Macular pigment

The role of xanthophylls in preventing AMD

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The carotenoids are a family of pigments that are divided into two main groups – carotenes and xanthophylls. Although not considered to be essential micronutrients, they have antioxidant and photoprotective properties, and these functions have prompted interest in their potential role in the prevention of disease. The focus of this article will be on the role of the xanthophylls, lutein, zeaxanthin and meso-zeaxanthin, in preventing the onset or progression of age-related macular degeneration (AMD).

Despite increasing evidence supporting the role of xanthophylls in preventing disease, recent data suggest that dietary intake levels have declined in Europe and the US[1,2]. Lutein and zeaxanthin are now "generally recognised as safe" (GRAS), which means that they can be added to foods such as cereals. This is important, as lutein and zeaxanthin are not formed within the body and so can only be obtained from the diet. They are abundant in dark green leafy vegetables such as spinach and kale[3], as well as yellow and orange fruits and vegetables such as peppers[4].

Dietary supplements containing lutein and zeaxanthin may be produced using marigold flowers (Tagetes erecta), which are grown in Asia, Mexico, and Central and South America[5]. In marigold petals, lutein dominates at approximately 93-95%, however, lutein can be metabolised to zeaxanthin within the body[6]. In flower petals, the pigments are stored as diesters, whereas they are found unesterified in most fruits and vegetables[7]. In fact, industrial research showed that 93% of the lutein and zeaxanthin found in fruits, vegetables and eggs is found as lutein, rather than lutein esters[8].

Lutein esters contain two fatty acid groups that must be cleaved off before the body can use the lutein[9]. The efficacy of this hydrolysis of lutein esters into lutein occurs with an efficacy that is well below 5%[10,11].

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Furthermore, a negative correlation between age and serum lutein levels in individuals consuming lutein esters has been reported, which was not found in people supplementing with lutein[12]. This may suggest that the ability to hydrolise lutein esters declines with age. The same group reported no difference in the absorption of 6mg lutein from spinach, 6mg lutein supplements or 10.23mg lutein esters supplements, but a significantly higher serum response following supplementation with lutein-enriched eggs providing 6mg lutein. They concluded that the bioavailability of lutein from eggs is higher than that from other sources, and that this may be related to the fact that within eggs, lutein is located in the digestible lipid matrix.

It should be noted that the eggs used in this study contained approximately five times the amount of lutein than conventional eggs. Nevertheless, the results provide useful information about the bioavailability of different sources, suggesting that a lipid base may be optimum for supplements.

The fact that no significant difference in serum response following supplementation with spinach, lutein supplements or lutein esters supplements contrasts with other reports that supplemental lutein is twice as bioavailable than lutein from spinach[13], and that supplemental lutein induces a 60% greater increase in serum concentrations of lutein than a daily vegetable intake containing a similar amount of lutein[14].

Oxidative stress and AMD

It is generally thought that oxidative damage is responsible for ageing and that this process has an important role in the pathogenesis of age-related conditions such as AMD[15]. Oxidation involves the removal of electrons, and is mediated by reactive oxygen species (ROS). ROS is an umbrella term and includes some free radicals, singlet oxygen and hydrogen peroxide. Free radicals have an unpaired electron in their outer orbits, which makes them unstable and harmful to cells of the body. In order to achieve stability, they pluck electrons from other molecules, producing further ROS and fuelling disease-generating cytotoxic chain reactions[16]. Examples of free radicals include the superoxide anion radical, formed from the reduction of molecular oxygen to water, and the hydroxyl radical. The hydroxyl radical is particularly damaging, as it can take electrons from almost any organic molecule.

The reduction of oxygen to water also produces hydrogen peroxide, which interacts with the superoxide anion radical to form the hydroxyl radical, another potent antioxidant. Singlet oxygen does not have an unpaired electron in its outer orbit, but it does have a peripheral electron that is excited to an orbital above that which it normally occupies, making it highly reactive[16].

