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Macular pigment and its putative protective effect for ARM

In the first of a major series looking at nutritional influence upon eye disease, **Mark Kirby**, **John M Nolan**, **Edward Loane**, and **Stephen Beatty** review our current state of knowledge about macular pigment and its potential for protecting against eye disease. C6263, two general CET points, suitable for OOs and DOs

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The macula is the central region of the retina with the highest density of photoreceptors, and is responsible for detailed central and colour vision. Age-related macular degeneration (AMD), the late stage of age-related maculopathy (ARM), is the leading cause of blindness in the Western world. The carotenoids lutein (L), zeaxanthin (Z) and meso-zeaxanthin (meso-Z) accumulate at the macula, where they are collectively referred to as macular pigment (MP).

This article reviews the evidence in support of the view that MP protects against ARM. Furthermore, we discuss the bioavailability of L and Z, and the importance of meso-Z as a macular carotenoid.

Prevalence of ARM

It is estimated that late ARM affects approximately 417,000 people in the UK[1] and 70,000 people in the Republic of Ireland, and more than 1.75 million individuals in the US, and this latter figure is expected to rise to almost 3 million by 2020.[2] The increasing prevalence of ARM worldwide is largely attributable to increasing longevity.

The pathogenesis of ARM

Although the pathogenesis of ARM remains poorly understood, there is a growing consensus that one or more of the following processes contribute to this condition: inflammation; oxidative stress; cumulative blue light damage; retinal pigment epithelial cell (RPE) dysfunction, and reduced foveolar choroidal circulation.

In this review, we focus on two of these proposed mechanisms of ARM pathogenesis, namely oxidative stress

Cross-section of the fovea
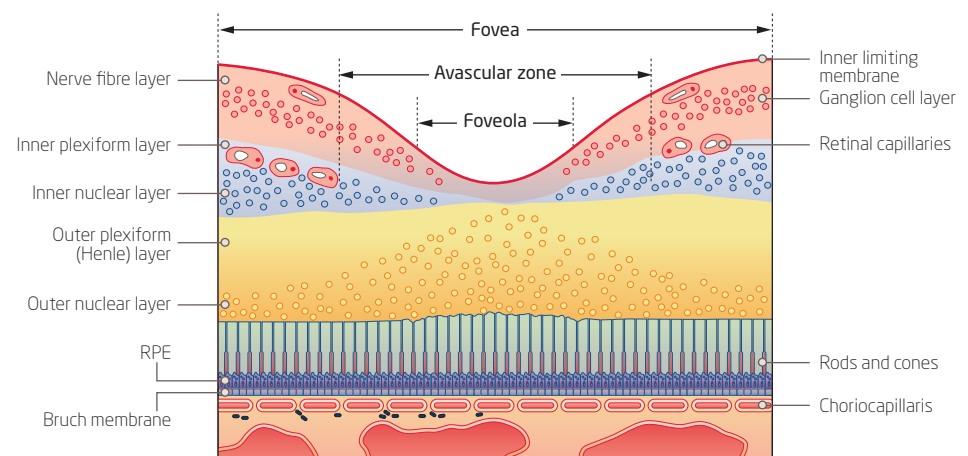
Figure 1 A simplified diagram of the macular region of the human retina

and cumulative blue light damage. It is important to note, however, that these proposed mechanisms are compatible with all of the other hypothesised mechanisms of ARM pathogenesis.

Oxidative stress

The hypothesis that oxygen, an essential requirement for all living organisms, is also a potentially toxic substance is becoming increasingly understood and accepted. Oxidative stress refers to damage caused by unstable and reactive oxygen intermediates (ROIs). There is a growing body of evidence to suggest that damage caused by ROIs play a role in the pathogenesis of ARM.

