Are lutein and zeaxanthin conditionally essential nutrients for eye health?

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Summary The carotenoids lutein and zeaxanthin are found in the macula in high concentrations and may play a role in the pathogenesis of age-related macular degeneration (ARMD). Lutein and zeaxanthin may protect the macula and photoreceptor outer segments throughout the retina from oxidative stress and play a role in an antioxidant cascade that safely disarms the energy of reactive oxygen species. Although lutein and zeaxanthin are not essential nutrients, studies are beginning to suggest that they fit the criteria for conditionally essential nutrients. Low plasma lutein and zeaxanthin concentrations or dietary intake are associated with low macular pigment density and increased risk of ARMD. Dietary deprivation of lutein and zeaxanthin in primates causes pathological changes in the macula. Should controlled clinical trials show lutein and/or zeaxanthin supplementation protects against the development or progression of ARMD and other eye diseases, then lutein and zeaxanthin could be considered as conditionally essential nutrients for humans.

INTRODUCTION

Lutein and zeaxanthin are two carotenoids that are distinguished from the other major dietary carotenoids because they are found in high concentrations in the retina of the human eye. The area of the retina that serves central vision is known as the macula lutea because of its yellow coloration from lutein and zeaxanthin. Lutein and zeaxanthin are oxygenated carotenoids (xanthophylls) that consist of 40-carbon hydroxylated compounds (1). The remaining four major dietary carotenoids in humans, α-carotene, β-carotene, β-cryptoxanthin, and lycopene, are not found in the retina in high concentrations.

Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet. Foods that are rich in lutein and zeaxanthin include egg yolk, corn, orange juice, honeydew melon, and orange pepper (2), and dark green leafy vegetables such as kale, spinach, collards, turnip greens, and broccoli (3). Recently, lutein and zeaxanthin have been implicated in the pathogenesis of age-related macular degeneration, the leading cause of visual loss in adults age 65 and older in the United States and Europe (4).

Currently there are no defined dietary reference intakes for the major dietary carotenoids, including lutein and zeaxanthin, but sufficient data exist to support existing recommendations for increased consumption of fruits and vegetables (5). Lutein and zeaxanthin do not fulfill the criteria to be considered as essential nutrients, as lutein and zeaxanthin have not been shown to be required for growth, health, and survival, and absence of lutein and/or zeaxanthin from the diet or inadequate intake does not appear to result in a characteristic deficiency disease and, ultimately, death (6). Although lutein and zeaxanthin are not essential nutrients, they may fulfill the criteria to be considered conditionally essential.
nutrients. The three criteria for conditional essentiality include: (1) decline of the plasma level of the nutrient into the subnormal range, (2) appearance of chemical, structural, or functional abnormalities, and (3) correction of both of these by dietary supplementation of the nutrient (6,7).

In this article, we discuss some of the mechanisms through which lutein and zeaxanthin may protect the retina against destructive reactions with photosensitizing agents and their possible interactions with other molecules such as vitamin E and vitamin C. Lutein and zeaxanthin may protect the retina from age-related macular degeneration and other eye diseases, and further clinical evidence may fulfill the criteria by which lutein and zeaxanthin could be considered conditionally essential for humans.

HISTORICAL BACKGROUND

A yellow pigment was described in the macula in 1782 by the Milanese ophthalmologist Francesco Buzzi (1751–1805) (8). Over a decade later, similar observations were made by Everard Home (1756-1832) (9) and Samuel Thomas von Soemmering (1775–1830) (10). After the development of the ophthalmoscope in the mid-nineteenth century, there were inconsistent observations of yellow pigmentation in the macula, and this variability was probably related to the wavelength of light that was used in the ophthalmoscope, as the yellow color was more readily visible with the use of red-free light (11). In 1869, Johann Ludwig Wilhelm Thudichum (1829–1901), a chemist at St. Thomas’s Hospital in London, found that parts of plants and animals contain a yellow crystallizable substance, which he named ‘luteine’ (12). In 1929, a new carotenoid was isolated from maize, named zeaxanthin, and characterized by the Swiss biochemist Paul Karrer (1889–1971) (13–16). In 1945, George Wald (1906–1997) observed that the macula pigment in humans had the same absorption spectrum as crystalline leaf xanthophyll. The extramacular portions of the retina were also noted to contain some xanthophyll, but at a lower concentration per unit area than the macula. Extraction of the yellow pigment from human maculas yielded a hydroxy-carotenoid that Wald believed was lutein or leaf xanthophyll itself, noting ‘this marks the first appearance of a carotenoid of this type in a mammalian retina’ (17).

