Transport and Retinal Capture of Lutein and Zeaxanthin with Reference to Age-related Macular Degeneration

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Abstract. Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the elderly population in the western world. The etiology and pathogenesis of this disease remain unclear. However, there is an increasing body of evidence supporting the hypothesis that the macular pigment carotenoids, lutein and zeaxanthin, play an important role in protection against AMD, by filtering out blue light at a pre-receptoral level, or by quenching free radicals. Lutein and zeaxanthin are dietary xanthophyll carotenoids, which are delivered to the retina via plasma lipoproteins. The biological mechanisms governing retinal capture and accumulation of lutein and zeaxanthin, to the exclusion of other carotenoids, are still poorly understood. Although these mechanisms remain unclear, it is possible that selective capture of these carotenoids is related to lipoprotein, or apolipoprotein, function and profile. Xanthophyll-binding proteins appear to play an important role in the retinal capture of the xanthophyll carotenoids. The Pi isoform of GSTP1 has been isolated as a specific binding protein for zeaxanthin. The binding protein responsible for retinal uptake of lutein remains elusive. This article reviews the literature germane to the mechanisms involved in the capture, accumulation and stabilization of lutein and zeaxanthin by the retina, and the processes involved in their transport in serum. (Surv Ophthalmol 53:68–81, 2008. © 2008 Elsevier Inc. All rights reserved.)

Key words. absorption • age-related macular degeneration • apolipoprotein E • lipoproteins • lutein • macular pigment • transport • xanthophyll-binding proteins • zeaxanthin

The central retina, known as the macula, is responsible for central and color vision because of its high concentration of cone photoreceptors. The macula is characterized by a yellow color, attributable to the presence of macular pigment. 81 Macular pigment (MP) is composed of lutein and zeaxanthin, two hydroxycarotenoids, which are entirely of dietary origin. 17

The concentration of MP peaks at the center of the macula, known as the fovea, where zeaxanthin is the dominant carotenoid. In contrast, lutein dominates in the parafoveal region. 16, 82 Dietary sources
of the carotenoids that make up MP include the yolk of eggs, and many fruits and vegetables, particularly maize, kiwis, spinach, and orange peppers.43

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in people over 50 years of age in the developed world.21 The pathogenesis of AMD is incompletely understood, however, there is a growing body of evidence to suggest that oxidative stress plays a role. At the macula, MP filters out blue light at a pre-receptoral level81 and quenches free radicals.44 These actions are consistent with the hypothesis that MP protects against AMD.

The Putative Protective Effect of Macular Pigment Against AMD

MP peaks at the center of the fovea, with optically negligible levels outside the macula.38,81,82 Within the layer structure of the retina, the highest concentration of MP is seen in the receptor axon layer and the inner plexiform layer.81

The peak absorbance spectrum of MP is at 460 nm, and MP therefore filters out damaging blue light at a pre-receptoral level. It has been estimated that MP absorbs approximately 40% of blue light before it is incident on the photoreceptors.81 This is a particularly important function, as exposure to high-energy wavelengths can result in photochemical retinal injury, as demonstrated by Ham et al in 1976.36 They exposed Rhesus monkey retinas to blue light for 1,000 seconds, which resulted in damage to the photoreceptor outer segments, cellular proliferation, and hypo-pigmentation of the retinal pigment epithelium (RPE). They found that the threshold for such retinal damage was lowest for blue light when compared with other wavelengths of the visible spectrum.

The peak concentration of MP at the center of the fovea is also consistent with its role as an optical filter. For example, it has been demonstrated that short wavelength cones (s cones) suffer a loss in sensitivity with increasing age.95 However, it has also been observed that this loss in sensitivity is less severe at the fovea, where MP peaks,35 suggesting a protective effect of the pigment.

MP also displays antioxidant properties, including the ability to quench singlet oxygen50 and inhibit the peroxidation of membrane phospholipids.55 Khachik et al reported the presence of direct oxidation products of lutein and zeaxanthin in the retina, thereby confirming the antioxidant activity of these carotenoids in the eye.44 The location of MP in the retina is of considerable importance, as it must be close to the site of high reactive oxygen intermediate (ROI) production, or to the tissues susceptible to oxidative damage, if it is to fulfil its role as an antioxidant. The photoreceptor outer segments contain chromophores, which act as photosensitisers, and therefore represent a site which is vulnerable to photo-oxidative damage. MP peaks at the fovea, where the density of cone photoreceptors is greatest, thus making its location suitable for quenching ROIs produced through irradiation of these chromophores.6