ROIs can be classified according to their reactivity towards biological targets, their site of production, their chemical nature, or their free radical or non-radical subgroups. In this review, we describe ROIs in terms of their free radical and non-radical subgroups. Free radicals are molecules that contain one or more unpaired electrons in their outer orbits.[3] In order to achieve a stable state, these unstable molecules 'steal' electrons from other molecules (for example, lipids, proteins and DNA), which are themselves rendered unstable by this reaction and a cascade of cytotoxic reactions ensues. Non-radicals, such as hydrogen peroxide, contain their full complement of electrons, but are nevertheless, in an unstable state. Antioxidants are molecules which neutralise ROIs. In brief, therefore, oxidative damage occurs when the level of ROIs overwhelms the antioxidant defence system,[3] with consequential cellular damage.
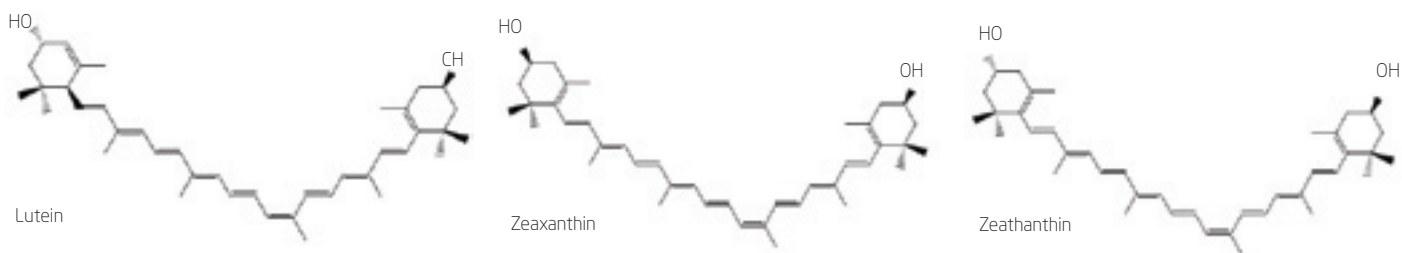
The age-related nature of ARM

Figure 2 Molecular structures for macular pigments

is consistent with 'The Free Radical Theory of Ageing'.[3] In brief, this theory states that with increasing age, there is a positive rise in oxidant load coupled with a decline in antioxidant load, both of which are known to be associated with an increased prevalence of age-related diseases.[4,5]

The retina is particularly susceptible to damage by ROIs, and there are several reasons which account for this vulnerability. Firstly, at the retina, there is an extremely high consumption of oxygen. Secondly, the photoreceptors of the retina contain high amounts of polyunsaturated fatty acids (PUFAs).

PUFAs are particularly susceptible to free radical damage because their conjugate double bonds are convenient sources of hydrogen atoms, which contain one electron. In addition, the retina is constantly exposed to visible light, which is known to generate ROIs via photosensitisation reactions.

Finally, the process of phagocytosis by the retinal pigment epithelium (RPE) is known to generate hydrogen peroxide (non-radical species) – Figure 1.[3,6; 6-8]

Cumulative blue light damage
Cumulative (short-wavelength) light damage represents an environmental factor, which is believed to play a role in ARM pathogenesis. Over a lifetime, exposure to visible light, specifically short-wavelength blue light, causes photic damage to the retina.[9,10] This damage is mediated through oxidative stress.[6] Indeed, the detection of lipid peroxidation products following irradiation of the retina provides strong evidence that cumulative retinal light damage is mediated through ROIs.[4,5;11-13]

It is generally accepted, therefore, that ROIs (generated by reactions involving short-wavelength irradiation and arterial oxygen) denature PUFAs in the photoreceptors, leading to cell death and, it is believed, to the development and/or progression of ARM.

Macular pigment
Carotenoids are naturally occurring plant pigments, with over 600 individual carotenoids identified to date.

MP is a yellow pigment composed of the xanthophyll carotenoids L and Z, which are of dietary origin, and a third non-dietary carotenoid, meso-Z, which will be discussed later in this article. The primary sources of L and Z in the diet are yellow, red and orange-coloured fruits and vegetables, with the highest quantities of these macular carotenoids being found in egg yolks and green leafy vegetables, such as cabbage, spinach and kale (See Figure 2 for molecular structures).