LUTEIN AND ZEAXANTHIN IN THE RETINA

Macular pigment consists primarily of two carotenoids, lutein and zeaxanthin (18–21). These carotenoids have an intense coloration due to extensive conjugation in the polyene chain (22) and give the macula its yellowish color. Zeaxanthin is found as two isomers, 3R, 3’-R-zeaxanthin and meso-zeaxanthin (23). Zeaxanthin and meso-zeaxanthin differ in relation to the stereochemistry of the secondary hydroxyl groups at the 3’ position. Lutein, zeaxanthin, and meso-zeaxanthin represent about 36%, 18%, and 18% of the total carotenoid content of the retina (22). Several minor carotenoids, consisting of additional isomers of both lutein and zeaxanthin, have also been identified in retinal extracts (24). In the inner macula, the concentration of zeaxanthin is approximately twice that of lutein, but lutein becomes the dominant carotenoid with increasing eccentricity from the fovea (25). Studies in primates show that there is a high degree of symmetry in the lutein and zeaxanthin concentrations in corresponding sections of the retinas between the left and right eyes of individual animals (26). In the adult retina, the concentration of lutein increases and the concentration of meso-zeaxanthin decreases with radial distance from the fovea (25). The concentration of macular pigment reaches almost 1 mM within the central macula, which is about three times the concentration of carotenoids in normal human sera (22).

In the primate retina, the highest concentrations of macular pigment are located in the inner retinal layers (27,28).

Serum lutein and zeaxanthin concentrations have been positively correlated with macular pigment density in human subjects (29). A high concentration of lutein has been described in subretinal fluid in subjects with rhegmatogenous retinal detachment, which supports the hypothesis that lutein is transported from the blood into the retina (30). The isomer meso-zeaxanthin is found in human serum in extremely low concentrations, and it is not clear whether meso-zeaxanthin in the plasma is the source for meso-zeaxanthin in the retina (22). It has been hypothesized that a yet undescribed isomerase converts lutein to meso-zeaxanthin by migration of the 4’,5’ double bond in lutein to the 5’,6’ position to form meso-zeaxanthin (25). In the human eye, iris, ciliary body, and retinal pigment epithelium and choroid also contain high concentrations of carotenoids, accounting for about one half the eye’s total carotenoids and about 30% of the total lutein and zeaxanthin found in the eye (31). Carotenoids have been reported to bind to tubulin with the receptor axon layer of the fovea (32), specifically to the paclitaxel-binding site of the β-tubulin subunit of microtubules in the primate retina (33). Other reports show that lutein and zeaxanthin are associated with rod outer segments in the peripheral retina of humans (34), and the concentrations of lutein and zeaxanthin in rod outer segment membranes is 2.7 times more concentrated in the perifoveal compared with the peripheral retinal region (35).
Macular pigment absorbs and attenuates blue light (36) and may protect the retina from excessive oxidative stress (22). The importance of blue light absorption for the protection of the outer retina and choroid should not be under estimated. In studies with rabbits, short wavelength (400–500 nm) light was 30 times as effective as long wavelength (510–740 nm) light in disrupting the integrity of the blood–retinal barrier at the pigment epithelium (37).

LUTEIN AND ZEAXANTHIN AS ANTIOXIDANTS

Carotenoids such as lutein and zeaxanthin absorb visible light and play a role in singlet–singlet energy transfer and the quenching of singlet oxygen (38). The absorption of light energy produces a transition $\pi \rightarrow \pi^*$ in which one of bonding $\pi$-electrons of the polyene chain is promoted to a previously unoccupied $\pi^*$ antibonding orbital (39). The $\pi$-electrons are delocalized over the polyene chain, and the energy that is needed to produce the transition of $\pi \rightarrow \pi^*$ is small and corresponds to light in the visible spectrum of 400–500 nm (39). Carotenoids have two low-lying electronic excited singlet states (40). The strong absorption of light in the visible region has been attributed to the transition from the ground state $S_0$ to the second singlet excited state $S_2$ (39). Carotenoids can also accept excitation energy from highly reactive singlet oxygen, $^1O_2$, and this property of carotenoids may protect against damage caused by a combination of light and oxygen (41,42). Singlet oxygen is highly reactive and can damage DNA and lipids. The reaction with singlet oxygen generates a triplet excited carotenoid:

$$^1O_2 + \text{carotenoid} \rightarrow ^3O_2 + ^3\text{carotenoid}$$

The triplet excited carotenoid then dissipates the energy harmlessly through rotational and vibrational interactions to recover the ground state:

$$^3\text{carotenoid} \rightarrow \text{carotenoid} + \text{thermal energy}$$

The precise mechanisms of interaction between carotenoids and reactive oxidants, and the possible roles of other molecules, especially in vivo, remain to be resolved (39). From an in vitro study of lipid peroxidation in photoreceptor outer segments and liposomes, there is evidence that lutein and zeaxanthin do not fulfill their antioxidant roles in isolation (43). Although lutein and zeaxanthin were more effective than other antioxidants in preventing lipid peroxidation, they were significantly more so, and were themselves better protected from secondary oxidative breakdown in the presence of melanin, glutathione, $\alpha$-tocopherol, and ascorbate.

While the mechanisms used to disarm singlet oxygen and other harmful oxidants in vivo remain to be elucidated, it is clear that carotenoids can serve to deactivate potentially harmful $^1O_2$ (42). The ability of lutein and zeaxanthin to protect against photo-oxidation is related to the number of conjugated double bonds, at least in vitro (44). For this reason, lycopene and $\beta$-carotene are more effective than lutein at quenching singlet oxygen. Zeaxanthin and meso-zeaxanthin, however, are almost as effective as lycopene and $\beta$-carotene (45), and their relative effectiveness may be different in vivo. Carotenoids can also quench peroxyl radicals (46), tocopheryl radicals (45), and can inhibit lipid peroxidation (47). The carotenoids were the first singlet oxygen quenchers to be characterized and are among the most effective quenchers known (48). Other functions of macular pigment may include the reduction of chromatic aberration (49) and improvement of visual resolution (50).

LUTEIN AND ZEAXANTHIN INTAKE AND AGE-RELATED MACULAR DEGENERATION

Large epidemiological studies have suggested an association between carotenoid intake or status and age-related macular degeneration. A high frequency of consumption of fruits and vegetables was associated with a lower risk of age-related macular degeneration in the first National Health and Nutrition Examination Survey (51). In the Eye Disease Case-Control Study, a high dietary intake of lutein and zeaxanthin was associated with a lower risk of age-related macular degeneration (Odds Ratio. 0.43, 95% Confidence Interval, 0.2–0.7) (4). Similarly, serum concentrations of carotenoids, vitamin C, vitamin E, and selenium were compared between 421 cases with neovascular age-related macular degeneration and 615 controls (52,53). A reduced risk of age-related macular degeneration was found in the upper two tertiles of carotenoids compared with the lowest tertile (52,53).

In a case-control study of 56 donor eyes with age-related macular degeneration and 56 control eyes without age-related macular degeneration, the concentrations of lutein and zeaxanthin in concentric regions centered on the fovea was significantly less in eyes with age-related macular degeneration than eyes that did not have the disease (54). Macular pigment density has been studied in detail in humans using non-invasive psychophysical measurements with tabletop devices that employ light-emitting diodes (55). An age-related decline in macular pigment optical density has been observed in healthy subjects (56). Subjects with age-related macular degeneration in one eye only had lower macular pigment density in the unaffected eye compared with healthy controls with no history of age-related macular degeneration (56).
MACULAR PIGMENT IN HEALTH AND DISEASE

In a study of 217 subjects, macular pigment density appeared to decline with age, and lower macular pigment density was significantly lower in women than men, was lower in those with light versus dark colored irises, and was low among current smokers who smoked >10 cigarettes per day (57). Macular pigment density appears to be well correlated between the two eyes of the same individual (58). Males have been shown to have higher macular pigment densities than females, despite similar plasma carotenoid concentrations (59). Plasma lutein and zeaxanthin concentrations were positively correlated with macular pigment density among both men and women. Visual sensitivity appears to be preserved in older adults who have high macular pigment density (60). Among individuals with stable dietary patterns, macular pigment densities appear to change little over time (61). Studies in monozygotic twins suggest that macular pigment density may vary according to dietary intake (62). Older adults showed a differential loss of sensitivity of short-wavelength-sensitive-cone (S-cone) in the retinal periphery compared with younger adults, suggesting that macular pigment may protect the fovea from light damage (63).