Thomson et al investigated the possible protective role of retinal zeaxanthin by manipulating the dietary intake of quail, and comparing the levels of light-induced photoreceptor death in supplemented versus non-supplemented birds. The number of apoptotic photoreceptor cells was inversely related to the concentration of retinal zeaxanthin, thus providing evidence that photoreceptors can be protected against photo-oxidative damage by increasing MP. The same study also reported that female quail, which have significantly higher levels of retinal zeaxanthin, exhibited less photoreceptor damage than did males.88

Dietary manipulation has also shown to have an effect on the RPE of primates. For example, dietary deficiency of vitamin E or n-3 fatty acids in monkeys has been shown to be associated with disruption of RPE cell integrity.39,59 Changes in the function of the RPE may lead to photoreceptor cell death and visual loss. Leung et al measured the RPE cell density in normal Rhesus monkeys and found that it peaks at the center of the fovea and declines gradually with increasing eccentricity.54 They examined the effects of age on the RPE cell density in a group of Rhesus monkeys, and found an increase in the RPE cell density with age. They also examined the effects of dietary supplementation with lutein or zeaxanthin on the RPE cell density in this group of monkeys. The effects of lutein or zeaxanthin supplementation were more complex, due to an interaction between xanthophyll status and fatty acid status. The small numbers of animals studied also made it difficult to determine whether xanthophylls alone can modulate RPE cell numbers in the central retina, or whether this effect is influenced to a greater extent by fatty acid status. However, they suggest that xanthophyll supplementation, for as little as 6 months, may stimulate migration of RPE cells towards the foveal region, which in turn would alter RPE profile. This suggests a role for MP in the regulation of foveal RPE cell density and distribution, factors that are important for photoreceptor integrity.

Several studies in humans have demonstrated the beneficial effects of xanthophylls in preventing the onset and progression of AMD. Gale et al examined the relationship between serum concentrations of...
Absorption and Transport of Lutein and Zeaxanthin

ABSORPTION

Absorption may be defined as the movement of a substance to the lymphatic or portal circulation from the gastrointestinal system. Several processes are required for optimal absorption of carotenoids. These include adequate digestion of the food matrix in order to release the carotenoids, formation of lipid micelles in the small intestine, uptake of carotenoids by intestinal mucosal cells, and transport of carotenoids to the lymphatic or portal circulation.

The release of carotenoids from digestion varies according to the form in which it is ingested. For example, β-carotene is released to a greater degree (up to 50% absorption) when present in oil dispersions, aqueous solutions or antioxidant-protected commercial beadlets, whereas the absorption of β-carotene may be as low as 2% from raw uncooked vegetables. In nature, carotenoids are found in protein complexes, which are thought to have inhibitory effects on their digestion, thus accounting for the improved absorption of lycopene from tomato juice after heating, as this helps to release lycopene from the protein complexes.

Dietary fat stimulates bile flow from the gall bladder, the primary function of which is the emulsification of fat and fat-soluble vitamins into lipid micelles within the small intestine. Without micelle formation, carotenoids are poorly absorbed. Consequently, a carotenoid-sufficient but fat-deficient diet can result in substantially reduced carotenoid absorption. The simultaneous appearance of β-carotene and newly absorbed fat in lymph following a meal suggests that carotenoids are transported from micelle to plasma membrane or cytoplasm with fatty acids. Of note, antagonism can be demonstrated when different carotenoids are concurrently given, suggesting competition for uptake by mucosal cells arising from the similarity in structure of different carotenoids.

Recently, Reboul et al examined the transport of lutein using Caco-2 TC-7 monolayers as an in vitro model for human intestinal epithelium, in an attempt to characterize the transport of lutein across human enterocytes. This in vitro model has previously been used by other investigators to evaluate the intestinal absorption of other carotenoids, and it has been shown to correlate well with in vivo results. Several interesting findings arose from these experiments: firstly, the rate of lutein uptake was saturable under physiological conditions; secondly, this rate of uptake was significantly impaired at 4°C; and thirdly, the rate of absorption was slower from the basolateral side of the cell monolayer to the apical side than in the opposite direction. These three findings support the argument in favor of a facilitated, protein-mediated, transport mechanism for lutein across the human enterocyte. This hypothesis is further supported by the findings of human studies in which the absorption of lutein is affected by β-carotene. However, the strongest evidence supporting the hypothesis of a protein-mediated absorption mechanism for lutein came from their use of an antibody raised against scavenger receptor class B type I (SR-BI) and from the use of a specific chemical inhibitor of SR-BI. Other investigators have suggested that SR-BI may be involved in the absorption of lipids in an indiscriminate manner and, in so doing, it may mediate the uptake of
compounds such as carotenoids, which are lipophilic. SR-BI is preferentially located at the apical side of cells, and Reboul et al found that the transport of lutein across the Caco-2 TC-7 monolayer was significantly reduced when cells were treated with the antibody, or the specific chemical inhibitor, of SR-BI. These findings, coupled with the fact that the transport of lutein occurred preferentially from the apical side of the cellular monolayer, support the view that SR-BI or other proteins mediate the intestinal absorption of lutein. Interestingly, they were not able to fully inhibit the absorption of lutein, suggesting that passive diffusion of lutein or other transport proteins is involved.

