

Possible contribution of lutein and zeaxanthin, carotenoids of the macula lutea, to reducing the risk for age-related macular degeneration: a review

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Abstract

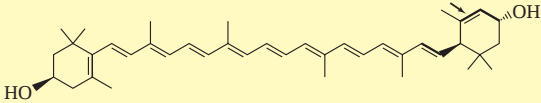
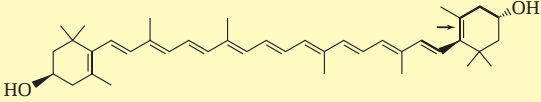
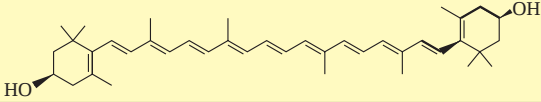
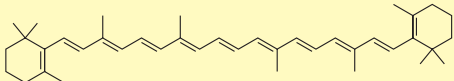
In the center of the retina, where visual acuity is highest, a yellow spot called the macula lutea is visible. The yellow color is due to the presence of the nutritional carotenoids, lutein and zeaxanthin, which accumulate there to a greater extent than in any other tissue. In the center of the macula lutea, the lutein-to-zeaxanthin ratio found in the plasma is inverted, with relatively more zeaxanthin than lutein, whereas in the more peripheral regions of the retina, lutein predominates, as in plasma. The physiological significance of this selective accumulation is based on filtration of potentially damaging blue light, quenching of photochemically induced reactive oxygen species, attenuation of chromatic aberration, and inhibition of apoptosis. It is believed that via these mechanisms, lutein and zeaxanthin can contribute to reduce the risk for age-related macular degeneration (AMD), the leading cause of irreversible loss of vision in aging Western populations. Epidemiological studies indicate that elevated dietary intake or blood concentrations of lutein and zeaxanthin are correlated with a reduction in risk for this disease. Furthermore, intake of these carotenoids can specifically increase their levels in the macula.

Key words: Age-related macular degeneration, Blue light, Carotenoids, Lutein, Macula, Reactive oxygen species, Retina, Zeaxanthin

Introduction

Lutein and zeaxanthin are carotenoids, a class of yellow to red substances whose chemical structure is similar to that of β -carotene (**Table 1**).¹⁻⁴ At present, approximately 600 naturally occurring carotenoid molecules are known.⁵ About 50 carotenoids are found in the nutritional chain, particularly in yellow, orange, and red fruits and dark green leafy vegetables.⁶ In contrast, only about 13 carotenoids have been identified to date in human plasma¹ and only six (α - and β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin) occur there in substantial quantities. The yellow carotenoids, lutein and zeaxanthin, together with 3R, 3'S (=meso)-zeaxanthin, a stereoisomer of zeaxanthin (see **Table 1** for formulas), are the constituents of the yellow pigment of the macula lutea. This pigment forms the yellow spot in the center of the human retina and is the most conspicuous accumulation of carotenoids in the human body.^{7,8} The carotenoid concentration in the most central part of the yellow spot is estimated to be around 1 mM and is thus three orders of magnitude higher than the typical carotenoid concentration in other human tissues.⁴ This specific accumulation of two carotenoids in the macula has led to hopes that dietary supplementation with these carotenoids may reduce the risk for age-related macular degeneration (AMD), the most common cause of irreversible vision loss in aging Western populations.

Table 1. Macular carotenoids in comparison with β -carotene — typical concentrations in human plasma and amounts in specific areas of the retina.¹⁻⁴ The arrows indicate how the chemical structures of lutein and 3R, 3'S (=meso)-zeaxanthin differ by virtue of the position of a double bond.

Carotenoid	Plasma concentration ($\mu\text{mol/L}$)	Content in retinal areas (pmoles) ⁴	Chemical structure
Lutein	0.29 ¹ 0.19 ² 0.28 ³	Central 17 Medial 20 Outer 22	
3R, 3'S (=meso)-zeaxanthin	Trace	Central 10 Medial 3 Outer 2	
3R, 3'R-zeaxanthin	0.04 ¹ 0.06 ² 0.07 ³	Central 12 Medial 9 Outer 7	
β -carotene	0.22 ² 0.46 ³	0	

The topic of AMD in connection with the carotenoids has attracted considerable attention in the scientific literature already and various aspects have been discussed in recent reviews.⁸⁻¹³ This article reviews how the idea of risk reduction for AMD by the macular carotenoids, lutein and zeaxanthin, is emerging from a chain of different observations. From experimental *in vitro* studies, through animal *in vivo* experiments to observational and epidemiological studies in humans, the avenue to clinical intervention trials will be shown.

Nutritional aspects

As can be seen in **Table 2**, the vegetables and fruits that are normally consumed in largish quantities contain more lutein than zeaxanthin,¹⁴⁻¹⁷ (for other compilations see Sommerburg *et al.*¹⁸ and USDA¹⁹). Therefore, it is not surprising to find that in human plasma the concentration of lutein is higher, by up to seven times, than that of zeaxanthin (**Table 1**). Zeaxanthin is the dominant carotenoid in red peppers and also in the small red berry *Lycium barbarum*, 'Gou Qi Zi', which can contain zeaxanthin in amounts of

up to 5 mg/100g.¹⁶ This berry is commonly used in home cooking in China and is a constituent of traditional Chinese herbal medicine, in which, interestingly, it is used to improve visual acuity.

Nutritionally, some carotenoids such as β -carotene act as precursors of essential vitamin A because they can be transformed by endogenous enzymes into retinol (vitamin A), which is instrumental not only in the vision process but also in the maintenance of conjunctival integrity. Therefore, these carotenoids can, at least partly, ameliorate vitamin A deficiency. However, lutein and zeaxanthin do not have substantial provitamin A activity²⁰ and therefore, cannot provide retinol to the retina.