There have not been many experimental animal studies of carotenoid deprivation, partly because the absorption of carotenoids is relatively poor in rodents such as rats, mice, and hamsters, and larger species such as chicks, rabbits, pigs, and sheep may break down carotenoids in the gastrointestinal tract (64). Monkeys raised on a xanthophyll-free diet showed a total loss of macular pigment that was accompanied by an increase in drusen-like bodies at the level of pigment epithelium (65). Another study showed that no detectable macular pigment and practically no plasma xanthophylls were found in monkeys raised on semipurified diets without carotenoids, and clinical histopathology showed vacuolated retinal pigment epithelial cells that corresponded to window defects in the retinal pigment epithelium (66).

EFFECT OF DIETARY SUPPLEMENTATION ON MACULAR PIGMENT

Several studies have shown that macular pigment density can be increased by dietary modification or supplementation with lutein. In a study involving a dietary modification, 13 subjects received spinach and corn, spinach alone, or corn alone, and increases in macular pigment density were seen in most, but not all, subjects after four weeks (67). In another study, two subjects consumed lutein esters, equivalent of 30 mg of free lutein per day, for 140 days. Over the first 40 days, serum concentrations of lutein increased ten-fold, and macular pigment density increased by 21% and 39% in the two subjects (68). The investigators estimated that lutein supplementation may have produced a 30–40% reduction in blue light reaching the photoreceptors, Bruch’s membrane, and the retinal pigment epithelium (68). In another dietary intervention study, seven subjects consumed spinach and corn daily for 15 weeks, and macular pigment density and carotenoid concentrations in serum, buccal mucosal cells, and adipose tissue were measured at baseline, 4, 8, 15 weeks and 2 months post-intervention (69). Daily consumption of spinach and corn resulted in a significant increase in macular pigment density at four weeks compared to baseline. In a cross-sectional study, lutein concentrations in adipose tissue and macular pigment were compared between 13 women and 8 men. There was a significant positive correlation between lutein concentrations and adipose tissue and macular pigment among men, but a negative correlation among women, suggesting that there may be sex differences in lutein metabolism (69).

In a small uncontrolled study, 14 male patients with atrophic age-related macular degeneration received five ounces of spinach, 4–7 times per week (70). Short term improvement in visual function was found in one or both eyes (70). Lutein supplementation (40 mg/day for 2 months followed by 20 mg/day 4 months) was associated with short-term improvement in visual acuity and central visual field diameter among subjects with retinitis pigmentosa and related retinal degenerations who were recruited and followed via the internet (71). In another study of lutein supplementation, 20 mg/day, for 58 patients with retinitis pigmentosa or Usher syndrome, serum lutein concentrations increased significantly by six months, but only about half the patients showed an increase in macular pigment density (72). Zeaxanthin supplementation has been studied in primates. Carotenoid extracts from the Gou Zi Qi berry (Lycium chinense) contain high concentrations of zeaxanthin, and retinal zeaxanthin increased after supplementation in rhesus monkeys (73).

CAROTENOIDs IN PHOTORECEPTOR OUTER SEGMENTS

When considering the antioxidant role of carotenoids in vivo, it may be important to distinguish between apolar molecules such as β-carotene and lycopene, which remain embedded in the lipid environment inside a membrane and generally interact with radicals within the membrane itself, and xanthophylls such as lutein and the zeaxanthins, whose polar end groups tend to protrude from the lipid cell membrane into the intra- and extra-cellular plasma and can therefore interact with radicals outside the membrane (39). This characteristic
makes lutein and zeaxanthin potentially very effective antioxidants in environments such as photoreceptor outer segments, where the phototransduction cascade takes place along the greatly enlarged membrane surface. The polyene chain in these carotenoids could also provide an excellent mechanism to carry charge introduced by radical species from the lipid to the aqueous milieu – such a mechanism has been postulated to reduce oxidative reactions inside the membrane (74).