Carotenoids enter the circulation via the lymphatic duct as a component of chylomicrons. Following uptake by the liver, carotenoids are re-secreted on lipoproteins, which are believed to deliver carotenoids to various tissues.

Transport of Lutein and Zeaxanthin

LIPOPROTEINS

Circulating lipoproteins consist of a complex of triglycerides, phospholipids and cholesterol, and one or more specific proteins, referred to as apolipoproteins. The association of lipoproteins with high affinity receptors on cell surfaces regulates lipid metabolism and transport in the body. Lipoproteins are classified into the following six groups: chylomicrons; chylomicron remnants; very low-density lipoproteins (VLDL); intermediate-density lipoproteins (IDL); low-density lipoproteins (LDL); and high-density lipoproteins (HDL).

Chylomicrons are synthesized by the intestine and deliver dietary triglycerides to muscle and adipose tissue, and dietary cholesterol to the liver. Lipoprotein lipase, located at capillary endothelial cell surfaces, hydrolyzes the triglyceride core of the chylomicron, thus liberating fatty acids and glycerol, which are used as energy sources by various cells, or are taken up by adipocytes and stored as triglycerides. Chylomicron remnants, which are rich in cholesterol, result from chylomicron metabolism, and are rapidly cleared by the liver.

Subsequently, the liver synthesizes a second class of triglyceride-rich lipoprotein, referred to as VLDL, which upon secretion functions as a transporter of lipids and cholesterol. In the bloodstream, VLDL undergoes progressive removal of triglycerides from its core by lipoprotein lipase, in a similar way to chylomicrons. The VLDL particles thus become increasingly smaller, leading to the formation of IDL and LDL. LDL are the final metabolic products of VLDL and are responsible for most of the cholesterol transport in serum.

HDL are the smallest lipoproteins, arising from several sources including the intestine and liver. HDL are involved in a process known as “reverse cholesterol transport,” whereby HDL acquire cholesterol from cells and deliver it to the liver. This is a particularly important mechanism in humans, as the quantities of cholesterol transported out of the gut and liver far exceed the quantities converted to steroid hormones, or those lost through the skin in sebum. Thus, unless the requirement for cell membrane repair or synthesis is high, excess cholesterol must be returned to the liver for excretion.

ASSOCIATION OF CAROTENOIDs WITH PLASMA LIPOPROTEINS

The majority of plasma carotenoids are transported on LDL, with 55% of total carotenoids associated with this lipoprotein, whereas HDL is associated with 33%, and VLDL is associated with 10–19%, of the total carotenoids.

However, hydroxycarotenoids, including lutein and zeaxanthin, are relatively equally distributed between LDL and HDL, with a progressive decrease in the content of lutein and zeaxanthin from light to dense LDL. This finding has prompted the suggestion that an individual’s lipoprotein, and apolipoprotein, profile may influence the transport and delivery of these carotenoids to the retina, with a consequential impact on MP.

MP is inversely related to percentage body fat. Interestingly, Viroonudomphol et al have demonstrated lower levels of HDL in overweight and obese subjects, consistent with the possibility that a relative lack of HDL may impair transport and/or retinal capture of the carotenoids. Of note, Seddon and co-workers have demonstrated a significantly increased risk of AMD in association with obesity.

However, it should be emphasized that there is a notable paucity of data on the mechanism(s) whereby lutein and zeaxanthin accumulate in the liver, are repackaged into lipoproteins, and transported via the circulatory system to specific target tissues such as the retina.