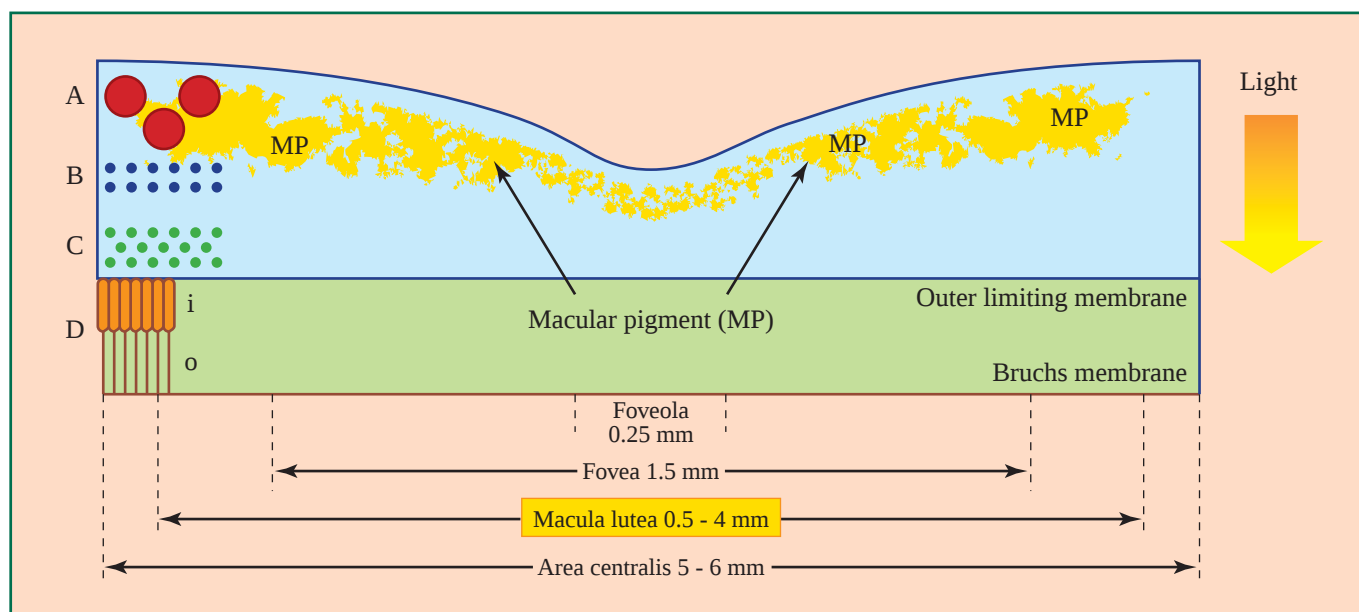
Occurrence and distribution of lutein and zeaxanthin in the retina and macula

In 1945, the macular pigment was tentatively described as consisting of the "xanthophyll lutein".²¹ However, it was not until 1985²² and 1988²³ that it was finally confirmed as

Table 2. Amount of carotenoids¹ (mg/100g) in selected plants¹⁴⁻¹⁷

	Lutein	Zeaxanthin	β -carotene	α -carotene	β -cryptoxanthin	Lycopene
<i>Vegetables (raw)</i>						
Spinach ¹⁵	9.5	0.4	3.3	np	np	np
Carrot	0.3	np	9.9	3.9	nd	np
Sweet corn	0.5	0.4	0.05	0.06	nd	np
Orange pepper	0.5	1.6	0.2	0.2	nd	np
Red pepper ¹⁵	np	2.2	3.3	0.5	1	0.1
Tomato	0.08	np	0.4	np	nd	1.6-5.6
<i>Fruits/berries/flowers</i>						
Mandarins	0.1	0.05	0.3	0.01	1.8	np
Peach	0.08	0.04	0.08	Trace	0.09	np
Gou Qi Zi ¹⁶	nr	4.3	nr	nr	nr	nr
Marigold ¹⁷	37	0.5	nr	nr	nr	nr

Abbreviations: nd = not done; np = not present; nr = not reported.



Abbreviations: A = blood vessels; B = inner nuclear layer; C = outer nuclear layer; D = photoreceptor layer; i = inner segments; o = outer segments.

* The capital letters at the left side indicate the approximate location of the respective retinal layer.

Figure 1. Diagram of approximate location of the macular pigment in the retina. Adapted from personal communication; B Daicker 1989.

consisting of lutein and zeaxanthin (see **Table 1** for chemical structures). In 1993, a third major macular carotenoid was identified, the zeaxanthin stereoisomer 3R,3'S (=meso)-zeaxanthin (**Table 1**).²⁴ Unlike lutein and zeaxanthin, this carotenoid is not found in food or plasma in any substantial amount.

In postmortem eyes, the macular yellow pigment appears macroscopically as an oval to round yellow area in the center of the macula, overlapping the avascular zone and covering the fovea. *In vivo*, when photographed in blue light or in fluorescein angiograms, the macular pigment appears as a dark spot. This is due to absorption of blue light by the yellow pigment. In terms of its distribution in the retina, the macular pigment appears to be localized in the Henle's fibers, the axons of the photoreceptors, an appropriate location for shielding the photoreceptors from blue light.^{25,26}

Figure 1 is a schematic representation of the localization of the macular pigment within the retina, relative to the incoming light and the photoreceptors. While the visibility of the yellow color marks just the regions of highest concentration of the macular pigment, carotenoids can also occur in other regions of the retina as indicated recently.^{27,28}

Sommerburg *et al.* and Rapp *et al.* reported that approximately 25% of the total retinal lutein is found in the rod outer segments (ROS), possibly in the extrinsic membranes.^{27,28} In the peripheral rod-dominated location these authors also found relatively more lutein than zeaxanthin. However, due to the technique employed, they could not make any conclusion regarding the carotenoid content of the cones. Nevertheless, this finding of significant amounts of lutein in the ROS is potentially relevant. If the macular pigment were exclusively located in the fibers of Henle, a direct antioxidant action at the level of the photoreceptors would be unlikely because the carotenoids could not exert their

antioxidant action over the relatively large distance from this layer to the rods. In the context of the cones, a hypothesis was put forward by Bone *et al.* that the more peripheral lutein may be associated with the rods and the more central zeaxanthin with the cones.²⁹ Another more recent hypothesis suggested the Müller cell as one of the locations of macular pigment, at least in the center of the foveola.³⁰ In summary, our knowledge of the detailed ultrastructural/subcellular localization of carotenoids in retinal tissue is still incomplete, but some interesting insights are emerging.