However, it should be noted that these carotenoids are fairly ubiquitous among green fruits and vegetables.

Bioavailability of the macular carotenoids
Of the 600 carotenoids that have been identified to date, two distinct groups exist: pro vitamin A and non-pro vitamin A carotenoids. The former group includes β-carotene, α-carotene and β-cryptoxanthin, all of which can be converted to retinol (the active form of vitamin A). Non-pro vitamin A carotenoids include L, Z and lycopene.

The stability of the macular carotenoids within the food matrix is dependent on many factors. The principal cause of carotenoid loss during processing and storage of food may be attributable to enzymatic oxidation or non-enzymatic oxidation of the highly unsaturated carotenoid molecules. Bioavailability is defined as the fraction of the nutrient utilised by the body out of the total ingested amount, and is an essential consideration when assessing nutrient intake and developing reference intakes (no reference intakes have been set for L or Z).

Several factors affect the bioavailability of L and/or Z, as follows:
● The nature of the food matrix in which they are ingested (raw or uncooked vegetables take longer to digest, rendering their various carotenoids less bioavailable)[14]
● The presence of dietary fat (lipids are required for the micellisation of fat-soluble vitamins)[15,16]
● The presence of competing molecules (the hydrophobicity of the carotenoid affects the efficiency with which it is transferred from lipid emulsion to micelle).[17]

Factors such as intestinal nutrient

malabsorption, lipid malabsorption, drug-nutrient interactions and liver disease may also affect bioavailability.

Effect of L and Z supplementation on the retina

There have been several studies of L and/ or Z and/or meso-Z supplementation in human subjects to date, with respect to MP levels.

In 1997, Hammond et al showed that dietary modification, for as little as four weeks, could augment MP, with this effect being maintained for several months following resumption of a normal, unmodified diet.[18] Of note, two of the 11 subjects involved in this study did not show a significant rise in MP optical density despite a significant increase in serum L. These subjects were termed 'retinal non-responders' and this phenomenon may be due to a compromised ability to capture and/or stabilise the macular carotenoids in these individuals.

Landrum et al investigated the effect of L supplementation in two individuals over a 140-day period.[19] They found an increase in serum L levels in both individuals, coupled with a parallel increase in MP optical density. Supplementation with the third component of MP, meso-Z, has also been shown to significantly increase MP levels in healthy subjects.[20] However, a comparison of the relative responses achieved following supplementation with the individual carotenoid constituents of MP is merited.

Functions of MP

The blue light filtering properties and/or antioxidant activity of MP are believed to confer protection against ARM.

MP peak absorption is at a wavelength of 460nm (blue light) and, therefore, it functions as an optical filter of blue light. It has been estimated that MP reduces blue light transmission at a pre-receptoral level by up to 40 per cent.[21] The importance of this rests on the fact that photo-oxidative damage, caused by short-wavelength blue light, is believed to play a role in the pathogenesis of ARM.

The antioxidant capability of MP is related to the structure of its carotenoids. Kirschfeld et al were the first to hypothesise that macular carotenoids act as antioxidants.[22]

However, firm evidence that the macular carotenoids act as antioxidants was provided by Khachik and co-workers by demonstrating the presence of direct oxidation products of the macular carotenoids in the human retina.[23] The structure of the carotenoids ensures that they themselves do not become radicalised. The importance of this rests on the fact

that oxidative damage caused by ROIs is also believed to play a role in the pathogenesis of ARM.