APOLIPOPROTEINS

Plasma lipoproteins include one or more protein constituents, known as apolipoproteins. Apolipoproteins have been classified into several subgroups, including apolipoprotein A (ApoA), apolipoprotein B (ApoB), apolipoprotein C (ApoC), and apolipoprotein E (ApoE). These subgroups are themselves further sub-classified, for example: ApoAI, ApoA-II, and so on. Each lipoprotein class is associated with certain apolipoproteins, for example: chylomicrons...
and VLDL are associated with ApoB; chylomicrons, VLDL and HDL are associated with ApoE. The primary role of apolipoproteins is the transport and redistribution of lipids amongst various tissues in the body. Specific apolipoproteins are recognised by cell surface receptors, and this facilitates the high affinity binding required for delivery to target tissues. Certain apolipoproteins also act as cofactors of enzymes involved in lipoprotein metabolic pathways, including those of lipoprotein lipase and lecithin-cholesterol acyl transferase (LCAT), which catalyze the formation of cholesterol esters. Another role of specific apolipoproteins is the maintenance of the structure of lipoproteins, by stabilizing the micellar structure of lipoproteins, and by providing a hydrophilic surface in association with phospholipids.

The function of apolipoproteins has provoked interest in their possible role in a range of degenerative conditions. In particular, several investigators have suggested an association between ApoE and various diseases, including Alzheimer disease (AD), atherosclerosis, and AMD. Abalain et al investigated the association between AMD and serum levels of lipoproteins and lipoparticles. They found that there was no difference in serum ApoA-I and ApoB levels between AMD patients and controls. However, they found that serum ApoE levels were higher, and that serum ApoC-III levels were lower, in AMD patients compared with controls. The higher level of serum ApoE in AMD patients is in agreement with the findings of Boerwinkle and Utermann, who found that the Apo ε4 allele is associated with lower serum ApoE levels, and that the Apo ε2 allele is associated with higher serum levels of ApoE. ApoC-III interferes with lipoprotein metabolism and, when associated with ApoB as a lipoparticle, it has been shown to be involved in atherogenesis. Abalain et al found no difference in the levels of this particular lipoparticle between AMD patients and controls. The evidence to date suggests that, of the apolipoproteins, ApoE has the strongest association with AMD.

**APOLIPOPROTEIN E**

ApoE is a structural component of plasma chylomicrons, VLDL, and a subclass of HDL. It is a 299 amino-acid protein, and is synthesized in a large number of tissues including spleen, kidneys, lungs, adrenal glands, liver, brain, and retinal Müller cells. ApoE is polymorphic, with three common isoforms: E2, E3 and E4, which are coded for by three separate alleles: ε2, ε3 and ε4. These alleles are differentiated on the basis of the number of cysteine-arginine residue interchanges at sites 112 and 158 in the amino acid sequence. As a result of this polymorphism, six common phenotypes exist: three homozygous phenotypes (ε3/ε3, ε2/ε2, ε4/ε4) and three heterozygous phenotypes (ε2/ε3, ε2/ε4, ε3/ε4).

ApoE is crucial to many processes, including: cholesterol transport and metabolism, receptor-mediated uptake of specific lipoproteins, heparin binding, formation of cholesteryl-ester-rich particles, lipolytic processing of type IIIb-VLDL, inhibition of mitogenic stimulation of lymphocytes, and transport of lipids within the brain.

**Cholesterol Transport and Metabolism**

ApoE is an important regulator of cholesterol metabolism because of its affinity for ApoE-specific receptors in the liver, and its affinity for LDL receptors in the liver and other peripheral tissues requiring cholesterol. ApoE-specific receptors are present on the membranes of hepatic parenchymal cells, and have a high binding affinity for chylomicron remnants, IDL and a sub-class of HDL. ApoE also regulates the activity of several lipid-metabolising enzymes, including lipoprotein lipase, and LCAT.

ApoE is found in greatest concentrations in the liver. However, it is also the dominant apolipoprotein in the brain, and is responsible for lipid transport and cholesterol regulation within the central nervous system (CNS). ApoE is a major component of plasma and cerebrospinal fluid, and plays a fundamental role after CNS injury.

ApoE polymorphisms result in differences in the metabolism of ApoE-containing lipoprotein particles. For example, it is possible that certain ApoE polymorphisms affect their ability to interact with lipoprotein lipase in the conversion of VLDL to LDL. Indeed, ApoE polymorphism influences plasma lipid levels both in sedentary states and in their response to exercise, and it is therefore believed to be related to risk for coronary artery disease. In general, carriers of the ε4 allele have higher levels of total cholesterol and LDL-cholesterol than those with the ε3 allele. ApoE polymorphism also appears to play a role in the responsiveness of blood lipids to dietary and lipid-lowering drug interventions. Thus, the ApoE gene-environmental interactions contribute to population variance in blood lipid-lipoprotein levels.