Ocular tissues are particularly susceptible to oxidative damage. The transparency of the cornea, aqueous humour, lens and retina allow continuous exposure to light, which along with ageing, inflammation, air pollutants and cigarette smoke, has been shown to increase production of ROS[17,18]. The role of oxygen in cataract formation has been demonstrated[19], and the retina is particularly vulnerable for the following reasons:

1. Polyunsaturated fatty acids are abundant in the retina, particularly the macular region. They are found in photoreceptor outer membranes and are readily oxidised[17,20,21].
2. The retina is subjected to high levels of light exposure. Light (particularly blue light) is a strong oxidising agent. The simultaneous presence of light and oxygen promotes production of free radicals[22].
3. Phagocytosis, which itself produces free radicals, occurs within the retinal pigment epithelium (RPE).
4. The retina is highly active metabolically and has a much higher blood flow than other tissues[22].

The body has several defence mechanisms against the production of ROS. The first involves antioxidant enzymes such as catalase and peroxidase[23]. Other micronutrients, such as selenium, zinc, manganese and copper, facilitate these antioxidant enzymes[23,24]. The second involves antioxidant nutrients such as vitamin E (alpha-tocopherol)[25-29], beta-carotene[30] and vitamin C (ascorbate)[23,31-34] and lutein and zeaxanthin[35]. Insufficient intake of dietary antioxidant vitamins and minerals can decrease the efficiency of the body's natural antioxidant systems and may allow cellular damage by ROS[17,36]. A review of the nutrients considered beneficial for ocular disease has been published[37].

Structure and function of lutein, zeaxanthin and meso-zeaxanthin

Interest has been raised into the protective role of the oxygenated xanthophylls group of carotenoids in the eye, particularly the retina. Lutein and its isomers are the only carotenoids present in the lens[38] and retina[39-42], and are known as macular pigment (MP). The proposed specific function of xanthophylls at the macula[40] is supported by the fact that macular levels are several thousand times higher than serum levels[43]. This may be explained by the discovery of a putative lutein-binding protein in the retinae of human eyes[44], which binds with high affinity and specificity to lutein and other xanthophylls.

Although possible binding proteins have been identified, the mechanism for uptake of xanthophylls into the bloodstream is still not clear. The efficacy of absorption from the gut depends on its original source, for example, lutein from egg yolk[45] is more readily absorbed than that derived from green leafy vegetables[46]. This relatively low absorption from green leafy vegetables may be due to complexing to proteins in chloroplasts within cell structures[47]. Xanthophylls that are associated with oil or fat may be more readily extracted during digestion[47].

The xanthophylls are packaged as plasma lipoproteins by the liver and released into the systemic circulation. Their major storage site is adipose tissue[48,49], so much so that a negative correlation between adipose tissue lutein concentration and the amount of lutein and zeaxanthin in the retina (macular pigment optical density, MPOD) has been reported in women[50].

In the central macula, lutein, zeaxanthin and meso-zeaxanthin are found in equal quantities, but the ratio of meso-zeaxanthin to zeaxanthin decreases with increasing eccentricity[51]. Meso-zeaxanthin has been found in the human macula, retina and RPE, but most recently has not been detected in the plasma or liver[52]. This forms the basis for the assumption that meso-zeaxanthin is formed via isomerisation of lutein[51], and it is thought that the conversion mechanism is concentrated at the macula. The xanthophyll binding protein mentioned earlier may also act as an enzyme for the conversion of lutein to meso-zeaxanthin.

In human retinae, the xanthophylls are concentrated mainly in the inner and outer plexiform layers. The ratio of lutein to zeaxanthin and meso-zeaxanthin within 0.25mm of the fovea is approximately 1:2.4[53], but the situation reverses at the retinal periphery where the ratio is 2:1[53]. There is a hundred-fold drop in the concentration of xanthophylls in the peripheral retina compared with the fovea, although levels vary vastly between donors[39,40]. The ratio of lutein:zeaxanthin and meso-zeaxanthin varies linearly with the ratio of rods:cones with increasing eccentricity[53]. The hypothesis that zeaxanthin is only found in the rods is refuted by the fact that the fovea contains predominately cones, as well as by the fact that squirrel monkey and macaque retinae have their highest concentration of lutein and zeaxanthin in the central fovea[54].

Xanthophylls have also been isolated in the rod outer segment [55,56] where there is a high concentration of polyunsaturated fatty acids that are particularly prone to oxidative attack. Within the rod outer segments, their highest concentration is found perifoveally, where it is 2.5 times higher than in the peripheral retina[56].

