AMD as the secondary outcome. In total, 4192 abstracts were screened, with only 12 studies (nine prospective cohort studies and three RCTs) meeting the inclusion criteria. All studies were conducted in developed countries and reported in the last 10 years. Only five cohort studies reported on the effects of L and Z, three of which reported no association,[62 70 71] one a positive (protective) association[72] and one a negative (deleterious) association.[68] However, measurement of MP optical density was not performed in any of these studies. Furthermore, a dietary questionnaire was administered only once in each study, at baseline. The authors conceded that meta-analyses of observational data are known to have more bias than those of RCTs, and that the duration of follow-up was short in the included studies.

Of the 11 observational studies that have examined the relationship between serum levels of antioxidants and risk for ARM, seven have found an inverse (protective) association (table 2).[59 73–83] 3

However, the protective effect of MP and other retinal antioxidants, if any, rests on their ability to defend against chronic and cumulative damage and, therefore, would need to be exerted in young and middle age, and decades before disease onset. Such an analysis prompted us to investigate, in a cross-sectional fashion, MP optical density and its relationship with known and putative risk factors for ARM in 828 healthy subjects (without any evidence of retinal pathology) aged 20–60 years. In brief, we found that there was a relative lack of MP in association with the three most important risk factors for ARM (age, family history of disease and tobacco smoking). In other words, a relative lack of MP in association with risk factors for ARM, decades before disease onset, has been demonstrated in healthy subjects.[84]

Table 1 Observational studies examining the relationship between dietary antioxidants and risk for age-related maculopathy (ARM)

Study	Year	No. of cases	Design	Age group	Nutritional data	ARM/nutrient relationship
NHANES I	1988	3082	Cohort	45 to 74	FFQ* (vitamins A and C)	Inverse
EDCCS	1994	1994	Case-control	55 to 80	FFQ (66-item)	Inverse
BDES	1996	1968	Cohort	45 to 86	FFQ (100-item)	None
BDES	1998	1586	Population-based cohort	43 to 86	FFQ (100-item)	Inverse
BMES	1999	3654	Cross-sectional	49+	FFQ (145-item)	None
NHANES III	2001	8222	Cross-sectional	40+	FFQ (carotenoids, L and Z)	Inverse
BMES	2002	2335	Population-based cohort	49+	FFQ (145-item)	None
NHS and HPFS	2004	118 428	Prospective follow-up	50+	FFQ (vitamins and carotenoids)	Inverse
AREDS	2007	4513	Case-control	55 to 80	FFQ (carotenoids, L and Z)	Inverse
BMES	2007	2454	Population-based cohort	49+	FFQ (145-item, L and Z)	Inverse

AREDS, Age-Related Eye Disease Study; BDES, XXXX; BMES, XXXX; EDCCS, XXXX; FFQ, XXXX; HPFS, XXXX; L, lutein; NHANES, XXXX; NHS, XXXX; Z, zeaxanthin.

Interventional studies

Koh *et al* reported that serum and macular carotenoid response in patients with ARM is similar to that of control subjects.[85] Richer *et al* have shown that ARM patients with the lowest MP optical density at baseline exhibit the greatest augmentation of MP optical density following supplementation with macular carotenoids.[86] Indeed, this finding is consistent with the recently published LUtein Nutrition effects measured by Autofluorescence (LUNA) study, where subjects with low-baseline MP optical density were more likely to exhibit a dramatic rise in MP optical density, or to exhibit no rise in MP optical density, in response to supplements than were subjects with medium- to high-baseline MP optical density values.[87] This latter finding suggests that low MP optical density values are attributable to a dietary lack of the macular carotenoids (where supplementation causes a dramatic rise in MP optical density) or to an inability of the retina to accumulate or stabilise these carotenoids (subjects who did not exhibit augmentation of MP optical density following supplementation, in spite of expected and observed increases in serum carotenoid concentrations).

Studies investigating supplemental β-carotene and vitamin E have failed to identify a beneficial effect of such supplements on the incidence and/or progression of ARM.[88 89] The AREDS investigated the use of a high-dose antioxidant formulation (vitamin C, vitamin E, β-carotene and zinc) on the progression of ARM in a 5-year prospective RCT.[90] Over 4700 patients, aged 55–80 years, were enrolled in this study, which demonstrated that patients with moderate to advanced ARM (extensive intermediate size drusen, at least one large drusen, non-central geographic atrophy in one/both eyes, or vision loss due to ARM in one eye) exhibited a 25% risk reduction in progression to advanced ARM when supplemented with zinc plus antioxidants. Unfortunately, however, the AREDS formulation did not contain the macular carotenoids (L, Z or *meso*-Z), as they were not commercially available at the inception of that study.

The first investigation of an L-fortified diet (consisting of dark green leafy vegetables and spinach) or supplementation with L-based formulations, in ARM patients, was conducted in 1999 by Richer.[91] This small pilot study, with short follow-up, reported a beneficial effect on visual function in one or both eyes of patients, and led the way for a formal RCT evaluation of L-based supplements in ARM.

