

Macular Carotenoids and Age-related Maculopathy

Eamonn O'Connell,¹*MRCOphth*, Kumari Neelam,¹⁻³*FRCS*, John Nolan,^{2,7}*MD*, Kah-Guan Au Eong,³⁻⁶*FRCS*,
Stephan Beatty,^{1,2}*MD*

Abstract

Lutein (L) and zeaxanthin (Z) are concentrated at the macula, where they are collectively known as macular pigment (MP), and where they are believed to play a major role in protecting retinal tissues against oxidative stress. Whilst the exact pathogenesis of age-related maculopathy (ARM) remains unknown, the disruption of cellular processes by oxidative stress may play an important role. Manipulation of dietary intake of L and Z has been shown to augment MP, thereby raising hopes that dietary supplementation with these carotenoids might prevent, delay, or modify the course of ARM. This article discusses the scientific rationale supporting the hypothesis that L and Z are protective against ARM, and presents the recent evidence germane to this theory.

Ann Acad Med Singapore 2006;35:821-30

Key words: Lutein, Macular degeneration, Oxidative stress, Zeaxanthin

Introduction

The macula lutea is an anatomic region of the posterior retina that measures approximately 5.5 mm in diameter, and is exquisitely specialised for sharp central vision.¹ Lutein (L) is a carotenoid, which, along with its stereo isomer zeaxanthin (Z), is concentrated at the macula lutea, to give it its eponymous yellow colour.² Together, these 2 carotenoids are referred to as macular pigment (MP).

Age-related macular degeneration (AMD), the advanced stage of age-related maculopathy (ARM), is a degenerative condition of the macula characterised by the dysfunction and death of photoreceptors secondary to an atrophic (geographic atrophy) and/or a neovascular (choroidal neovascularisation) event. At present, AMD is the leading cause of blind registration in the developed world,³ with choroidal neovascularisation accounting for 90% of these cases.

In the future, the prevalence of AMD is likely to rise because of increasing longevity, and the associated demographic shift towards an elderly population means that this disease will represent an increasing socioeconomic

problem. Furthermore, the currently available therapeutic interventions are limited to a small subgroup of AMD sufferers. Therefore, the delay, prevention, modification or arrest of ARM progression represents the best means of minimising the impact of this degenerative disorder on vision-related quality of life in the modern world.

While the exact aetiopathogenesis of ARM remains uncertain, there is a growing body of evidence that oxidative damage, which refers to tissue damage by reactive oxygen intermediates (ROIs), may play a causal role.^{4,5} MP is purported to prevent or retard the development or progression of ARM because of its ability to absorb blue light at the prereceptorial level, and its capacity to quench ROIs via its powerful antioxidant activity.⁴ MP cannot be synthesised *de novo* in primates, and is entirely of dietary origin. Furthermore, it has been observed that increased intake of dietary L and Z results in augmentation of MP,⁶ consistent with the possibility that appropriate dietary supplementation or modification would confer protection against development and/or progression of ARM.

This review article aims to furnish the reader with an up-

¹ Department of Ophthalmology, Waterford Regional Hospital, Ireland

² Waterford Institute of Technology, Ireland

³ The Eye Institute, Alexandra Hospital, National Healthcare Group, Singapore

⁴ Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

⁵ Singapore Eye Research Institute, Singapore

⁶ The Eye Institute, Tan Tock Seng Hospital, Singapore

⁷ Medical College of Georgia, Augusta, Georgia, USA

Address for Reprints: Dr Eamonn O'Connell, Department of Ophthalmology, Waterford Regional Hospital, Dunmore Road, Waterford, Republic of Ireland.
Email: dreamonnoconnell@iolfree.ie

to-date analysis of the scientific rationale, and the evidence base, with respect to the putative protection that MP confers against ARM.

Historical Background

The first evidence that yellow pigment at the macula might be a carotenoid appeared in 1945, when George Wald⁷ observed that the absorption spectrum of the MP closely resembled that of a preparation from a leaf xanthophyll (lutein). Based on this property, as well as its solubility, he concluded that the pigment was the xanthophyll known as lutein.

Bone et al⁸ carried out the first chromatographic characterisation of MP using high-performance liquid chromatography, and concluded that MP is composed of 2 xanthophylls, L and Z. Subsequently, Handelman and co-investigators⁹ reported that there were differences in the ratios of L to Z in the central and peripheral retina, with Z predominating in the fovea and L dominant in the parafoveal region. Later, Bone and co-investigators¹⁰ established the complete identification and stereochemistry of macular carotenoids.

Biochemical Structure of Macular Carotenoids

Macular carotenoids belong to a group of fat-soluble coloured pigments known as carotenoids. The carotenoids are characterised by a common $C_{40}H_{56}$ -isoprenoid backbone, and are subdivided into carotenes and their oxygenated derivatives, xanthophylls. L and Z are xanthophylls, and are distinguished from other carotenoids by the presence of 2 hydroxyl groups, one on either side of the molecule, that

are believed to play a critical role in their biological function.^{11,12}

Although L and Z are constitutional isomers, their biochemical structures differ in many subtle ways (Fig. 1). First, L differs from Z in the position of the double bond present within the six-carbon (ionone) ring located on the right side of the carbon chain. Second, L has one ionone ring as β type and another as ϵ type, whereas both the ionone rings in Z are of β type. Third, the 3'-hydroxyl group of the ϵ -ionone ring in L is folded back from the horizontal plane, while the corresponding group in Z projects forward from this plane. Finally, L is characterised by the presence of 3 stereocentres while Z has 2.¹⁰

It is noteworthy that L exists as a single stereoisomer (3R, 3'R, 6'R- β , ϵ -Carotene-3, 3'-diol) at the macula, whereas Z subsists as 2 separate isomers, (3R, 3'R zeaxanthin: Z; 3R, 3'S zeaxanthin: mesozeaxanthin). The retinal concentration of the third isomer of Z that exists in nature (3S, 3'S zeaxanthin) is negligible.