Meso-zeaxanthin

The constituent carotenoids of MP are of dietary origin as L and Z cannot be synthesised endogenously. However, there is a third macular carotenoid, known as meso-zeaxanthin (meso-Z),[24] which has not been identified in either a normal diet or in serum. Indeed, this fact is borne out following a review of current literature and food composition tables.[25]

Interestingly, it has been shown that L is the dominant carotenoid in diet,[26] whereas Z/meso-Z have been shown to be the dominant carotenoids at the macular.[24]

In 1993, Bone et al hypothesised that meso-Z was a metabolic product formed in the retina from L (namely, L undergoes isomerisation of one of its double bonds and is oxidised to meso-Z).[24]

To test this hypothesis, Neuringer et al [27] performed a supplementation study in monkeys. In this study, monkeys that were completely deficient in MP, as a result of lifelong dietary deprivation of the macular carotenoids, exhibited carotenoid accumulation at the macula following supplemental L or Z for a period of many weeks. Those given pure Z were found to have only that carotenoid present in the retina, whereas those fed L were found to have both L and meso-Z, supporting the hypothesis that meso-Z is derived from L. However, a complete understanding of the pathway by which L converts to meso-Z remains elusive.

Given that the putative importance of MP rests on its antioxidant properties and its ability to filter blue light, it is interesting to note that meso-Z has been suggested to be more important than L and Z, in relation to these functions.

Indeed, studies have shown that meso-Z is a more potent antioxidant than Z when bound to its retinal binding protein, glutathione S-transferase (GSTP1).[28]

Also, it is believed that meso-Z facilitates a wider range of blue light filtration (due to its orientation within retinal cell membranes).

Risk factors for ARM, and their relationship with MP

The three most important and established risk factors for ARM include:
● Increasing age
● Cigarette smoking
● Family history of ARM.

Other possible risk factors for this disease include:

● Obesity
● Female gender
● Low macular pigment levels.

Increasing age

The prevalence, incidence and progression of ARM have been shown to rise exponentially with increasing age.[29,30]

As mentioned previously, the 'Free Radical Theory of Ageing' is consistent with the notion that oxidative stress contributes to age-related disorders, including ARM.[3]

To date, studies that have reported on the relationship between age and MP have been inconsistent due to the differing methodologies used and differences in sample sizes. However, the largest study to investigate this relationship to date has found a modest, but statistically significant, age-related decline in MP levels.[31]

Cigarette smoking

There is now a consensus that tobacco use represents an established risk for ARM, as almost all epidemiological studies have shown that cigarette smoking is associated with an increased incidence and prevalence of this condition.[32,33] A meta-analysis of epidemiological data, published in 2005 by Thornton et al, showed that in 13 of 17 population-based studies, a statistically significant association was seen between tobacco use and late ARM.[34]

Despite the lack of clarity regarding the mechanisms responsible for such findings, two main rationales have been suggested.

Firstly, cigarette smoking is believed to promote the same vascular changes in the eye, as those seen in cardiovascular disease.[35] Therefore, cigarette smoking may simply represent an antecedent common to both atherosclerosis and ARM, as ARM has been putatively linked with cardiovascular disease.

Secondly, cigarette smoking has been shown to increase oxidative stress levels, and to be associated with attenuated antioxidant defences within the body.[3]

Studies reporting on the association between tobacco use and MP levels have consistently shown a relative deficiency of MP among cigarette smokers.[31,32]

Family history

Having a family history of ARM has been consistently associated with an increased risk for developing this condition.

Seddon et al reported a significantly higher prevalence of ARM among first-degree relatives of patients with this condition when compared with first-degree relatives of control subjects.[36]

Interestingly, recent investigations into

specific genes involved in the pathogenesis of ARM have identified an association between a variant of the Complement Factor H gene and this condition.[37,38]

Of note, only one study in the current literature has reported on the relationship between family history of ARM and MP levels. In brief, this study found that the sons and daughters of ARM sufferers had a significant lack of MP compared to individuals without such a family history.[31]

Obesity
Obesity is a suggested risk factor for ARM. Obesity is known to be associated with a poor diet,[39] increased oxidative stress, an unfavorable lipoprotein profile (which may affect antioxidant transport), and increased inflammation. However, studies investigating the relationship between obesity and the progression of ARM have resulted in conflicting findings.