The exact mechanisms of uptake of lutein and zeaxanthin into the retina have also remained elusive, and the existence or non-existence of binding proteins that are instrumental in this process is still unclear. Based on the results of an attempt at biochemical isolation, Bernstein *et al.* suggested that retinal tubulin may be the principal carotenoid-binding substance in the retina.³¹ However, the lack of specificity of this substance suggests that it may only passively stabilize zeaxanthin and lutein in the fovea, probably in the same way as actin stabilizes the carotenoid astaxanthin in salmon muscle. Recently, the same group reported the purification of a 28 kDa membrane-associated protein from human retinas that has xanthophyll-binding properties.³²

Knowledge of the distribution of the macular carotenoids in the retina was further refined by the work of Bone *et al.*²⁹ In this study, specific areas were cut from human retinas by three trephines of 3, 11, and 21 mm diameter (**Figure 2**). This dissection produces a central disc containing most of the yellow spot ('central' area) plus two concentric annuli, of which one is the middle ('medial') area and one contains peripheral retina ('outer' area). These areas were subjected to high-pressure liquid chromatography (HPLC) analysis for carotenoids. As can be seen from **Table 1**, the two zeaxanthin stereoisomers, 3R, 3'R-zeaxanthin and 3R,3'S (=meso)-zeaxanthin

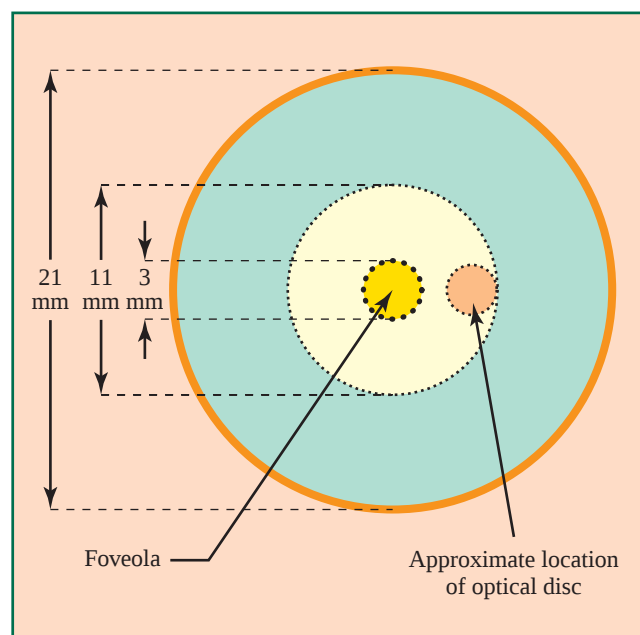


Figure 2. Dissection of retina for high-pressure liquid chromatography analysis. Adapted from Bone *et al.*²⁹

dominate the central area, while lutein is relatively more abundant in the peripheral retina (medial and outer areas). The ratio of lutein to total zeaxanthin is about 0.8 in the center of the macula and about 2.4 in the peripheral retina (**Table 1**), whereas in plasma, lutein-to-zeaxanthin ratios of from 7 to 4 are observed (**Table 1**). Thus, it appears that nature, by still unknown mechanisms, not only accumulates lutein and zeaxanthin specifically in the macula, but also inverts the concentration ratio of lutein to zeaxanthin normally found in plasma, making zeaxanthin dominant in the center of the macula and lutein dominant in the peripheral retina.

Further evidence that lutein predominates peripherally was provided by the finding that subretinal fluid extracts from patients with rhegmatogenous retinal detachment, a disease that primarily involves the peripheral retina, show retinol and lutein, but no zeaxanthin, on HPLC analysis.³³ It is important to note that the apolar carotenoids, β -carotene and lycopene, two major carotenoids of human plasma, are not found in the human retina.²³ This indicates that the polarity of a molecule may determine its potential to have access to the retina. Interestingly, the apolar β -carotene was not detected in a postmortem retina from a subject who had been taking very high doses of a combination of β -carotene and canthaxanthin, whereas the highly-polar carotenoid canthaxanthin was present in substantial amounts.³⁴

Age-related macular degeneration

AMD is a multifactorial degenerative disease of the central part of the retina and the retinal pigment epithelium that manifests itself in an atrophic ('dry') and a neovascular ('wet') form.³⁵ The latter form is characterized by the presence of fluid accumulation with a gradual loss of central high-acuity vision due to hemorrhagic maculopathy. Ultimately

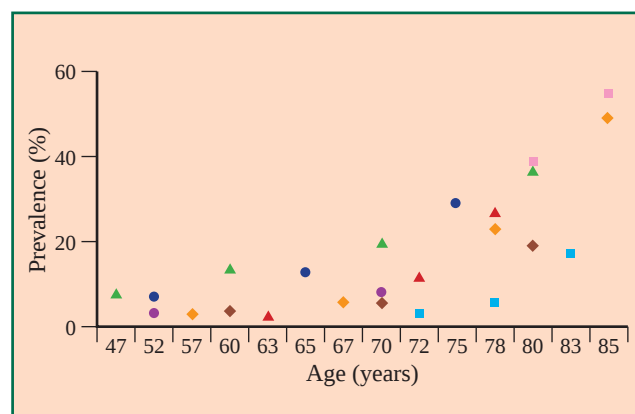


Figure 3. Prevalence of macular degeneration — from eight different studies as indicated by the different symbols (n=12,000). Adapted from Vingerling *et al.*³⁶

this decline in visual acuity can lead to absolute loss of vision, and AMD is the leading cause of irreversible blindness in aging Western populations. Vingerling *et al.* compiled data from eight epidemiological studies containing prevalence data from more than 12,000 individuals in five countries (Figure 3).³⁶ As can be seen from **Figure 3**, the prevalence of macular degeneration increases dramatically from about 65 years of age.