Spatial Distribution of Lutein and Zeaxanthin

The concentration of L and Z at the macula represents the most conspicuous accumulation of carotenoids in the human body. The estimated concentration rises to almost 1 mM within the central macula, 3 orders of magnitude above that existing in normal serum.¹³ L, Z, and meso-zeaxanthin represent about 36%, 18% and 18% of total retinal carotenoids, respectively.¹³

The macular carotenoids are distributed in a retinotopic fashion. The concentrations of L and Z peak in the central 1 to 2 degrees of the fovea, and decline in an exponential fashion to negligible levels by 5 to 10 degrees radial eccentricity. In terms of their vertical distribution, L and Z are localised mainly in the plexiform layers of the retina¹⁴ and in the outer segments of the photoreceptors.^{15,16} It is important to note that although the concentrations of individual carotenoids are highest in the central fovea, the absolute quantity of each carotenoid is greater in the non-macular retina.

Z predominates in the macula to a radial distance of 2.5 mm from the fovea (L:Z ratio = 1:2.4), while L is found in greater abundance than Z in the peripheral macula (L:Z ratio = 2:1).⁸ This variation in the ratio of L:Z mirrors the linear variation in the ratio of rods: cones, with increasing eccentricity from the foveal centre.⁸ It has also been proposed that a conversion mechanism may exist in the cones whereby L is isomerised to mesozeaxanthin, and may thus explain the predominance of zeaxanthin at the cone-enriched fovea.

Dietary Sources of Macular Carotenoids

Humans and primates do not synthesise L and Z de novo, and therefore depend entirely on dietary sources of these

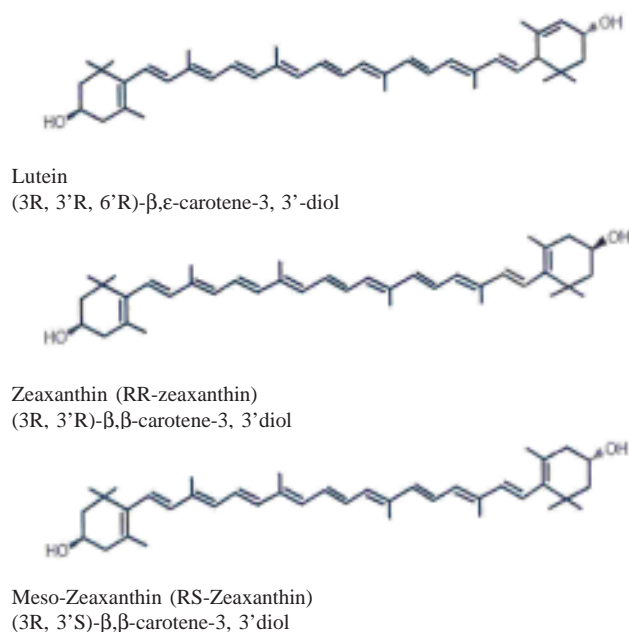


Fig. 1. Biochemical structure of macular carotenoids.

compounds. The highest concentration of L is found in food sources with yellow colour, such as maize (60 Mole%) and egg yolk (54 Mole%), the intense coloration being due to the extensive conjugation in the polyene chain.¹⁷ Dark leafy green vegetables, such as spinach and kale, are also good sources of L; however, the yellow colour of L is masked by high concentrations of chlorophyll. Orange peppers, followed by egg yolk, maize, and orange juice, also serve as good sources for Z (Table 1).¹⁸

On average, fruits and vegetables contain 7 to 10 times more L than Z. It is worth mentioning that mesozeaxanthin is virtually non-existent in food sources originating from plants.

Uptake, Distribution and Stabilisation of the Carotenoids at the Macula

Naturally occurring macular carotenoids exist as protein complexes called carotenoproteins. Heating food denatures the carotenoproteins and facilitates the absorption of L and Z, thus improving their bioavailability. However, excessive heating results in the isomerisation of all trans double bonds to cis configurations, destroying up to 60% of xanthophylls during the cooking process. Interestingly, of all xanthophylls, L appears to be the most heat-stable.¹⁹

L and Z are absorbed from the duodenum via chylomicrons, and this process is dependant on the presence of bile and pancreatic juices. Following absorption, these carotenoids reach the liver via the portal circulation where they are repackaged as plasma lipoproteins for subsequent release into the systemic circulation. In the plasma, it has been suggested that they are carried predominantly by the Apo E containing high-density lipoprotein.

Several factors influence the bioavailability of macular carotenoids from the gastrointestinal tract. These include food preparation and storage (Table 2), interaction among different types of carotenoids, physiological variations and various disease states.¹⁹

The processes governing the capture and stabilisation of L and Z at the macula remain elusive. Intracellularly, L and Z are evenly distributed between the cytosolic and membrane fractions. The cytosolic fraction is water-soluble, and may be passively deposited by tubulin in a non-specific manner.²¹ The abundance of tubulin in the axonal layer of the retina may account for the heavy concentration of MP within the Henle’s layer. The uptake of the membrane fraction is thought to be actively mediated by highly specific xanthophyll-binding proteins.²² This mechanism may account for the presence of L and Z in the rod outer segments, which are devoid of tubulin.