ApoE receptors also play an important role in lipoprotein metabolism. This is seen in conditions such as familial hypercholesterolaemia, which is caused by dysfunctional receptors, arising from mutations in LDL receptors. This disorder is characterised by an accumulation of LDL in the circulation resulting in a longer exposure to oxidative processes, with consequential modification of LDL to atherogenic lipoproteins.
**ApoE and the Retina**

The primary physiological role of ApoE is to facilitate the binding of lipoproteins to LDL receptors, thereby regulating the uptake of cholesterol required by the cell. For instance, large amounts of lipids are released from degenerating cell membranes after nerve cell loss, thus stimulating astrocytes to synthesise ApoE, which binds these excess lipids and distributes them appropriately for reuse in cell membrane biosynthesis. This observation prompted Klaver et al to speculate that a high degree of ApoE biosynthesis is required to support the high rate of photoreceptor renewal in the macular region. Indeed, it has been demonstrated that mice, which were fed a high-fat diet, or which were deficient in ApoE, exhibit an increase in the thickness of Bruch’s membrane, which is seen in association with ageing and with AMD.

Ishida et al identified the presence of ApoE and lipids at the inner aspect of the RPE, and proposed that both compounds may be secreted by the RPE. The role of ApoE in reverse cholesterol transport prompted the authors to suggest that this apolipoprotein may also facilitate the efflux of lipids from the RPE into the adjacent Bruch’s membrane, and they proposed a possible pathway for RPE cell-secreted lipids to cross Bruch’s membrane, where partially digested or undigested photoreceptor outer segments are secreted across the basal surface in association with ApoE. Subsequent binding with HDL at Bruch’s membrane may then facilitate desorption of the lipid particles into circulation.

In the retina ApoE is synthesised in Müller cells and in the RPE, and the presence of ApoE has been demonstrated in drusen. It has been suggested, therefore, that age or disease-related disruption of normal ApoE function may result in the accumulation of lipoproteins at the interface between the RPE and Bruch’s membrane, consistent with observations that lipid deposits in drusen are largely composed of cholesteryl esters and unsaturated fatty acids.

These findings are consistent with the view that ApoE plays an important physiological role in the maintenance of macular health, and that an impaired ApoE system may affect the functional integrity of Bruch’s membrane. Furthermore, there is a biologically plausible rationale whereby the ApoE profile might influence the transport, capture, and stabilization of key compounds, such as lutein and zeaxanthin, at the macula.

**Apo ε4 Allele Status and AMD**

ApoE has been shown to be a component of soft drusen, and the ε4 allele is therefore an intuitive candidate gene for AMD genetic studies. For example, a decline in permeability of Bruch’s membrane has been demonstrated in association with ageing and with AMD, and this decline in permeability is believed to result from the accumulation of lipids and other debris within Bruch’s membrane. Due to the lack of cysteine residues at positions 112 and 158, preventing the formation of disulphide bridges with ApoA-II or other peptide components, the ε4 allele has an inability to form dimers. It has been suggested that this inability of the ε4 allele to form dimers, when compared with the ε2 and ε3 alleles, favors easier transport of lipids through Bruch’s membrane because of a smaller size of lipid particles, thus protecting against a loss of permeability of Bruch’s membrane.

In addition, the hydrophobic nature of the age-related thickening of Bruch’s membrane has been implicated in the aetiopathogenesis of AMD. It is noteworthy that ApoE4 presents a positive charge relative to both ApoE2 and ApoE3. ApoE4 possesses arginine at residue 112 of the amino acid sequence, whereas ApoE3 possesses cysteine at this position, and in the case of ApoE2, the most frequent variant has cysteine instead of the normally occurring arginine at residue 158. Thus, ApoE3 presents a neutral charge, and ApoE2 a negative charge, relative to ApoE4. Souied et al suggested that this difference in charges between the ApoE isoforms may also contribute to differences in the clearance of debris through Bruch’s membrane.