In 2004, the Lutein Antioxidant Supplementation Trial (LAST) was undertaken to investigate whether nutritional supplementation with L alone, or L together with antioxidants, vitamins and minerals, improved or stabilised visual function in patients with advanced atrophic ARM.[92] This study was a prospective, 12-month, double-masked RCT involving 90 patients with ARM. The investigators reported that visual function improved with L supplementation alone, or with L supplementation in combination with other antioxidants, when compared with control subjects.[86] However, it should be noted that none of the above studies was designed to investigate whether antioxidant supplements have any effect on the primary prevention of ARM, as subjects without disease were not enrolled in these studies.

The AREDS II trial, which is currently in progress, is a placebo-controlled RCT involving 4,000 subjects with moderate to advanced ARM that is investigating the effects of supplementation with high doses of the macular carotenoids and omega-3 PUFAs, in addition to the original AREDS formulation (with the exception of β-carotene). Unfortunately, however, measurement of MP levels is not forming part of the investigation of AREDS II (with the exception of a few study

Table 2 Observational studies examining the relationship between serum antioxidants and risk for age-related maculopathy (ARM)

Study	Year	No. of cases/ no. of controls	Design	Age group	Nutritional data	ARM/serum antioxidant relationship
Blumenkranz *et al*	1986	26/23	Case-control	–	Vitamins A, C and E	None
Tsang *et al*	1992	80/86	Case-control	–	Vitamin E and selenium	None
EDCCS	1992	421/615	Case-control	–	Carotenoids	Inverse
EDCCS	1993	421/615	Case-control	55 to 80	Vitamins C, E carotenoids and zinc	Inverse
BLSA	1994	870	Cohort	40+	Vitamins, retinol and β-carotene	Inverse
BDES	1995	167	Case-control	43 to 86	Vitamin E and carotenoids	None
BMES	1997	156/156	Case-control	–	Vitamin E and β-carotene	None
Belda *et al*	1999	25/15	Case-control	60+	Vitamin E and zinc	Inverse
POLA	1999	2584	Cross-sectional	–	Vitamin E	Inverse
NHANES III	2001	8222	Cross-sectional	40+	L and Z (combined)	Inverse
Gale *et al*	2003	380	Cross-sectional	66 to 75	L and Z (separate)	Inverse (Z only)

BDES, XXXX; BLSA, XXXX; BMES, XXXX; EDCCS, XXXX; L, lutein; NHANES, XXXX; POLA, XXXX; Z, zeaxanthin.

sites). Another study that is currently in progress is the Carotenoids in Age-Related Maculopathy (CARM) study, which is a placebo-controlled, double-masked RCT, designed to investigate the potential benefits of supplementation with L, Z and coantioxidants on the progression of ARM.[93]

COMMENT

Although the notion that MP protects against ARM remains a hypothesis, the rationale in support of this view is biologically plausible and supported by the findings of in vitro and animal studies, in which L and/or Z have been shown to protect photoreceptors against oxidative injury.

We understand that ophthalmologists currently find themselves in a difficult position when attempting to make sound and evidence-based recommendations to patients with ARM. It is true that the AREDS formulation remains the only formulation that has been shown, in the context of a well-designed RCT, to be of benefit in ARM. However, the AREDS formulation contains β-carotene, which is associated with an increased risk of lung cancer among smokers.[94] Also, the doses of vitamin C, vitamin E and zinc in the AREDS formulation far exceed the European Union (EU) upper safety limits.[95] However, it is difficult to ignore the basic implication of the AREDS, namely that antioxidants are beneficial for patients with ARM. It is such an interpretation that has encouraged the nutraceutical industry to promote the use of antioxidant supplements that do not include β-carotene, are EU-compliant and contain the macular carotenoids. One may understand why an ophthalmologist, in the absence of an evidence base but in the presence of a biologically plausible rationale, might recommend such a supplement in view of the lack of other available putative or proven preventive measures against ARM. The patient, who may have already lost vision in one eye, often explains how they wish to participate actively in risk reduction against further visual loss, and how they are unwilling to wait for a conclusive evidence base. Nevertheless, under these circumstances, it is incumbent upon the ophthalmologist to inform patients with ARM that such supplements have not been proven to protect against development, or progression, of ARM.

In conclusion, we await the outcomes of several RCTs before a meaningful comment can be made upon the potential beneficial effects of supplemental L and Z in patients with ARM. However, the benefits of L and Z, if any, relate to the ability of these compounds to protect against chronic and cumulative damage, and therefore MP may indeed be important in preventing and delaying the onset of ARM. The ongoing RCTs are not designed to test this hypothesis, which would require longitudinal data involving serial MP measurements in a large number of subjects over a period of at least 20 years.

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