Adipose tissue is a major storage site for the macular carotenoids. A study of tissue-specific distribution of carotenoids in female quails suggests that fat tissue has a

Table 1. Carotenoids in Fruits and Vegetables Expressed as Mole %

Fruits/Vegetables	L+Z	L	Z
Egg yolk	89	54	35
Maize (corn)	86	60	25
Kiwi	54	54	0
Red seedless grapes	53	43	10
Zucchini squash	52	47	5
Pumpkin	49	49	0
Spinach	47	47	0
Orange pepper	45	8	37
Yellow squash	44	44	0
Cucumber	42	38	4
Pea	41	41	0
Green pepper	39	36	3
Red grape	37	33	4
Butternut squash	37	37	0
Orange juice	35	15	20
Honeydew	35	17	18
Celery (stalks/leaves)	34	32	2
Green grapes	31	25	7
Brussels sprouts	29	27	2
Scallions	29	27	3
Green beans	25	22	3
Orange	22	7	15
Broccoli	22	22	0
Apple (red delicious)	20	19	1
Mango	18	2	16
Green lettuce	15	15	0
Tomato juice	13	11	2
Peach	13	5	8
Yellow pepper	12	12	0
Nectarine	11	6	6
Red pepper	7	7	0
Tomato (fruit)	6	6	0
Carrots	2	2	0
Cantaloupe	1	1	0
Dried apricots	1	1	0

Reproduced with permission¹⁸

preference for L, whereas the retina has a preference for Z.²³ The observed differences in capture of these compounds suggest that tissue-specific xanthophyll binding proteins may mediate L and Z capture in each tissue.

Aetiopathogenesis of ARM

The pathogenesis of ARM is believed to be multifactorial, and includes cumulative light damage,^{24,25} free radical injury,²⁶ genetic factors,²⁷ and various haemodynamic

Table 2. Effect of Processing on Carotenoid Content (μg per 100g) of Various Fruits and Vegetables

Fruit/Vegetables	Preparation	L + Z content (μg per 100 g)
Kale	boiled	15,798
	raw	39,550
Spinach	boiled	7043
	raw	11,938
	frozen	830
Broccoli	boiled	2226
	raw	2445
Corn	boiled	1800
	canned	884
Brussels sprouts	boiled	1290
	raw	1590
Celery	boiled	250
	raw	232
Tomatoes	boiled	150
	raw	130

Adapted with permission²⁰

processes.²⁸ It is increasingly believed that oxidative stress may be a final common mediator for all of these factors.

Oxidative stress occurs when the level of cytotoxic reactive oxygen intermediates (ROIs) in a system exceeds the detoxifying capacity of the antioxidants, thus causing oxidative damage to cells. ROIs are unstable molecules produced in tissues throughout the body, largely by the mitochondria (during the process of oxidative phosphorylation), and by the cytochrome P450 enzyme system in the liver. Even at low concentrations, prolonged exposure to ROIs results in tissue injury, DNA mutation and disease.²⁹

The retina is vulnerable to oxidative damage for several reasons. First, the retina is constantly exposed to light and high levels of oxygen that provide a favourable environment for the generation of ROIs. Second, the lipid bilayer of the photoreceptor outer segments contains high concentrations of polyunsaturated fatty acids, which are readily oxidised by ROIs. Third, the retina and the retinal pigment epithelium (RPE) have an abundance of photosensitisers. And, finally, the phagocytic function of the RPE itself is an oxidative stress and results in the production of ROIs.⁵ ROIs react with adjacent integral cellular molecules, oxidising them, and thereby changing their structure and function. Oxidised molecules of lipid and protein are phagocytosed by the RPE, and accumulate as lipofuscin,³⁰ which, in turn, generates additional ROIs in response to irradiation with blue light.³¹

The cytotoxic effect of ROIs in the retina is modulated in vivo by 3 mechanisms. Cellular compartmentalisation (limiting exposure of sensitive structures to the damaging

effect of ROIs), repair of damaged macromolecules, and quenching and/or removal of ROIs by the antioxidant defence system.³² Of these 3 mechanisms, the most biologically important is the antioxidant defence system. This system consists of the endogenous antioxidant enzymes (glutathione, superoxide dismutase and catalase) and the exogenous antioxidants (vitamins A, C, E, the carotenoids, bioflavonoids, selenium and zinc).

Evidence that Oxidative Stress is Responsible for ARM

ROIs are produced in all cells as a by-product of metabolism,³³ and additionally, in the retina, by photochemical reactions between light and oxygen.³⁴ ROIs are likely to be particularly abundant in the retina because of its high metabolic rate, and because of the abundance of photosensitisers⁵ which increase photochemical production of ROIs in response to incident light energy. Oxidative stress in the retina results in the formation of quantifiable by-products, evidence of which has been directly demonstrated in animal models of ARM³⁵ and in animal models with light induced retinal damage.³⁶ Similarly, by-products of oxidative stress have been demonstrated in the retinae of human subjects with ARM.³⁷⁻³⁹ Studies have shown that vitamin C, a powerful antioxidant, can protect against light induced retinal damage in animal models,⁴⁰ and that higher dietary intake, and serum levels, of antioxidants may be associated with a lower incidence of ARM in humans.^{3,41-44} Finally, several studies have demonstrated a link between the pro oxidant effects of light exposure,^{23,45} dietary fatty acids⁴⁶ and retinal lipofuscin,^{47,48} and an increased risk for ARM.

Functions of Macular Carotenoids

The 2 main properties of macular carotenoids, which are particularly attractive in terms of their putative protective effect for ARM, include blue light filtration and antioxidant capacity.

Blue Light Filtration

Ham et al⁴⁹ first demonstrated that the magnitude of light induced retinal damage is a function of wavelength, and that there is an exponential rise in the retinal injury with decreasing wavelength.

In the retina, short-wavelength blue light initiates photosensitisation with the consequential generation of ROIs.⁵⁰ L and Z, by virtue of absorbing blue light en route to the photoreceptors, may prevent this short-wavelength light from producing ROIs. From this perspective, the blue light filtering property of the macular carotenoids can be considered as a passive, or indirect, antioxidant function.