It appears that Müller cells are the most prominent biosynthetic sources of ApoE in the neural retina, and RPE cells are the most prominent sources in the RPE/choroid. However, it remains unclear whether the concentration of ApoE in the cytoplasm of some RPE cells, especially those in close proximity to drusen, is the result of biosynthesis or selective accumulation. It has been shown that, in both the central and peripheral nervous systems, ApoE expression by astrocytes is up-regulated in response to neuronal injury and neuro-degenerative disease. Indeed, there is evidence for ApoE up-regulation by Müller cells in degenerating human retina, where increased ApoE immuno-reactivity is found in the sub-retinal space of detached retinas and in the Müller cells of retinas affected by glaucoma or AMD. Furthermore, the relatively high levels of ApoE mRNA detected in the retina, especially in the eyes of older donors and in an individual with documented AMD, support the view that up-regulation by retinal glia may be responsible for the observed increase in ApoE expression. In the same way, it is possible that the neurosensory retina and the RPE respond to conditions of high oxidative injury by up-regulation...
of ApoE synthesis or accumulation, with implications for selective capture and stabilisation of lutein and zeaxanthin in the retina. In other words, there is a biologically plausible rationale why ApoE polymorphism might affect age-related morphological and functional changes in the retina, especially in Bruch’s membrane.

Several studies have investigated the possible association between ε4 allele status and AMD. Recently, Schmidt et al investigated the combined effects of smoking and Apo ε genotype in AMD. They studied these effects in a group of 377 patients with early- and late-AMD, and 198 age- and ethnically matched controls. The main effects of Apo ε genotype did not reach statistical significance in the overall analysis, however, they did show a trend towards a protective effect of Apo ε4 compared to ε2 or ε3. A study by Zareparsi et al in 2004 demonstrated a significant association between the ε4 allele and AMD in a large cohort of subjects, where this allele was found to be protective against AMD. This particular study examined the ε4 allelic distribution in sporadic and familial cases of AMD (632 affected patients versus 206 control subjects). The frequency of the ε4 allele was found to be significantly lower in subjects with AMD. Furthermore, another recent study has reported a protective role for the ε4 allele, and also identified that the ε2 allele is associated with earlier onset of AMD. This study also demonstrated that the protective effect of the ε4 allele was greatest in men, and in subjects with the atrophic form of this condition. Schmidt and co-workers found that the ε4 allele was associated with a reduced risk of AMD, and also suggested that the putative increased risk for the condition seen in subjects with the ε2 allele may be restricted to males only. Klaver et al also found that the ε4 allele was protective for AMD, whereas Schultz found no such protective effect in sporadic cases. However, Schultz observed a trend for a decreased risk of AMD in association with the ε4 allele in a set of unrelated patients with a family history of the condition. Two studies undertaken to investigate the putative protective effect of the ε4 allele for AMD in non-white subjects failed to demonstrate any such protective effect.

Table 1 provides a summary of the studies investigating the association between Apo ε allele status and the risk for AMD in humans.

### Table 1

<table>
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<th>AMD Association</th>
<th>Author, Year</th>
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<tr>
<td>A trend towards a protective effect of the ε4 allele was demonstrated. This protective effect modified the highly significant association between cigarette smoking and AMD.</td>
<td>Schmidt et al, 2005</td>
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<tr>
<td>A significant association between the ε4 allele and a reduced risk of AMD was observed.</td>
<td>Zareparsi et al, 2004</td>
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<tr>
<td>A protective role of the ε4 allele was observed for AMD, whereas the ε2 allele was associated with earlier onset of the disease.</td>
<td>Baird et al, 2004</td>
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<td>A trend for a decreased risk of AMD in association with the ε4 allele was observed in a set of unrelated patients with a family history of AMD.</td>
<td>Schultz et al, 2003</td>
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<tr>
<td>The ε4 allele, or an allele in linkage disequilibrium with it, was associated with reduced risk of AMD. The authors also suggested that the effects of the ε2 allele may confer an increased risk to males only.</td>
<td>Schmidt et al, 2002</td>
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<td>A lower frequency of the ε4 allele was found in the AMD subjects when compared with the general population, whereas the frequency of the ε2 allele was higher in AMD subjects versus controls.</td>
<td>Simonelli et al, 2001</td>
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<td>A significantly lower frequency of the ε4 allele was observed in subjects with the neovascular form of AMD when compared with control subjects. The ε4 allele was associated with a significantly reduced risk of AMD, whereas the ε2 allele was associated with a slightly increased risk for this condition. No association between the ε4 allele and AMD in Chinese subjects.</td>
<td>Souied et al, 1998</td>
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<td>Japanese subjects with AMD had a lower frequency of the ε2 and ε4 alleles when compared with controls subjects, but Gotoh et al, 2004 the differences did not reach statistical significance.</td>
<td>Klaver et al, 1998</td>
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<td>Asian subjects with AMD had a lower frequency of the ε2 and ε4 alleles when compared with controls subjects, but Pang et al, 2000 the differences did not reach statistical significance.</td>
<td>Simonelli et al, 2001</td>
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ApoE Polymorphism and the Transport of Lutein and Zeaxanthin