It has been suggested that xanthophylls play a similar role in humans as in plants, as antioxidants and screeners of high-energy blue light[57]. The MP may prevent light-initiated oxidative damage to the retina, and therefore protect against subsequent age-related deterioration[58]. The presence of MP in the inner retinal layers[59] supports a photoprotective role. The absorbance spectrum of MP peaks at 460nm and it is purported to act as a broadband filter, reducing the sensitivity of the macular region to short wavelength light which is most damaging in the 440-460nm range[60,61]. Lutein is reported to be a superior filter[62] due to the fact that it is orientated both parallel and perpendicular to the plane of the membrane[63]. Zeaxanthin is orientated perpendicular to the membrane plane only, and so may not be able to absorb the excitation beam from all directions. Zeaxanthin, however, is reported to be a superior photoprotector during prolonged light exposure; the shorter time-scale of protective efficacy of lutein has been attributed to oxidative damage of the carotenoid itself[63].

Carotenoids are also able to quench singlet oxygen (a potent oxidant)[64], scavenge reactive oxygen species[65], limit peroxidation of membrane phospholipids[66] and reduce lipofuscin formation[67]. The presence of MP in the rod outer segments and RPE[55,56] is suggestive of a ROS-quenching function. The fact that lutein and zeaxanthin have been found in higher concentration in the rod outer segments of the perifoveal retina than the peripheral retina, lends support to their proposed protective role in AMD[55].

In vivo measurement of MPOD

MPOD in the central 1-2° of the macula lies in the range 0.1-0.9 for most people[68,69]. For a person with MPOD at the low end of this range, structures posterior to the MP will be exposed to approximately six times the blue light flux, compared to a person with MPOD at the higher end of the range[70]. It follows that there is a suspected increased risk of AMD development for those with low MPOD levels. It has also been noted that geographic atrophy tends to spare the very central macula, where MPOD peaks, until the disease is well advanced[71,72].

Psychophysical methods

The psychophysical approach to MPOD measurement is based on the fact that the MPOD acts as a broadband filter in the 440-460nm range. In heterochromic flicker photometry[73-75], a blue reference light, close to the optical peak density of MP (450nm) is alternated with a light of variable wavelength. This is set to a value which is not absorbed by the MP, such as 560nm[76]. Whilst viewing this flickering stimulus, the luminance of one of the lights is altered until the perceived flicker is minimised. At the minimum flicker point, the perceived luminance of the two lights is equalised. The perceived intensity of the blue reference light will be relatively low when viewed at the fovea (where MP is relatively high), compared with a point outside the fovea (where there is less MP). The difference between the ratios of the luminance of the two lights obtained at foveal and parafoveal points is used to derive the MPOD.

Although this technique is reproducible and exhibits good test-retest reliability[77], it is difficult for the subject to perform[78,79], and requires good visual acuity. It is also associated with high variability in subjects with low levels of MPOD[80]. A commercial instrument that employs this technique for measurement of MPOD is the MacuScope™ from the Birmingham Optical Group (**Figure 1**).

» **Figure 1**
The MacuScope
(by courtesy of Birmingham Optical Group)

Imaging techniques

Fundus reflectometry involves measuring the reflectance of short wavelength light (462nm) that has passed through pigment containing layers of the retina twice[81]. A digitised image obtained at an illuminating wavelength of 559nm is subtracted from one taken at 462nm in order to correct for the absorptive effects of melanin and oxyhaemoglobin. This provides the spatial variation of the MP.

Scanning laser ophthalmoscopy (SLO) can also be used to produce fundus reflectance maps, and this method is reported to be more resistant to light scatter than conventional fundus reflectometry[82]. Digital subtraction of the maps at 488-514nm, with adjustments made for absorption of the lens, provides a mean value of MPOD[83]. A disadvantage of this technique is that it requires a normal retinal structure, and therefore is not suitable for use in patients with advanced AMD.

Raman spectroscopy

This technique is based on the Raman effect, which is the inelastic scattering of photons by the molecules under investigation. In other words, the wavelength of a small fraction of the radiation scattered by certain molecules differs from that of the incident beam, and the shift in wavelength depends on the chemical structure of the molecules responsible for the scattering. This phenomenon has been used in the assessment of MPOD because when carotenoids are excited with a monochromatic laser beam, they exhibit characteristic wavelength shifts of the back-scattered light. A blue/green argon laser is used to excite the electronic absorption of carotenoid pigments[84]. The resultant Raman signals are recorded and analysed by a spectrometer. This technique has the advantage that it can be used to assess MPOD in AMD-affected eyes. This technique is reported to be highly reproducible and not subject to meaningful test-retest variability[85], although it has only been used in a research setting.