Macular carotenoids are well-suited to act as an optical filter to the potentially damaging blue light for numerous reasons. First, the absorbance spectrum of macular

carotenoids peaks at 460 nm, which corresponds to the wavelength of blue light. Second, the distribution of macular carotenoids is such that they reach their highest concentration in the prerenceptorial axon layers of the retina, and therefore, absorb blue light before it reaches the photoreceptors. Finally, MP is distributed throughout the photoreceptor cell, and thus, each receptor cell screens itself as well as the adjoining cells due to the lateral course of the axons.¹⁴

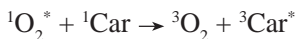
L has greater filtering efficacy than Z due to the difference in the orientation of the respective molecules. The molecules of Z are orientated perpendicular to the photoreceptor membrane, whereas those of L have both parallel and perpendicular orientation within the membrane.⁵¹ The two orthogonal positions of the L molecule allow absorption of light from all possible directions, and thus make L a better optical filter than Z. Furthermore, L and Z exert equally protective effects against short-term photochemical damage; however, under conditions of prolonged UV exposure, Z is more effective than L at diminishing UV-induced lipid peroxidation.

According to Snodderly et al,⁵² it has been estimated that macular carotenoids decrease the incident blue light by approximately 40%. This is particularly important in young individuals with a transparent lens, which allows virtually all the incident visible light to reach the macula.

Antioxidant Function

In 1997, Khachik et al³⁹ demonstrated the presence of oxidative products of macular carotenoids in the retina, providing the first evidence that L and Z have antioxidant activity in this tissue. Evidence exists to support a dual mechanism for this antioxidant activity, namely singlet oxygen quenching, and free radical scavenging.

Singlet oxygen quenching: The quenching of singlet oxygen is primarily by a physical mechanism, in which the molecule of a carotenoid accepts the excitation energy from singlet oxygen. The added energy causes excitation of the carotenoid molecule, resulting in the generation of a “triplet” state (³Car*¹⁷).



This triplet state of carotenoid dissipates energy, harmlessly, through rotational and vibrational interactions, and relaxes into its ground state (¹Car).¹⁷



Because this is a physical mechanism (as opposed to a chemical reaction), the structure of the carotenoid molecule remains unchanged.

The relative singlet oxygen quenching ability of a given carotenoid is related to the number of conjugated double bonds, at least in vitro. Therefore, macular carotenoids are

very effective quenchers of singlet oxygen.¹⁷

Reactive oxygen intermediate scavenging: Carotenoids can scavenge free radicals in 2 ways. Firstly, the free radical obtains its missing electron by removing an electron from the electron-rich molecule of the carotenoids. Secondly, the free radical adds itself to the carotenoid molecule to pair its single electron, thus forming a covalent bond. In either case, the electron-rich structure of the carotenoid molecule attracts free radicals, thus sparing the cell components such as lipids, proteins and DNA from oxidative damage.⁵³

Of note, L and Z are more effective quenchers of singlet oxygen when exposed to ROI-initiated oxidative conditions than are lycopene and beta-carotene. The main reason being that the polar end-groups of the macular carotenoids protrude from the lipid cell membrane into the intra- and extra-cellular plasma, and therefore, can interact with the ROI outside the membrane.⁵⁴ This property makes L and Z highly effective antioxidants, in contrast with apolar carotenoids such as lycopene, in the photoreceptor outer segment where the photo transduction cascade takes place along the vast membrane surface.¹⁷ Also, the antioxidant capacity of L and Z is dependent on the site of ROI generation, with greater efficacy observed in the lipid phase environment of the photoreceptor membranes.

Macular Carotenoids and ARM; Evidence For and Against

Macular Pigment

The evidence to support a protective role of MP in ARM exists in the form of histopathological, clinical, circumstantial, and epidemiological (observational and interventional) studies.

Histopathological evidence: Landrum and Bone¹³ analysed the levels of macular carotenoids in donor retinæ from subjects with and without AMD, using high-performance liquid chromatography. These authors found that the mean concentration of the macular carotenoids in the peripheral retina was lower in eyes with AMD than control eyes. The importance of this observation rests on the fact that any observed lack of L and/or Z in peripheral retina, as opposed to central retina, cannot be attributed to AMD. Similarly, the same investigators went on to determine L and Z levels in 3 concentric regions centred on the fovea in 56 donor eyes with AMD and in 56 controls without AMD, and found that those peripheral retinæ in the highest quartile of L and Z concentration had an 82% lower risk for AMD compared with those in the lowest quartile.⁵⁵

Clinical evidence: A protective effect of macular carotenoids has been suggested by the clinical picture of atrophic AMD, which has been observed to spare the fovea (where the concentration of MP peaks) until advanced disease. A similar pattern is observed in other annular

macular degenerations such as Bull’s eye maculopathy, drug-induced maculopathy, and photic lesions consequential to ophthalmic surgery.

Circumstantial evidence: The term circumstantial evidence refers to parallels between the risk of developing AMD and factors associated with low levels of macular carotenoids. These include smoking,^{4,56} female gender,^{4,57} light iris colour,⁴ and increased lens density.⁴

Smoking is a modifiable risk factor that has been strongly associated with an increased risk of AMD. According to a recent review, there is a two to threefold higher risk of AMD in current smokers versus never-smokers. It is known that smoking is associated with an increase in oxidative stress and a decrease in antioxidant levels, which may result in the depletion of macular carotenoids, with a consequential increase in the risk of ARM.⁵⁸

With age, there are various morphological and functional changes in the macula, the principal changes being a reduction of cone density and preferential loss of sensitivity of the short wavelength cone (*s*-cone) pathway. Past studies have observed an accelerated decline in the sensitivity of the *s*-cone pathway in elderly subjects who have relatively lower levels of MP, suggesting that this pigment protects against such functional deterioration (which is known to precede ARM).⁵⁹ Thus, it has been postulated that the macular carotenoids may retard ageing changes that are known to precede the clinical manifestation of AMD.