The localization of carotenoids in the microstructure of retinal cells begs further characterization. The high concentration of lutein in subretinal fluid in subjects with rhegmatogenous retinal detachment, interpreted as evidence that lutein is transported from the blood into the retina (30), could rather be interpreted as evidence of lutein arising from ruptured photoreceptor outer segment membranes. As noted above, the presence of lutein and zeaxanthin in rod outer segments has been firmly established (34,35), and the hypothesis of their antioxidant role is generally accepted, based on the high concentration of polyunsaturated fatty acids in rod outer segment membranes and their risk of oxidative damage in this photochemically active environment. Yet the precise location, function, and interactions of lutein and zeaxanthin with other antioxidants remain to be elucidated. Human cone outer segment membranes have not been studied separately, and it is unknown whether similarly high concentrations of lutein and zeaxanthin are found there. However, residual retinal membrane, that is, all membranes combined after isolation of the rod outer segment membranes, contain approximately half the lutein and one third the zeaxanthin concentration of rod outer segment membranes, suggesting that cone outer segments may also contain these two xanthophylls in high concentration.

There is also circumstantial functional evidence for a role of lutein and zeaxanthin in photoreceptor function. The finding that increased lutein in the diet in age-related macular degeneration (70) or lutein supplementation in retinitis pigmentosa (71) both improved visual acuity could possibly be attributed to increased filtering of short wavelength light by the macular pigment. A direct supportive role of the nutrients in the disease photoreceptors is more plausible, and, in addition, the enlargement of the central visual field in retinitis pigmentosa patients who received lutein (71) clearly suggests a biological mechanism outside the range of macular pigment.

As was noted earlier, in vitro studies of photoreceptor outer segments and liposomes have indicated improved antioxidant function and preservatin of lutein and zeaxanthin in the presence of \( \alpha \)-tocopherol, glutathione, ascorbate, and melanin (43). A protective role for \( \alpha \)-tocopherol has also been suggested for rat photoreceptors in vivo (75) and for frog photoreceptors in eye cup preparations (76). It is plausible that lutein and zeaxanthin could fulfill their antioxidant role in human photoreceptors in cascade with other antioxidants such as \( \alpha \)-tocopherol, ascorbate, and glutathione.

**FUTURE RESEARCH NEEDS**

The roles of lutein and zeaxanthin in the human eye are not completely understood, and the potential effects of a lack of lutein and zeaxanthin on human eye disease remain to be more firmly established. Further research is needed to elucidate whether lutein and zeaxanthin play a role in retinal cones as well as rods. Such work could include studies of the presence and structural localization of lutein and zeaxanthin in photoreceptor outer segments, for example, photoreceptors could be harvested from primates fed radio-labelled carotenoids and comparing the membrane structures from carotenoid-depleted and normally fed animals. Raman spectroscopy and other techniques should be used to study the molecular signatures of carotenoids in the membrane, and their absorption bands should be compared to those in solution to study shifts in energy level caused by the membrane environment. Further research is needed to determine whether lutein and zeaxanthin interact in a cascade with other antioxidants such as \( \alpha \)-tocopherol, ascorbate, and glutathione through nutritional supplementation and deprivation, and by modulating their concentrations in solution in tissue culture. Long-term synergistic protection of antioxidant combinations could be studied in animal models with appropriate dietary modifications and high intensity light exposure, followed by histological study.

Once the effects of low lutein and zeaxanthin concentrations on the photoreceptors are better characterized, careful epidemiological studies of visual function and clinicopathological studies of retinal carotenoids in populations with a history of low lutein and zeaxanthin status may help to fulfill the third criterion for conditional essentiality: that plasma concentrations of lutein and zeaxanthin return to normal and there is no appearance of chemical, structural, or functional abnormalities in the retina with dietary supplementation of lutein and zeaxanthin (6,7). Controlled clinical trials will be needed to establish whether lutein and zeaxanthin supplementation can prevent the early stages or progression of age-related macular degeneration and other photoreceptor diseases (77).

**CONCLUSIONS**

The macula contains the highest concentrations of lutein and zeaxanthin found in the human body, and
existing data support the role of lutein and zeaxanthin in the reduction of photo-oxidative stress in the retina. In vitro studies suggest that they carry out this role in cascade with α-tocopherol, ascorbic acid, and other antioxidants, and a possible interactive role in photoreceptor outer segments may be underappreciated. Epidemiological and clinical studies suggest that low dietary intake or plasma concentrations of lutein and zeaxanthin are associated with age-related macular degeneration. Studies in primates show that dietary deprivation of lutein and zeaxanthin results in structural abnormalities in the retina. Should future controlled clinical trials show that improvement of lutein and zeaxanthin reduces the progression of age-related macular degeneration, then lutein and zeaxanthin will fulfill the criteria to be considered as conditionally essential nutrients for humans.

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