The most common ApoE is the E3 isoform, whereas ApoE2 and ApoE4 arise from a mutational variation of a single amino acid located within the receptor-binding region of the protein, with consequentially altered receptor binding properties. ApoE2 has a much lower, and ApoE4 a much higher, receptor binding affinity than ApoE3. Selective binding of certain receptors within the CNS to HDL particles enriched with ApoE, and
a lack of binding of these receptors to HDL particles deficient in ApoE, has been demonstrated. Should this selectivity of the uptake mechanism be dependent on the ApoE polymorphism of the transporting lipoproteins, and given that the ε4 allele is putatively protective for AMD, it is tempting to hypothesise that retinal capture of lutein and zeaxanthin may be related to apolipoprotein profile. In other words, the apolipoprotein composition as well as the lipoprotein profile may play an important role in the transport and delivery of lutein and zeaxanthin, and their subsequent accumulation and stabilization within the retina. We have referred to the growing body of evidence suggesting that the ε4 allele protects against AMD, reflected in the significantly reduced prevalence of this allele among sufferers of AMD. It is possible, therefore, that the putative protective effect of the ε4 allele against AMD is attributable, at least in part, to the role its phenotypic expression (ApoE4) plays in the transport and delivery of the macular carotenoids to the retina, and to their stabilization within the retina.

A direct relationship has yet to be shown between MP and apolipoprotein profile. The results of such a study would provide further information regarding the possible role apolipoproteins play in the delivery and accumulation of the retinal carotenoids. Because the ε4 allele is putatively protective against AMD, a study investigating the relationship between ε4 allele status and the response to lutein or zeaxanthin supplementation is merited.

Accumulation of Lutein and Zeaxanthin in the Retina

Of the approximately 600 known carotenoids, only 34 dietary carotenoids (including 13 geometrical isomers) and 8 of their metabolites have been identified in human serum. It is assumed that the gastrointestinal absorption of these carotenoids, and their uptake by tissues, is governed by specific mechanisms. For instance, supplementation studies have demonstrated that the accumulation of lutein and zeaxanthin at the macula, to the exclusion of other carotenoids, indicates a high degree of selectivity. However, the discovery of xanthophyll-binding proteins (XBP) has led to a greater understanding of the mechanisms involved in the capture and stabilization of these carotenoids at the macula.

Effect of Lutein and Zeaxanthin Supplementation on the Retina

Within the macula, zeaxanthin comprises a mixture of dietary (3R,3’S-meso)-zeaxanthin and non-dietary (3R,3’R)-zeaxanthin. Meso-zeaxanthin is thought to result from a series of oxidation-reduction and double-bond isomerization reactions of dietary (3R,3’S-meso)-lutein, and is not found in the diet, serum, liver or adipose tissue, suggesting that this xanthophyll is produced, either enzymatically or photochemically, within retinal tissue. Therefore, idiosyncratic differences in the conversion of lutein to meso-zeaxanthin within the retina may account for individual variability in response to lutein supplementation, as zeaxanthin represents the dominant carotenoid at the fovea where MP peaks.

Recent reports of nutritional manipulation on animal models provide strong evidence that supplementary lutein and zeaxanthin results in augmentation of MP, and is protective against oxidative stress. Studies by Neuringer et al and Johnson et al using Rhesus monkeys found that the relationship between dietary intake of zeaxanthin and retinal concentration of this carotenoid was more variable than the relationship between dietary intake of lutein and retinal concentration of lutein. This group of Rhesus monkeys, reared on a xanthophyll-free diet since birth, had no detectable levels of lutein or zeaxanthin in serum, retina, liver, or adipose tissue. However, upon supplementation with these carotenoids, xanthophyll concentrations rose rapidly within the first four weeks in all of these tissues. Furthermore, animals on both lutein-fortified and zeaxanthin-fortified diets had significantly more MP (2.97 ± 0.39 and 2.58 ± 0.27, respectively) than those on a regular, unfortified diet (0.18 ± 0.12). Despite consuming eight times less lutein and zeaxanthin, the standard stock-fed monkeys had similar amounts of total retinal zeaxanthin compared to the animals supplemented with either lutein or zeaxanthin, suggesting that only a limited number of sites are available for the accumulation of zeaxanthin. In contrast, however, dietary intake of lutein was reflected in the retina, where an increase in lutein through supplementation resulted in a subsequent and parallel rise in retinal lutein.