The etiology of AMD is only poorly understood and both genetic and environmental factors have been hypothesized as playing a role. One environmental factor seems to be ocular exposure to sunlight,³⁷ in particular a history of exposure to blue light in the preceding 20 years.³⁸ In the presence of photosensitizers, light can induce oxidative damage. However, oxidative damage can also be mediated independently of light by endogenous metabolic processes. The retina is highly active metabolically and has a much higher blood flow than other tissues. In such an environment, characterized by the simultaneous presence of light and oxygen,¹⁰ numerous reactive oxygen species, including singlet oxygen and the superoxide radical, can be generated. These ultimately induce peroxidation of polyunsaturated fatty acids, in particular of docosahexenoic acid, a major lipid constituent of vertebrate photoreceptor outer segments.³⁹ Through such damage, the integrity of the complex of photoreceptors and the retinal pigment epithelium is impaired, as well as the cyclic process of photoreceptor phagocytosis and renewal. Ultimately, this can lead to the accumulation of cell debris and lipofuscin in Bruch's membrane, drusen formation, and finally neovascularization and retinal detachment. Since there is no effective management for the neovascular form of the disease other than laser⁴⁰ and verteporfin treatment,⁴¹ which, however, seem to be effective only in selected patient populations, preventive strategies are of the greatest importance.

The following section presents some of the indirect evidence collected *in vitro*, in animals, and in humans suggesting that lutein and zeaxanthin may reduce the risk for AMD. Firstly, however, the mechanistic basis of this putative efficacy must be discussed.

Lutein and zeaxanthin in risk reduction of AMD

The mechanistic basis

The yellow color of lutein and zeaxanthin

Carotenoids appear yellow because they absorb blue light (blue being the complementary color of yellow). On the other hand, blue light can damage the retina,^{42,43} and this property of carotenoids is one basis of their physiological action in the retina. The relationship between the wavelength of blue light and its potential to induce damage in the retina is expressed by the 'blue light hazard function'.⁴² This function is maximized at around 450 nm, the wavelength at which lutein and zeaxanthin absorb light (**Figure 4**). Thus, these carotenoids can absorb blue light before it can initiate damaging reactions in the photoreceptors. Their location in Henle's fiber layer (**Figure 1**), just in front of the photoreceptors, is appropriate to their filter action and also explains the classical function of macular yellow pigment, namely the attenuation of chromatic aberration.

Blue light filtration by the macular pigment is probably of particular importance in the young, until the age of 30 to 40 years, namely at times when the lens is virtually clear. During the normal aging process, however, the lens gradually yellows,⁴⁴ leading to age-related reduction of blue light transmission. Therefore, in later life, the antioxidant properties of lutein and zeaxanthin that are described in the next section may become relatively more important since the body's antioxidant system deteriorates with age.⁴⁵

Antioxidative properties of lutein and zeaxanthin

The screening effect described above attenuates blue light and thus indirectly limits the photochemical generation of reactive oxygen species mediated via endogenous or exogenous photosensitizers. However, carotenoids in general, and lutein and zeaxanthin in particular, also have intrinsic properties that result in direct quenching of these potentially

damaging reactive entities.^{46,47} This quenching capability of carotenoids depends on the number of conjugated double bonds. As can be seen from **Table 1**, lutein has 10 conjugated double bonds, while zeaxanthin and 3R, 3'S (=meso)-zeaxanthin, the two other macular carotenoids, contain 11 such bonds and are indeed better singlet oxygen quenchers than lutein.⁴⁷ From this point of view, the preponderance of zeaxanthin and 3R, 3'S (=meso)-zeaxanthin over lutein in the macular center, where the incident light is most intense and the likelihood of formation of reactive oxygen species is greatest, seems logical. Given that 3R, 3'S (=meso)-zeaxanthin is not present in the plasma in any substantial amount, it may be generated from lutein in the retina. Via still unknown mechanisms, such a conversion could be effected by a shift of the double bond in the lutein molecule, as indicated by the arrows in **Table 1**, and would generate the better singlet oxygen quencher.

An important consideration when discussing the antioxidant activities of the carotenoids in the retina is whether they are close enough to where oxidative reactions are most likely to occur. The region of highest macular pigment concentration is approximately 50 μm away from the area of highest aerobic metabolism,⁴ with the effect that carotenoids in the fibers of Henle are unable to quench singlet oxygen and other reactive oxygen species that are generated in the outer segments. The findings by Sommerburg *et al.* and Rapp *et al.* of substantial quantities of lutein in ROS is therefore of particular relevance in this respect.^{27,28} The hypothesis that the macular carotenoids are indeed involved in antioxidant reactions is supported by the identification of oxo-lutein [**Figure 5**] by Khachik *et al.* in 58 pairs of postmortem human retinas and one pair of monkey retinas.¹ While the amount of this molecule in the retina is substantial (up to 24% of total retinal lutein), whether its occurrence in the retina is specific or due to passive transport from the plasma is not yet known.

In vivo evidence in animals

Animal studies can be used to test whether mechanistic ideas developed on the basis of *in vitro* experiments are clinically relevant. The problem regarding AMD is that no good animal model that would also allow the evaluation of the effect of carotenoids in the context of this disease exists. Only primates qualify for such a model because only they have a macula lutea and are reported to develop drusen and age-related macular changes similar to that of human AMD.^{48,49} Primates, however do not seem to have been widely utilized as animal models.