Serum Levels of Macular Carotenoids and ARM

To date, 7 studies have investigated the relationship between serum levels of L (and Z) and the risk of developing ARM^{3,41,42,60-62} (Table 3). Of these, 3 studies have observed a significant protective effect of at least one of these carotenoids.^{3,41,42}

The EDCCS demonstrated a significant inverse relationship between the prevalence of AMD and the serum concentrations of L and Z ($P = 0.0001$).⁴² Furthermore, this

study also demonstrated a progressive decrease in the risk of exudative ARM with increasing serum levels of carotenoids and increasing antioxidant index (a composite score based on serum carotenoids, selenium, vitamin C and E).⁴²

Similarly, NHANES III⁴¹ found an inverse, though marginal, association between serum levels of L and Z and risk of ARM. In addition, recently, Gale et al³ have demonstrated a twofold higher risk of ARM in patients within the lowest versus the highest tertile for serum Z levels. However, no such relationship was observed for serum L in that study.

It is important to remain cognisant of the fact that several studies have failed to show any relationship between serum levels of L (or Z), and ARM (Table 3).⁶⁰⁻⁶³ It is worth noting, however, that these studies⁶⁰⁻⁶³ had fewer subjects than those which reported that low serum levels of these carotenoids represented increased risk for ARM,^{3,41,42} and may, therefore, have had less statistical power to detect small differences between cases and controls.

The measurement of serum levels of antioxidants has some advantages over dietary assessment, because incomplete food composition databases and variation in absorption between individuals do not influence the results of the former. However, serum levels may not always reflect the macular concentrations of the corresponding carotenoids, due to the fact that serum L and Z are poor indicators of tissue status.

Dietary Intake of L and Z with Respect to ARM

Observational studies: Of 5 studies which examined the relationship between dietary L and risk of ARM (Table 4), 3 found an inverse correlation,^{41,43,44} whereas 2 failed to demonstrate any significant relationship.^{64,65}

The Eye Diseases Case Control Study (EDCC) was the first epidemiological study to observe a direct relationship between the dietary intake of L (and Z) and the risk of

Table 3. Observational Studies Examining the Relationship between Serum Concentration of L and Z and Risk of Age-related Maculopathy

Study/Author (Year of publication)	Design	No. of participants	Carotenoid examined	Relationship
Saunders et al (1993) ⁶²	Case control	65 cases, 65 controls	L	none
EDCCS (1993) ⁴²	Case control	356 cases, 520 controls	L + Z	inverse
BDES (1995) ⁶¹	Case control	167 cases, 167 controls	L + Z	none
NHANES III (2001) ⁴¹	Cross-sectional	8222	L + Z	inverse
Simonelli et al (2002) ⁶⁰	Case-control	48 cases, 46 controls	L + Z	none
Gale et al (2003) ³	Cross-sectional	380	L + Z; L, Z independently	inverse (Z only)
Cardinault et al (2005) ⁶³	Case control	34 cases, 21 controls	L, Z independently	none

BDES: Beaver Dam Eye Study; EDCCS: Eye Disease Case Control Study Group; L: Lutein; NHANES III: The Third National Health and Nutrition Examination Survey; Z: Zeaxanthin

Table 4. Observational Studies Examining the Relationship between Dietary Intake of L and Z and Risk for Age-related Maculopathy (ARM)

Study/Author (Year of publication)	Design	No. of participants	Carotenoid examined	Relationship
EDCCS (1994) ⁴³	Case-control	356 cases, 520 controls	L + Z	Inverse
BDES (1996) ⁶⁴	Cohort	1968	L + Z	None
NHANES III (2001) ⁴¹	Cross sectional	8222	L + Z	Inverse*
BMS (2002) ⁶⁵	Cohort	2335	L + Z	None
Snellen et al (2002) ⁴⁴	Case control	72 cases, 66 controls	L	Inverse

BDES: Beaver Dam Eye Study; BMS: Blue Mountain Eye Study; EDCCS: Eye Disease Case Control Study Group; L: Lutein; NHANES III: The Third National Health and Nutrition Examination Survey; Z: Zeaxanthin

* Inverse relation between dietary intake of L + Z and soft drusen, and between dietary intake of L + Z and the presence of pigmentary abnormalities, in the lowest age group at risk of ARM.

ARM.⁴³ Of all the carotenoids, L and Z were most strongly related to a reduced risk of ARM, reflected in a 57% lower risk for highest quintile of L intake (6 mg per day) when compared with the lowest quintile (0.5 mg per day).

Subsequently, the National Health and Nutritional Examination Survey (NHANES III) found a significant association between the consumption of L (and Z) and the risk of advanced AMD in the youngest age group at risk for this condition.⁴¹ Likewise, Snellen and coworkers⁴⁴ demonstrated an association between early ARM and low intake of L in the diet. It is important to note that this latter study did not assess the relationship between dietary intake of Z and the risk for ARM.

All of these studies assessed the dietary intake of L and Z with the help of a food frequency questionnaire (FFQ). It is worth mentioning that FFQs do not take into account the effect of cooking on nutrient content, food interactions affecting bioavailability, and the influence of memory and social desirability, which may affect the precise assessment of dietary intake of L and Z.