MP has been shown to be inversely, and significantly, related to measures of obesity, such as body mass index and percentage body fat.[39,40]

At present, the Macular Pigment Research Group, at the Waterford Institute of Technology in Ireland, is conducting a study entitled *Changes in macular pigment optical density and serum concentrations of its constituent carotenoids, in response to weight loss*. This study aims to investigate whether a decrease in adiposity would result in a parallel increase in MP, as it has been shown that obesity is inversely related to MP levels.[39] Such a finding may have important implications for advising patients with ARM, or at increased risk of developing this condition.

Sex
Female sex is a putative risk factor for ARM; however, reports regarding this association are inconsistent. In keeping with this, studies investigating sex-related differences in MP are also conflicting.[31,41]

Conclusion
In conclusion, there is a biologically plausible rationale whereby MP may protect against ARM. This hypothesis rests on the antioxidant properties and the optical properties of this dietary pigment, and is rendered all the more provocative by its dietary origins.

Indeed, there is a growing body of evidence that subjects at high risk for ARM have a relative deficiency of MP in young and middle age. The importance of this finding rests on the fact that a protective effect of MP, if any, relates to the ability of this pigment to defend the central retina from chronic and cumulative damage. In other words, such a protective effect would need to be exerted in young to middle age, thus prompting the following question: When is the ideal time for dietary modification and/or supplementation in an attempt to augment an individual's MP?

Finally, meso-Z, a constituent of MP which is not found in a typical diet, and which is derived from retinal L, appears to be the most potent antioxidant component of MP and ideally suited to maximally filter out damaging blue light at a pre-receptoral level. The role of this fascinating carotenoid, hitherto largely ignored by investigators in this field, warrants further study. ●

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MULTIPLE-CHOICE QUESTIONS

1 How many people in the UK are estimated to have late ARM?
A 70,000
B 417,000
C 1.75 million
D 3 million

2 Which of the following processes is unlikely to contribute to ARM?
A Inflammatory response
B Oxidative stress
C Infection
D Choroidal circulatory compromise

3 Which of the following statements relating to oxidative effects and the retina is NOT true?
A The retina has a very high oxygen demand
B Photoreceptors contain high amounts of saturated fatty acids
C Free radicals have a cytotoxic effect
D Hydrogen peroxide is not a free radical reactive oxygen intermediate

4 Which of the following is a pro vitamin A carotenoid?
A Alpha-carotene
B Lutein
C Lycopene
D Zeaxanthin

5 Which of the following is TRUE concerning bioavailability of lutein and zeaxanthin?
A Ingesting raw vegetable matter improves bioavailability
B Ingesting vegetable matter in a lipid substrate improves bioavailability
C Bioavailability is the calorific value of a food item
D The gastrointestinal tract is the sole organ dictating bioavailability

6 What is meant by the term 'retinal non-responders'?
A Patients failing to show improvements in ARM signs at the retina
B Patients who show no improvement in acuity after nutrient supplementation
C Patients showing low serum levels of macular pigments despite elevated macular levels
D Patients showing no change in macular pigment density despite elevated serum levels

7 Which of the following is the peak absorption wavelength for macular pigment?
A 420nm
B 460nm
C 520nm
D 555nm

8 By how much is it estimated macular pigment will reduce blue light transmission?
A 10 per cent
B 40 per cent
C 60 per cent
D 100 per cent

9 Where is the most likely site of origin of meso-zeaxanthin?
A Diet
B Liver
C Blood
D Retina

10 Which of the following is the greatest controllable risk factor for ARM?
A Smoking
B Age
C Family history
D Gender

11 Which of the following is TRUE concerning obesity and ARM?
A There is no link between obesity and ARM
B Macular pigment has been shown to be directly proportional to obesity
C Obesity reduces oxidative stress
D Higher body mass index is linked with lower macular pigment levels

12 Which of the following is TRUE regarding cigarette smoking and ARM?
A All epidemiological studies have suggested a link between smoking and ARM
B Smoking causes dilation of choroidal vessels leading to leakage
C Smoking is linked with atherosclerosis, itself associated with ARM
D Smoking reduces oxidative stress

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