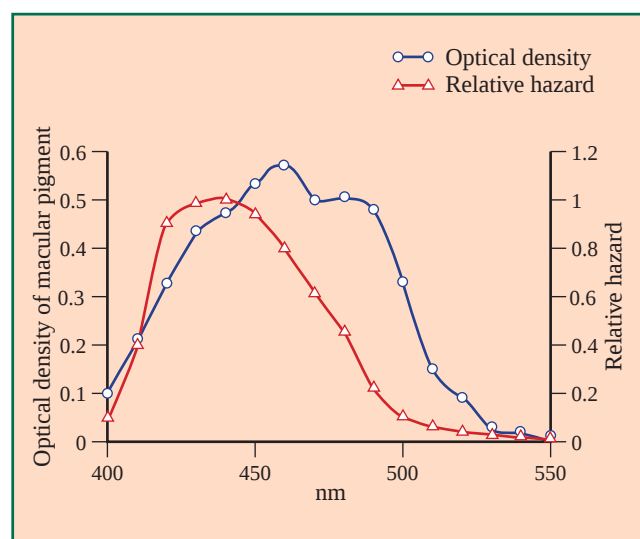


Figure 4. Blue light hazard function and absorption spectrum of macular pigment. Adapted from Ham & Müller.⁴²

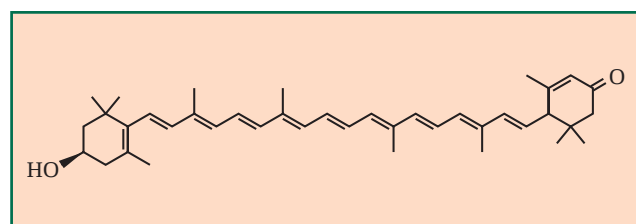


Figure 5. Oxo-lutein [(3R,6'R)-3-hydroxy- β,ϵ -carotene-3'-one].¹

Studies in rats and mice

Recently, a supplementation experiment in female BALB/c mice fed different doses of lutein extracted from marigold was described.¹⁷ For 28 days, lutein 0.15 to 1.2 mg per day (equivalent to 7 to 52 mg/kg/day) was administered with the normal feed that was freely available to the mice. After 3 days, peak plasma levels of between 2.5 and 3.2 $\mu\text{mol/L}$ were attained. Plasma levels then decreased to approximately 2.5 $\mu\text{mol/L}$, irrespective of dose, and remained stable throughout the study. This study demonstrates that lutein from marigold is readily available to mice but, unfortunately, no ophthalmological parameters were determined in this study, nor was the carotenoid content of the retina measured.

Li and Tso reported what was probably the first animal study to evaluate the potential protective effect of zeaxanthin on light retinal damage.⁵⁰ These researchers fed *Gou Qi Zi* berries, that have a very high content of zeaxanthin (**Table 2**), to rats before, during, and after 24-hour exposure to intense fluorescence light (250 foot-candles). At the end of the experiment, photic damage to the retina was assessed by histology and compared to that of the control group. In the control group, the rods and cones were severely injured. The number of nuclei in the outer nuclear layer (ONL) was significantly decreased and degeneration and necrosis of the retinal pigment epithelium was noted. In the animals that had received the zeaxanthin-containing berries, however, the rods and cones appeared normal, with only the number of photoreceptors being slightly decreased. This study could not exclude whether the observed protective effect was indeed caused by zeaxanthin or by some other ingredients of the berry; furthermore, the rat is not an ideal animal for study of carotenoids and, ophthalmologically, is widely different from humans.

Studies in quails

Although quails lack a macula lutea, their cone-rich retina has some characteristics similar to the human retina in that it accumulates carotenoids⁵¹ and can form drusen.⁵² Dorey *et al.* presented evidence supporting the idea that zeaxanthin has a preventive potential in regard of light damage.⁵³ They fed carotenoid-free diets or diets supplemented with 5 mg/kg zeaxanthin from flavobacter to Japanese quails (*Coturnix coturnix japonica*) for 3 months. The animals were then exposed to intermittent white light (3200 lux) for 28 hours in order to induce general photic damage to the retina. After 14 hours in the dark, the eyes were excised for HPLC determination of zeaxanthin in the retina and measurement of the extent of apoptosis, including analysis by TdT-mediated dUTP nick end-labeling (TUNEL) staining. The number of light-induced apoptoses of rod and cone photoreceptor cells was drastically reduced in treated animals. Furthermore, the retinas containing more zeaxanthin as assessed by HPLC seemed to be better protected. In an extension of the study, the authors found indicators that zeaxanthin also slowed the natural aging of the quails' retinas.⁵⁴

Studies in other non-primate animals

Another animal that may prove useful for the study of lutein and zeaxanthin in the eye is the frog. Recently, 3R, 3'S (=meso)-zeaxanthin was detected in the retina but not in the liver of the frog (*Rana pipiens*),⁵⁵ indicating that the metabolism of macular carotenoids in this aquatic animal may be similar to that in humans.

Studies in primates

The best animal to study to investigate questions related to carotenoids in the macula are primates because they have a macula lutea very similar to that of humans. One of the first questions to be asked was whether the yellow spot is of nutritional origin and if it disappeared when a carotenoid-depleted diet was given. For this purpose, macaque monkeys were fed a carotenoid deficient diet for 2 to 6 years.⁵⁶ This led to disappearance of plasma carotenoids and more gradual disappearance of the yellow pigmentation of the retina. Furthermore, fluorescence angiography documented various defects of the retina including window defects. In comparison with normally fed monkeys of the same age, the carotenoid-deficient monkeys also showed more drusen — one of the hallmarks of AMD in humans.

Investigations in humans

Observational studies

In 1988, Haegerstrom-Portnoy reported that age-related decline of retinal sensitivity of the blue-sensitive cones is slower in areas where macular yellow pigment is present.⁵⁷ Consistent with this observation, Hammond *et al.* found an accelerated decrease in short-wavelength (blue) cone sensitivity in older individuals with lower macular pigment concentrations.⁵⁸ Furthermore, individuals with high levels of macular yellow pigment had a retinal sensitivity similar to that of young individuals, as if the presence of macular pigment had conserved their retinal sensitivity. The authors felt that these results supported the hypothesis that carotenoids reduce the risk for AMD since the deterioration in retinal sensitivity and acceleration of blue cone loss that accompanies aging are known to precede the clinical manifestation of AMD and other macular diseases.⁵⁹