Interventional studies: Several randomised placebo-controlled clinical trials have investigated the role of antioxidants in individuals with AMD. However, the Age-Related Eye Disease Study (AREDS) has reported the firmest evidence that antioxidants are protective against ARM. This research group has demonstrated that antioxidant supplementation reduces the risk of ARM progression and visual deterioration by 25% and 19%, respectively.⁶⁶

However, the AREDS supplement did not contain L and Z, as these were commercially unavailable at the time of that study’s inception. Therefore, it is possible that the beneficial effect of antioxidant supplement, as seen in the AREDS trial, would have been even greater if L and/or Z had been included in the supplement given. This is because L and Z are specifically concentrated at the macula, and have a putative additional beneficial effect of filtering blue light before it reaches the photoreceptors.

To date, 3 interventional studies have examined the effect of dietary L supplementation on the risk for ARM (Table 5). The data from all 3 studies report improvement in visual function and/or visual acuity as a result of L supplementation.⁶⁷⁻⁶⁹ Although the statistical power of these studies is limited by their small size, they are, nonetheless, encouraging. It is worth mentioning that all interventional studies used L for supplementation, as preparations of pure Z are not commercially available. However, preparations of L isolated from marigold (*Tagetes erecta*) petals contain approximately 5% Z.

Safety of Macular Carotenoids

Animal Studies

The 4 studies which have investigated L toxicity in animal models using purified crystalline L in supplemental form are summarised in Table 6.¹⁹ The results from these studies demonstrate that there were no observed adverse clinical or histopathological changes suggestive of toxicity following the administration of high doses of L (up to 639 mg L per kg body weight per day for 4 weeks).

Furthermore, genotoxic potential of purified crystalline L was assessed in different strains of *Salmonella typhimurium* using the Ames test, and it was observed that L does not induce mutagenicity.⁷¹ Indeed, a number of studies have revealed an antimutagenic effect of natural xanthophylls.^{73,74}

Human Studies

Among the general population, the mean intake of L and Z is significantly lower than the usual dietary recommendation, and, therefore, there are no reported adverse effects of long-term exposure to these carotenoids from dietary sources. However, a number of human interventional studies, involving supplementation with high doses of L for extended periods of time, have failed to demonstrate toxicity.¹⁹

The only undesirable effect reported as a result of L

Table 5. Human Intervention Studies Investigating the Influence of Supplemental L on Visual Function in Age-related Maculopathy

Study/Author (Year of publication)	Design	Participants (n)	Supplement given/ dose	Period of supplementation	Outcome
Richer et al (1999) ⁶⁷	Case-series	AMD patients (14)	14 mg L/day	3 to 12 months	Improvement in visual acuity, and several other parameters of visual function
Massaccesi et al (2001) ⁶⁸	Randomised, placebo-controlled	ARM patients (50)	15 mg L/day	18 months	Improvement in visual acuity
LAST (2004) ⁶⁹	Randomised, placebo-controlled	AMD patients (90)	10 mg L/day	12 months	Improvement in visual acuity, and several other parameters of visual function

AMD: age-related macular degeneration; ARM: age-related maculopathy; L: Lutein; LAST: the Veterans LAST study (Lutein Antioxidant Supplementation Trial)

Table 6. Summary of Lutein Toxicity Studies in Animals

Author (Year of publication)	Animal model	Study design	Conclusion
Jenkins et al (2000) ⁷⁰	Rat	Up to 35 mg L per day for 8 weeks	No exposure-related toxicity except reduced lung and brain weight
Kruger et al (2002) ⁷¹	Rat	Up to 639 mg L/kg body weight per day for 4 weeks	No exposure-related toxicity or adverse events
Kruger et al (2002) ⁷¹	Rat	Up to 208 mg L/kg body weight per day for 13 weeks	No exposure-related toxicity or adverse events
Goralczyk et al (2002) ⁷²	Monkey	Up to 20 mg L/kg body weight per day for 52 weeks	No exposure-related toxicity or adverse events

L: Lutein

Reproduced with permission¹⁹

supplementation in humans has been carotenedermia, a harmless and reversible cutaneous hyperpigmentation analogous to jaundice.⁷⁵⁻⁷⁷ This condition results from excessive intake of carotenoids, and is not solely attributable to high intake of lutein.

Lutein is unlikely to exert a pro-carcinogenic effect similar to high doses of beta-carotene or other vitamin A toxicity. The reason is that L does not interact with the retinoic acid receptors or AP-1 complex, and possesses no provitamin A activity. Indeed, a diet with nearly 25 g of L per day consumed by the population in the Fiji islands is associated with a lower rate of lung cancer relative to other South Pacific islands.⁷⁸ Furthermore, the data from a few observational studies suggest that L tends to have a protective effect against lung cancer.⁷⁸⁻⁸⁰

Conclusion

In conclusion, the macular carotenoids may play a vital role in protecting the macula against oxidative stress. Currently, an increasing number of observational studies, and a small number of intervention trials, suggest that L and/or Z may prevent ARM, or arrest its progression.

Furthermore, the data from animal and human studies report that long-term supplementation with pharmacological doses of L is safe. However, the results from ongoing large randomised clinical trials are awaited, and should clarify the role of macular carotenoids with respect to protection against ARM.

REFERENCES

1. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr* 1995;62:1448S-1461S.
2. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. *Vision Res* 1985;25:1531-5.
3. Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2003;44:2461-5.
4. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol* 1999;83:867-77.
5. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115-34.