Apparent motion photometry

A more recent development in MPOD measurement is based on an apparent motion technique[86] for matching the luminance of different colours. This technique has the advantage of simplicity when used for adjusting colour luminance on television displays. If a red/green square-wave grating is suddenly replaced with a dark yellow/light yellow square-wave grating, which is displaced by one-quarter of a cycle to the right, then the grating will appear to jump to the left if the green bars are lighter than the red bars, or to the right if the reverse is true[86]. If, however, the red and green bars are made equiluminous, no consistent apparent motion is seen.

A MPOD measurement technique developed by Cambridge Research Systems uses a stimulus made up of four consecutively presented square wave gratings, each 90° out of phase with the next (**Figure 2**).

The first grating is a chromatic grating of red and blue bars. The luminance of the blue is fixed whilst the red luminance can be varied. The second grating is a purely luminance modulated grating, modulated around the mean luminance of the blue/red chromatic grating. If the luminance of the red component in the chromatic grating is greater than the blue, the observer correlates that with the brighter of the bars of the luminance grating when it is presented. However, if the luminance of the red is less, then it is correlated with the darker bar in the luminance grating. This continues in successive grating presentations, so that the sequence of gratings appears to move in one direction or the other, the direction being solely dependent upon the relative luminance of the two components in the chromatic gratings.

When the red luminance is greater, the grating appears to drift upwards, when the blue is greater it drifts downwards. The subject is simply required to decide whether the grating is drifting upwards or downwards in a 2AFC weighted up/down staircase procedure.

Epidemiological and clinical evidence

A cross-sectional sample taken from the National Health and Examination Survey (NHANES) in the late 1980s revealed an inverse association between fruit and vegetable intake and AMD[87]. Serum carotenoids (lutein, zeaxanthin, beta-carotene, alpha-carotene, cryptoxanthin and lycopene) were associated with reduced risk for AMD in 1992[88], and prevalence of AMD in this sample was 66% lower in those in the highest quintile of carotenoid intake compared with those in the lowest. In the early 1990s, however, subsequent epidemiological studies reported only marginal relationships[89,90].

More recently, serum concentrations of lutein and zeaxanthin and MP density have been found to be responsive to dietary modifications[50,75,91]. It has been reported that 55% of the variability in serum concentrations of lutein and zeaxanthin can be explained by their dietary intake of these carotenoids; 30 % of the variability on MPOD can be explained by serum levels[92]. Although retinal response to a change in dietary intake of lutein and zeaxanthin is much slower that the serum response[75,93], both serum and dietary lutein were significantly positively correlated with MPOD in a study involving 278 healthy volunteers[94].

A cross-sectional study reported that people with plasma concentrations of lutein in the lowest third of the distribution have a significant odds ratio for risk of AMD of 2.0 (95% CI: 1.0-4.1) compared with those in the highest third after adjustment for other risk factors[95]. There were no significant trends between plasma concentrations of lutein or lutein plus zeaxanthin.

There is evidence for selective deposition of lutein in the retina[55,96], increase of retinal and serum levels of lutein with supplementation[75,83,91] and an increased risk of AMD with low serum[97] and retinal[98,99] lutein levels. Lutein/zeaxanthin supplementation has been linked with improved visual function in patients with congenital retinal degenerations[100] and with AMD[101]. Monkeys fed a lutein-free diet eventually lost all macular pigment and developed pathology consistent with macular degeneration[102,103]. Repletion of these monkeys led to restoration of macular pigment levels[103].

Studies investigating the effect of unesterified lutein dosage on MPOD levels found a general increase in MPOD response with dose[104,105]. In one study, those supplementing with 10mg or 20mg of lutein, but not 5mg lutein, for 120 days had an increased response compared with those taking a placebo[104]. Another study showed that in patients with varying stages of AMD, doses of 2.5mg, 5mg and 10mg lutein all induced an increase in serum levels by one month, and a peak by three months. Three-month levels ranged from 104% to 339% change from baseline. MPOD levels, however, remained largely unchanged over the six-month supplementation period. No adverse reactions to lutein were reported[105].