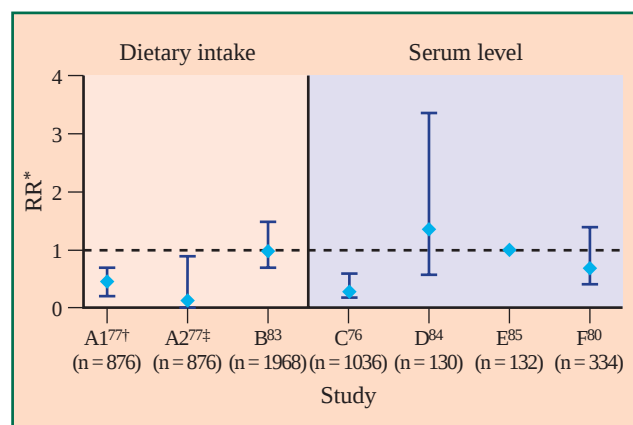
A number of toxic (such as photosensitivity from chloroquine⁶⁰) and degenerative changes in the retina show an annular pattern called Bull's-eye maculopathy. In this condition, a circular ring of structural change surrounding the macula can be seen. The macula itself does not seem to be affected. In a study involving 95 patients, Weiter *et al.* found that the area spared degenerative changes corresponded closely to the area with the highest concentration of macular pigment.⁶¹ The authors concluded that the presence of macular pigment may have provided some protection from the degenerative changes. A more direct link of carotenoids with AMD is provided by the following study.

In a postmortem HPLC study of eyes from 12 normal subjects and 12 with AMD, Landrum *et al.* found average lutein and zeaxanthin levels to be approximately 30% lower in the

retinas of people with AMD than in the retinas of normal subjects.⁶² This difference was greatest in the central disc, decreasing in the medial and outer annulus (see **Table 1**). Therefore, it is possible that AMD at least partly contributes to the preferential loss of lutein and zeaxanthin in the macula. With this reasoning, Landrum *et al.* have recently refined their analyses and investigated the retinas of a total of 56 patients with AMD and 45 control patients.⁶³ The eyes of the control subjects were divided into quintiles of carotenoid concentration in the outer annulus, which is normally unaffected by AMD, and the number of cases of AMD in each quintile was determined so as to calculate the relative risk ratio. Comparison of individual relative risk ratios revealed a substantial and statistically significant reduction of risk in the highest quintile of carotenoid concentration in the outer annulus. While it is still possible that decreased carotenoid levels even in the outer annulus could be a result, rather than the cause, of AMD, these data are consistent with some of the epidemiological studies discussed in the following section.

An interesting correlation of macular pigment density with a number of known risk factors for AMD appears to emerge. Interestingly, subjects who smoke,⁶⁴ have blue irises,⁶⁵ or are female⁶⁶ appear to have a reduced macular pigment density when compared with non-smokers, subjects with dark irises, or males. On the other hand, factors that increase macular pigment optical density such as dietary ingestion of carotenoid containing vegetables seem to decrease the risk for AMD, as will become evident in the discussion of two epidemiological studies in the following section. Nevertheless, whether and how the individual amount of yellow pigment in the macula contributes to determining the risk for AMD of individuals or populations is still open to debate.

Macular pigment density varies widely between individuals even if corrected for differences in measuring technique.⁶⁷ Environmental, probably dietary, rather than genetic factors seem to be responsible for this variability because macular pigment can vary among homozygotic twins,⁶⁸ while having similar values in the two eyes of the one individual.⁶⁹ Recent larger surveys of macular pigment density indicate the existence of populations with quite different mean macular pigment levels, demonstrating variability among populations. Thus, mean macular pigment densities of approximately 0.2 were reported for populations in Indianapolis,^{70,71} Phoenix,⁷² and New York.⁷³ These values are smaller than the average macular pigment density of approximately 0.35 generally found in populations in the North-eastern USA.⁷⁴ The existence of these variations may indicate that a larger-than-expected proportion of the general population could be at increased risk for AMD because of low macular pigment levels. In this context it might be interesting to study South Pacific populations. Men from the Fiji islands are reported to have dietary lutein intakes of up to 25.7 mg per day⁷⁵ as compared with an average total carotenoid intake of only 1 to 6 mg per day in the USA.³ The implications of this elevated and probably long-term



* Relative risk and 95% confidence intervals.

† Spinach 2-4/week.

‡ Spinach daily.

Figure 6. Relative risk for age-related macular degeneration from different studies investigating dietary intake or serum levels of lutein or zeaxanthin.

intake of lutein in terms of increased macular pigment levels and potentially lowered risk for AMD in comparison with individuals or populations that differ in these respects have yet to be evaluated.

Epidemiological studies

The next pieces in the puzzle of an emerging role of carotenoids in risk reduction of AMD are epidemiological studies. Such studies generally determine the risk for AMD in specifically selected populations relative to a control group. The study that is probably most relevant in this respect is a case control trial that compared plasma levels of 356 subjects with neovascular AMD with 520 control subjects. This study found a statistically significant inverse relationship between plasma levels of lutein and zeaxanthin and the risk for neovascular AMD⁷⁶ i.e. higher plasma levels were correlated with a lower AMD risk. These results are consistent with those of a later study by the same group showing a lower risk for AMD in subjects with a higher dietary intake of lutein and zeaxanthin.⁷⁷ In this later study, subjects who consumed a medium-sized portion of spinach (approximately 75 g containing approximately 7.2 mg lutein and 0.3 mg zeaxanthin; **Table 2**) two to four times a week were found to have a statistically significant 45% reduction in the risk for AMD (study A1 in **Figure 6**) as compared with the control group, while subjects who ate spinach daily had their risk reduced by more than 80% (study A2 in **Figure 6**). To put these numbers into context, the average daily intake of lutein is about 0.97 mg and that of zeaxanthin about 0.14 mg.⁷⁸ Even a diet rich in fruit and vegetables provides only 2.3 mg lutein and 0.3 mg zeaxanthin per day,⁷⁹ i.e. only one-third of the lutein present in a medium-sized portion of spinach.