6. Johnson EJ, Hammond BR, Yeum KJ, Qin J, Wang XD, Castaneda C, et al. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr* 2000;71:1555-62.
7. Wald G. Human vision and the spectrum. *Nature* 1945;101:653-8.
8. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC—retinal distribution and age study. *Invest Ophthalmol Vis Sci* 1988;29:843-9.
9. Handelman GJ, Snodderly DM, Adler AJ, Russett MD, Dratz EA. Measurement of carotenoids in human and monkey retinas. *Methods Enzymol* 1992;213:220-30.
10. Bone RA, Landrum JT, Hime G W, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci* 1993;34:2033-40.
11. Johnson EJ. The role of carotenoids in human health. *Nutr Clin Care* 2002;5:56-65.
12. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vis* 1999;5:32.
13. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 2001;385:28-40.
14. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. 1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci* 1984;25:660-73.
15. Sommerburg O, Siems WG, Hurst JS, Lewis JW, Kliger DS, van Kuijk FJ. Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Curr Eye Res* 1999;19:491-5.
16. Rapp LM, Seema SS, Choi JH. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol* 2000;41:1200-9.
17. Semba RD, Dagnelie G. Are lutein and zeaxanthin conditionally essential nutrients for eye health? *Med Hypotheses* 2003;61:465-72.
18. Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol* 1998;82:907-10.
19. Alves-Rodrigues A, Shao A. The science behind lutein. *Toxicol Lett* 2004;150:57-83.
20. Holden JM, Eldridge AL, Beecher GR. Carotenoid content of US foods: An update of the database. *J Food Comp Anal* 1999;12:169-96.
21. Bernstein PS, Balashov NA, Tsong ED, Rando RR. Retinal tubulin binds macular carotenoids. *Invest Ophthalmol Vis Sci* 1997;38:167-75.
22. Yemelyanov AY, Katz NB, Bernstein PS. Ligand-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retinas. *Exp Eye Res* 2001;72:381-92.
23. Toyoda Y, Thomson LR, Langner A, Delori FC, Garnett KM, Craft N. Effect of dietary zeaxanthin on tissue distribution of zeaxanthin and lutein in quail. *Invest Ophthalmol Vis Sci* 2002;43:1210-21.
24. Cruickshanks KJ, Klien R, Klien BE. Sunlight and age-related macular degeneration. The Beaver Dam Eye Study. *Arch Ophthalmol* 1993;111:514-8.
25. Taylor HR, Munoz B, West S, Bressler NM, Bressler SB, Rosenthal FS. Visible light and risk of age-related macular degeneration. *Trans Am Ophthalmol Soc* 1989;88:163-73.
26. Nicolas MG, Fujiki K, Murayama K, Suzuki MT, Shindo N, Hotta Y, et al. Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. *Exp Eye Res* 1996;62:399-408.
27. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277:1805-7.
28. Friedman E. A haemodynamic model of the pathogenesis age-related macular degeneration. *Am J Ophthalmol* 1997;124:677-82.
29. Mccord JM. The evolution of free radicals and oxidative stress. *Am J Med* 2000;108:652-9.
30. Boulton M, Dontsov A, Jarvisevans J, Ostrovsky M, Svistunenکو D. Lipofuscin is a photoinducible free-radical generator. *J Photochem Photobiol B* 1993;19:201-4.
31. Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ. Cell loss in the aging retina—relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci* 1989;30:1691-9.
32. Seis H. Oxidative stress: from basic research to clinical application. *Am J Med* 1991;91:31-7.
33. Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. *Cardiovasc Res* 1992;26:651-5.
34. Dargel R. Lipid peroxidation—a common pathogenetic mechanism? *Exp Toxicol Pathol* 1992;44:169-81.
35. Olin KL, Morse LS, Murphy C, Paul-Murphy J, Line S, Bellhorn RW, et al. Trace element status and free radical defence in elderly rhesus macaques (*Macaca mulatta*) with macular drusen. *Proc Soc Exp Biol Med* 1995;208:370-7.
36. Wiegand RD, Giusto NM, Rapp LM, Anderson RE. Evidence for rod outer segment lipid peroxidation following constant illumination of the rat retina. *Invest Ophthalmol Vis Sci* 1983;24:1433-5.
37. Hammes HP, Hoerauf H, Alt A, Schleicher E, Clausen JT, Bretzel RG, et al. N (epsilon) (carboxymethyl) lysine and the AGE receptor RAGE colocalise in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 1999;40:1855-9.
38. Ishibashi T, Murata T, Hangai M, Nagai R, Horiuchi S, Lopez PF, et al. Advanced glycation end products in age-related macular degeneration. *Arch Ophthalmol* 1998;116:1629-32.
39. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 1997;38:1802-11.
40. Organiskiac DT, Wang HM, Li ZY, Tso MO. The protective effect of ascorbate in retinal light damage of rats. *Invest Ophthalmol Vis Sci* 1985;26:1580-8.
41. Mares-Perlman JA, Fisher AI, Klein R, Palta M, Block G, Millen AE, et al. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol* 2001;153:424-32.
42. Eye Disease Case Control Study Group. Antioxidant status and neovascular age-related macular degeneration. *Arch Ophthalmol* 1993;111:104-9.
43. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA* 1994;272:1413-20.
44. Snellen EL, Verbeek AL, van den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand* 2002;80:368-71.
45. Taylor HR, West S, Munoz B, Rosenthal FS, Bressler SB, Bressler NM. The long term effects of visible light on the eye. *Arch Ophthalmol* 1992;110:99-104.
46. Mares-Perlman JA, Brady WE, Klein R. Dietary fat and age-related maculopathy. *Arch Ophthalmol* 1995;113:743-8.
47. Feeney-Burns L, Eldred GE. The fate of the phagosome: conversion to age pigment and impact in human retinal pigment epithelium. *Trans Ophthalmol Soc UK* 1983;103:416-21.
48. Feeney-Burns L, Hilderbrand ES, Eldridge S. Aging human RPE: morphometric analysis of macular, equatorial and peripheral cells. *Invest Ophthalmol Vis Sci* 1984;25:195-200.