The Lutein and Antioxidant Supplement Trial (LAST) was a 12-month RCT designed to evaluate the effect of 10mg unesterified lutein alone, or 10mg lutein combined with additional carotenoids and antioxidants and minerals, on MPOD and objective visual

Figure 2

Measuring MPOD using wave gratings

(by courtesy of Cambridge Research Systems)

Constituents (mg) per daily dose	**Vitalux® Plus** (Novartis)	**ICaps®** (Alcon)	**Ocuvite® PreserVision®** (Bausch & Lomb)	**Ocuvite® Lutein** (Bausch & Lomb)	**Visionace®** (Vitabiotics)	**Retinex** (Healthspan)	**RDA (mg)**
Beta-carotene	-	-	17.2	-	-	-	0.8
Vitamin A	-	0.67	-	-	0.3	-	-
Vitamin C	60	400	452	60	150	-	60
Vitamin E	20	150	268	20	60	-	10
Zinc	10	60	69.6	15	15	-	15
Copper	0.25	4	1.6	2	1	-	1.15
Vitamin B3	10	-	-	-	18	-	18
Lutein	10 (unesterified)	3.6 (unesterified)	-	6 (unesterified)	3.8 (esterified)	10 (unesterified)	None established
Zeaxanthin	-	0.4	-	-	0.2	0.44	None established
Selenium	-	0.04	-	-	0.15	-	0.055
Vitamin B2	-	10	-	-	4.8	-	1.6
Manganese	-	10	-	-	4	-	-
Other antioxidants, vitamins and minerals	-	-	-	-	131.35	-	-
Dosage/day	One capsule	Two tablets	Four capsules	One capsule	One tablet	One tablet	

» **Table 1**
Formulations of a selection of commonly available ocular nutritional supplements

outcome measures in 90 subjects with atrophic AMD. Glare recovery and contrast sensitivity significantly improved with both interventions, although it is worth noting that the study population was 95.6% male[106].

Macular pigment and normal visual function

With respect to healthy eyes, it has been hypothesised that the blue-light filter effect of xanthophylls may reduce longitudinal chromatic aberration[107]. The acuity hypothesis states that MP may improve visual acuity for images that are illuminated by white light by absorbing poorly focused short wavelengths before this light is processed by the retina[108]. Despite a lack of empirical evidence, lutein/zeaxanthin supplements are being taken by the public in an attempt to improve retinal health and vision[109].

Modification of xanthophylls intake

The characteristics of nutritional ocular supplements commonly available to optometrists can be found in **Table 1**. Practitioners who discuss nutrition with their patients may find a review of the possible contraindications and adverse effects of nutritional supplements[110] helpful. It is also important to remember that patients can modify their diets to increase lutein and zeaxanthin intake, and information about different foods can be found in **Table 2**.

Conclusion

There is compelling evidence for a role of lutein and zeaxanthin in the protection of the retina against oxidative damage. The Lutein Antioxidant Supplementation Trial reported a positive effect of 10mg unesterified lutein supplementation on measures of visual function in atrophic AMD patients. Recent work on the optimum dosage levels of lutein and zeaxanthin suggest that a daily intake of 10mg or more is required to increase MPOD levels.

In summary, a review of the current evidence suggests that for optimum benefit, lutein should be taken in its unesterified form, dissolved in lipid, and at a level of at least 10mg per day. Patients should be advised to talk to their GP about nutritional supplementation if they are taking prescribed medication.

References

Visit www.optometry.co.uk/references

Food	**mg / serving**
Kale (cooked)	33.8 / 1 cup
Kale (raw)	22.1 / 1 cup
Turnip Greens (cooked)	18.1 / 1 cup
Collard Greens (cooked)	17.2 / 1 cup
Spinach (cooked)	15 / 1 cup
Spinach (fresh, raw)	6.7 / 1 cup
Broccoli (cooked)	3.4 / 1 cup
Corn (cooked)	2.9 / 1 cup
Green peas (canned)	2.3 / 1 cup
Lettuce (Romaine)	1.5 / 1 cup
Corn (canned)	1.4 / 1 cup
Eggs (two)	0.5 / 2 medium
Green beans	0.76 / 1 cup
Orange juice (frozen concentrate)	0.50 / 12oz
Oranges	0.49 / 2 medium
Papayas	0.45 / 2 medium
Tangerines (fresh)	0.40 / 2 medium

» **Table 2**
Lutein content of various foods