However, the epidemiological relation of lutein and zeaxanthin to the risk of AMD is not yet totally clear. The Beaver Dam Study examined a largely Caucasian community in south-central Wisconsin, comparing 167 subjects with retinal pigment epithelial abnormalities, soft drusen, and

exudative AMD with an equal number of normal control subjects.^{79,80} Individual plasma concentrations of lutein and zeaxanthin were found to be slightly, though not significantly, lower in subjects with exudative macular degeneration. A more recent epidemiological study that investigated vitamin E but not the macular carotenoids concluded that there is some evidence that vitamin E may provide protection against AMD.⁸¹ An evaluation of the Blue Mountains Eye Study provided no evidence of a protective association of plasma levels of vitamin E and β -carotene.⁸² In the case of β -carotene this is not surprising since this carotenoid is not found in the retina.

Results from the few epidemiological studies that have evaluated the relative risk for AMD in respect to lutein or zeaxanthin plasma or intake levels are compared in **Figure 6**.^{76,77,80,83-85} While the epidemiological evidence may appear to be conflicting, it has to be appreciated that AMD is multifactorial and is therefore a difficult disease to study. AMD has a long time-course and, etiologically, may be initiated early in life. Plasma levels and dietary intake of lutein and zeaxanthin appear to be good parameters for delineating the influence of present nutrition on the development and progression of AMD. Thus, the information they provide relates to current dietary intake, whereas long-term dietary history may be of particular significance, especially as AMD is a disease of senescence.

Epidemiological studies therefore cannot provide definite proof of the efficacy of lutein and zeaxanthin in AMD. Such studies can provide evidence of possible relationships but cannot determine whether an effect is causal. The situation is different with intervention studies in which agents are administered on a double-masked, placebo-controlled, randomized basis and results are evaluated using predefined efficacy parameters. In the case of supplementation with lutein and zeaxanthin, where only small to moderate responses can be expected, only studies such as these are likely to provide a definite answer as to an effect of lutein and zeaxanthin on AMD.⁸⁶ However, the specific time-course and nature of this disease makes the design of such trials difficult.

Intervention trials

To date, no well-controlled intervention trials with lutein or zeaxanthin have been published. One reason is that, until recently, lutein and zeaxanthin supplements were not widely available for human consumption. In the case of lutein, this situation has now changed, for instance in the USA, with the marketing of a number of preparations containing up to 25 mg lutein per unit. The lutein is normally manufactured by extraction of marigold (*Tagetes erecta*) petals and also contains approximately 5% zeaxanthin. However, no pure zeaxanthin preparation has been marketed to date.

Neither of these carotenoids is being used in the ongoing Age-Related Eye Disease Study.⁸⁷ This study was started by the National Eye Institute in 1992, before lutein became readily available. The epidemiological part is investigating

the natural history of AMD and cataract, while the clinical part is evaluating the effect of high-dose vitamin (including β -carotene) and mineral supplements on AMD and the effect of high-dose vitamin supplements on cataract.

The results of two recent studies could be interpreted as supporting the early hypothesis that macular pigment improves visual performance by absorbing blue light and attenuating chromatic aberration. This is thought to have a direct influence on some visual function parameters such as visual acuity. Zorge *et al.* reported significantly improved visual function, including visual acuity, for 20 patients with congenital retinal degeneration who were supplemented with lutein.⁸⁸ In a case study of a small number of subjects, Richer investigated the effect on various ophthalmological diseases involving the macula of dietary supplementation including consumption of 5 ounces of spinach, which is rich in lutein (**Table 2**).⁸⁹ These researchers also found improvements in a number of visual function tests such as contrast sensitivity.

Measurement of macular pigment in humans

Because of the potential importance of lutein and zeaxanthin for the health of the retina, particular importance is attached to measurement of carotenoids in the retina and macula, the target organ of AMD. In postmortem retinas, carotenoids can be measured very precisely by HPLC, and the distribution of the various carotenoid molecules in the retina has been determined using this technique (see **Table 1**). However, only by using noninvasive techniques is it possible to directly relate disease outcome parameters to macular carotenoid levels in epidemiological studies and intervention trials. For this noninvasive measurement of macular pigment, a number of different techniques are available. Only some should be mentioned here:

- fundus reflectometry⁹⁰ and its adaptation to use of the scanning laser ophthalmoscope⁹¹
- resonance Raman scattering⁹²
- a method based on the fluorescence of lipofuscin in the RPE⁹³ for objective measurements.

Heterochromatic flicker photometry is a subjective measurement⁹⁴ and this method appears to be the most widely used technique. In brief, subjects view a narrow spot of light whose color alternates between blue and green (460 nm and 550 nm, respectively; the maximum and minimum absorbances of the macular yellow pigment). When the spot of light falls within the area of the macula lutea, the blue light is absorbed by the yellow macular pigment and therefore attenuated, whereas the green light is not. This results in a difference in luminance that in turn causes the light impression to flicker. The flicker can be minimized by manually increasing the luminance of the attenuated blue light until it matches the luminance of the non-attenuated green light. When corrected by the luminance at a parafoveal location (usually at around 5.5° eccentricity) where it is assumed that no macular pigment is present, the increment in

luminance is a measure of the optical density of the macular yellow pigment at the location targeted by the spot of light. The validity of heterochromatic flicker photometry has been repeatedly demonstrated. In particular, it is possible to reconstruct the absorbance spectrum of the macular pigment and to show that it is identical to the spectrum measured directly in excised retinal tissue by microspectrophotometry.²⁵ While the devices for this technique used to be optically complex and difficult to handle, simplified apparatus, based on light emitting diodes, have recently been described^{95,96} and may enable the technique to be used in larger field-type studies.

Modulation of macular pigment density and plasma concentrations of lutein and zeaxanthin

One of the important questions to be answered before initiating human intervention trials is the specific bioavailability of the test substance at the target organ. Is it possible to increase the amount of lutein and/or zeaxanthin in the macula by means of dietetic manipulation or by supplementation with the pure compounds? This question has been investigated in various studies.