49. Ham WT Jr, Mueller HA, Ruffolo JJ Jr, Clarke AM. Sensitivity of the retina to radiation damage as a function of wavelength. *Photochem Photobiol* 1979;29:735-43.
50. Ruffolo JJ, Ham WT Jr, Mueller HA, Millen JE. Photochemical lesions in the primate retina under conditions of elevated blood oxygen. *Invest Ophthalmol Vis Sci* 1984;25:893-8.
51. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the "protective role of lutein and zeaxanthin in the eye. *Annu Rev Nutr* 2003;23:171-201.
52. Snodderly DM, Auran JD, Delori FC. The macular pigment. 2. Spatial-distribution in primate retinas. *Invest Ophthalmol Vis Sci* 1984;25:674-85.
53. Eperjesi F, Beatty S. *Nutrition and the Eye – A Practical Approach*. Toronto: Elsevier, 2005:91-8.
54. Britton G. Structure and properties of carotenoids in relation to function. *FASEB J* 1995;9:1551-8.
55. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD. *Invest Ophthalmol Vis Sci* 2001;42:235-40.
56. Hammond BR Jr, Caruso-Averi M. Macular pigment optical density in a Southwestern sample. *Invest Ophthalmol Vis Sci* 2000;41:1492-7.
57. Hammond BR, Curran-Celentano J, Judd S, Fuld K, Krinsky NI, Wooten BR, et al. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res* 1996;36:2001-12.
58. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. Smoking and age-related macular degeneration: a review of association. *Eye* 2005;19:935-44.
59. Haegerstrom-Portnoy G. Short-wavelength-sensitive-cone sensitivity with ageing: a protective role for macular pigment? *J Opt Soc Am A* 1988;5:2140-4.
60. Simonelli F, Zarilli F, Mazzeo S, Verde V, Romano N, Savoia M, et al. Serum oxidative and antioxidant parameters in a group of Italian patients with age-related maculopathy. *Clin Chim Acta* 2002;320:111-5.
61. Mares-Perlman JA, Brady WE, Klein R, Klein BE, Bowen P, Stacewicz-Sapuntzakis M, et al. Serum antioxidants and age-related macular degeneration in a population based case control study. *Arch Ophthalmol* 1995;113:1518-23.
62. Saunders TA, Haines AP, Wormald R, Wright LA, Obeid O. Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. *Am J Clin Nutr* 1993;57:428-33.
63. Cardinault N, Abalain JH, Sairafi B, Coudray C, Grolier P, Rambeau M, et al. Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients. *Clin Chim Acta* 2005;357:34-42.
64. Mares-Perlman JA, Klein R, Klein BE, Greger JL, Brady WE, Palta M, et al. Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch Ophthalmol* 1996;114:991-7.
65. Flood V, Smith W, Wang JJ, Manzi F, Webb K, Mitchell P. Dietary antioxidant intake and incidence of early age-related maculopathy. *Ophthalmology* 2002;109:2272-8.
66. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss – AREDS Report No. 8. *Arch Ophthalmol* 2001;119:1417-36.
67. Richer S. ARMD – pilot (case series) environmental intervention data. *J Am Optom Assoc* 1999;70:24-36.
68. Massacesi KR, Faletra R, Gerosa F. The effect of oral supplementation of macular carotenoids (lutien and zeaxanthin) on the prevention of age-related macular degeneration: a 18 months of follow up study. *Assoc Res Vision Ophthalmol* 2001;42:S234.
69. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, et al. Double-masked, placebo-controlled, randomised trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* 2004;75:216-30.
70. Jenkins MY, Mitchell GV, Grundel E. Natural tocopherols in a dietary supplement of lutein affect tissue distribution of tocopherols in young rats. *Nutr Cancer* 2000;37:207-14.
71. Kruger CL, Murphy M, DeFreitus Z, Pfannkuch F, Heimbach J. An innovative approach to the determination of safety for a dietary ingredient derived from a new source: case study using a crystalline lutein product. *Food Chem Toxicol* 2002;40:1535-49.
72. Goralczyk R, Barker F, Froescheis O, Aebiacher JC, Niggeman B, Kotte U, et al. Ocular safety of lutein and zeaxanthin in a long term study in Cynomolgous monkeys. *Assoc Res Vision Ophthalmol* 2002;43:2546.
73. Gonzalez de Mejia E, Ramos-Gomez M, Loarca-Pina G. Antimutagenic activity of natural xanthophylls against aflatoxin B1 in *Salmonella typhimurium*. *Environ Mol Mutagen* 1997;30:346-53.
74. Gonzales de Mejia E, Loarca-Pina G, Ramos-Gomez M. Antimutagenicity of xanthophylls present in Aztec Marigold (*Tagetes erecta*) against 1-nitropyrene. *Mutat Res* 1997;389:219-26.
75. Granado F, Olmedilla B, Gil-Martinez E, Blanco I. Lutein ester in serum after lutein supplementation in human subjects. *Br J Nutr* 1998;80:445-9.
76. Olmedilla B, Granado F, Gil-Martinez E, Blanco I. Supplementation with lutein (4 months) and alpha-tocopherol (2 months), in separate or combined oral doses, in control men. *Cancer Lett* 1997;114:179-81.
77. Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Martinez E, et al. A European multicentre, placebo-controlled supplementation study with alpha-tocopherol, carotene-rich palm oil, lutein or lycopene: analysis of serum responses. *Clin Sci (Lond)* 2002;102:447-56.
78. Le Marchand L, Hankin JH, Bach F, Kolonel LN, Wilkins LR, Stacewicz-Sapuntzakis M, et al. An ecological study of diet and lung cancer in the south pacific. *Int J Cancer* 1995;63:18-23.
79. Holick CN, Michaud DS, Stolzenberg-Solomon R, Mayne ST, Pietinen P, Taylor PR, et al. Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the alpha-tocopherol, beta-carotene cohort study. *Am J Epidemiol* 2002;156:536-47.
80. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Spiezer FE, Willett WC, et al. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 2000;72:900-97.