Modulation by diet

The response of macular pigment density to dietary administration of lutein and zeaxanthin was investigated by Hammond *et al.*⁷⁴ Volunteers were given a diet that was rich in spinach (providing 10.8 mg lutein and 0.3 mg zeaxanthin per day) and/or corn (providing 0.4 mg lutein and 0.3 mg zeaxanthin per day) for up to 15 weeks. The carotenoid concentrations in plasma and optical densities in the retina were measured. One volunteer showed no increase in the levels of the carotenoids in either plasma or macula. Two volunteers were found to have increased concentrations in plasma, but not in the macula. The remaining nine volunteers, however, showed increased concentrations of lutein and zeaxanthin in both plasma (up 33% from baseline) and macula (up to about 19%). Plasma zeaxanthin increased by 70% and macular pigment by 25% in one of the two volunteers who received the corn diet, while the other did not respond at all. It was therefore concluded that although the response to dietary lutein and zeaxanthin ingestion varies considerably, the amount of carotenoids in the macula can be increased by dietary modification.

A recent paper investigated the effect on macular pigment density of ingesting daily portions of spinach and corn containing a total of 11.2 mg lutein and 0.6 mg zeaxanthin in addition to the usual diet.⁹⁷ After 4 weeks, plasma lutein had increased almost two-fold, while plasma zeaxanthin had increased by only a small, though statistically significant, amount. The latter finding is not surprising given the relatively small amount of zeaxanthin ingested. To put this into perspective in comparison with lutein, the authors report the mean peak serum concentration per amount of carotenoid

ingested as 20 nM/ μ mol for lutein and 24 nM/ μ mol for zeaxanthin, indicating that the bioavailability of these two carotenoids from food cannot be very different. Concomitantly with the increase in plasma levels of lutein and zeaxanthin, there was also a small but statistically significant increase in macular pigment as measured by heterochromatic flicker photometry. While plasma levels of lutein returned to baseline levels 2 months after cessation of the additional dietary lutein and zeaxanthin intake, macular pigment density was still significantly higher than at baseline. The conclusion of similar availability of lutein and zeaxanthin from food sources is supported by another recent investigation in which diets supplemented with egg yolks containing known amounts of lutein and zeaxanthin were given to volunteers for 4.5 weeks.⁹⁸ The specific increments in the group ingesting beef tallow supplemented with egg yolk were almost identical for lutein and zeaxanthin.

If the fact that the concentration of zeaxanthin in the center of the macula is higher than that of lutein also reflects a more important physiological role of zeaxanthin there, it could be advantageous to ingest more zeaxanthin than is present in the normal diet. However, given the small amount of zeaxanthin present in most foods (**Table 2**), this could only be easily realized by supplementation.

Modulation by supplementation

The plasma response of monkeys to feeding with synthetic zeaxanthin formulated into a beadlet formulation was studied in three animals. The plasma concentration of zeaxanthin increased dose-dependently, reaching a level of around 500 nM approximately 3 weeks after starting supplementation with a daily dose of 2.5 mg (equivalent to 2.8 mg/kg/day).⁹⁹ In this study, macular pigment of the supplemented animals was not assessed. Khachik *et al.* purified lutein from marigold flowers and zeaxanthin from *Gou Qi Zi* (**Table 2**), and administered suspensions in olive oil to three volunteers.¹⁰⁰ Daily doses of 10 mg were given for 18 (lutein) or 21 (zeaxanthin) days. Analyses by HPLC showed that the serum levels of both carotenoids peaked after 1 week: lutein at 1.4 μ mol/L zeaxanthin considerably lower at 0.1 μ mol/L. No explanation was offered for this large difference, however it could be that other substances in the berry inhibited the uptake of zeaxanthin into the plasma. Another study measured uptake of lutein into the plasma after supplementation with capsules containing an extract of marigold flowers in corn oil.¹⁰¹ For 3 months, nine volunteers were given supplements of 15 mg lutein. After 1 month, independently of initial lutein levels (mean, 0.3 μ mol/L), plasma concentrations increased three- to five-fold.

Landrum *et al.* measured plasma carotenoids concurrently with macular pigment density in a supplementation study of two subjects receiving 30 mg lutein (as a marigold lutein ester extract suspended in canola oil) daily for 140 days.¹⁰² Serum lutein levels rapidly increased 10-fold from 0.2–0.3 μ mol/L in the first week and maintained that level

for the remainder of the study. Macular pigment density, as estimated by heterochromatic flicker photometry, showed a slower response than the serum, starting to increase after approximately 20 days. For one subject, macular pigment density increased by approximately 41% and 37% in the right and left eyes, respectively, and by 21% bilaterally for the other by the end of the supplementation period, after which the density continued to increase for another month. The authors concluded that an increase in macular yellow pigmentation appears to be a slow process with considerable interindividual variation. The same authors also gave a small number of volunteers supplements of 30 mg per day of pure zeaxanthin from flavobacter that was formulated into gelatin/starch beadlets for 4 months.¹⁰³ Ten to 20 days after the start of the supplementation period, plasma levels had reached a plateau at a concentration of approximately 0.5 μM , almost six-fold higher than at baseline. Approximately 40 days after starting the supplementation, macular pigment densities had also started to increase.

Smaller doses of lutein increase plasma and retina levels only slightly as reported by Chen *et al.*¹⁰⁴ In this study, volunteers were given supplements of 2.4 mg lutein daily. While plasma levels increased by 132%, macular pigment showed

statistically insignificant small increases after 2 months that became statistically significant only after 6 months.

Though there appears to be considerable variability, these results demonstrate that macular pigment can indeed be altered by supplementation or by diet. This finding may be important, as macular pigment appears to be very stable over time when analyzed chemically postmortem¹⁰⁵ or psychophysically *in vivo*.⁶⁷ In this respect, Hammond *et al.* reported on a subject whose macular pigment was very stable over 5 years, yet increased by 50% after only 14 weeks of a test diet rich in lutein and zeaxanthin, and remained elevated for 9 months after the diet was discontinued.⁷⁴

Conclusion

On the basis of the scientific ideas presented in this paper, it seems plausible that lutein and zeaxanthin may significantly contribute to reducing the risk for age-related macular degeneration, though in a way that cannot be precisely quantitated as yet. Pending the results of further experimental, epidemiological, and, most importantly, well-controlled clinical intervention trials, lutein and zeaxanthin already appear to be valid candidates as ingredients for food intended to maintain retinal function.

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