The ability of the monkey retina to capture lutein and zeaxanthin, even after lifelong denial of these dietary carotenoids, suggests that late intervention through supplementation could result in MP augmentation in humans. Using the same group of xanthophyll-free animals, Leung et al investigated the effect of age and dietary manipulation on the cell density of the RPE. These Rhesus monkeys were supplemented with pure lutein or zeaxanthin, but were fed a diet with either low or adequate amounts of n-3 fatty acids. After a prolonged period of such supplementation (between 6 and 24 months), a change in the peak symmetry of RPE cell density was observed. The lutein or zeaxanthin
supplemented animals on a low n-3 fatty acid diet had a lower RPE cell density than unsupplemented animals on the same low n-3 fatty acid diet. This change in peak symmetries, after supplementation with either lutein or zeaxanthin, prompted the authors to suggest that macular xanthophylls could stimulate the movement of RPE cells away from the central retina, which is seen as an important part of shaping and maintaining the RPE. This migration may arise from an altered light distribution caused by the increase in concentration of macular xanthophylls in the region, or alternatively, in response to a change in cell metabolite distribution due to xanthophyll occupation.

There has been a paucity of lutein or zeaxanthin supplementation studies in human subjects to date, in whom these effects have been investigated with respect to MP optical density. In 1997 Hammond et al showed that dietary modification, for as little as four weeks, could augment MP, with this effect being maintained for several months following resumption of a normal, unmodified diet. Of note, two of the 11 subjects involved in this study did not show a significant rise in MP optical density despite a significant increase in serum lutein. These subjects were termed “retinal non-responders” and this may be due to the fact that the retinal binding sites in these individuals were already saturated. Landrum et al investigated the effect of lutein supplementation in two individuals over a 140 day period. They found an increase in serum lutein levels in both individuals, coupled with a parallel increase in MP optical density. Interestingly, they also demonstrated maintenance of the interocular asymmetry of MP optical density in one of these individuals, suggesting that local retinal processes may play a role in the capture and stabilization of the xanthophyll carotenoids. Supplementation with 10 mg of lutein alone in a small number of subjects by Berendschot et al showed similar results, with MP optical density increasing by 4–5% after just 4 weeks of supplementation. Johnson et al investigated the effects of a diet modified with increased amounts of spinach and corn, representing a seven-fold increase in xanthophyll carotenoid intake, in a group of seven subjects over a period of 15 weeks. Once again, these investigators showed a significant increase in both serum and MP optical density levels after a period of just 4 weeks. However, both serum and MP optical density levels dipped after the first 4 weeks of supplementation and then remained on a plateau for the rest of the study period. Consistent with the findings of Hammond et al in 1997, this may represent a saturation of retinal binding sites, or a critical stage of redistribution of carotenoids amongst various body tissues. A pilot study by Koh et al also showed serum and MP optical density augmentation in a group of AMD sufferers, as well as control subjects (subjects without AMD), on a diet supplemented with 20 mg of lutein ester over an 18- to 20-week period. This study provided the first evidence that serum and MP optical density levels could be increased in an already diseased retina. Recent work by Rodriguez-Carmona et al in 24 healthy subjects demonstrated an increase in MP following supplementation with lutein and/or zeaxanthin. The subjects in this study were young males who received lutein, zeaxanthin, a combination of lutein and zeaxanthin, or placebo for 12 months. Each of the supplementation groups showed a significant rise in MP optical density.

XANTHOPHYLL-BINDING PROTEINS (XBP)

Toyoda et al investigated the effect of prolonged xanthophyll supplementation on a group of xanthophyll-free quail. They found that liver and adipose tissue captured lutein more efficiently than zeaxanthin, whereas the retina captured zeaxanthin more efficiently than lutein. This selectivity for lutein and zeaxanthin has prompted the suggestion that carrier proteins may play a specific role in the transport of these compounds, and their capture by the retina.

The carotenoid-binding proteins, or carotenoproteins, involved in binding and stabilization of carotenoids have been extensively characterized in invertebrates, plants, and micro-organisms. Examples include crustacyanin, an astaxanthin-binding protein responsible for the colour of lobster shells. However, limited knowledge exists regarding carotenoproteins in vertebrates.

Bernstein and co-workers investigated the biochemical mechanisms involved in the retinal capture of lutein and zeaxanthin, and suggested that it may be regulated by non-specific passive interactions, but also by a strong binding affinity with XBP. In 1997, Bernstein et al mixed soluble extracts of bovine retina with radioactive carotenoids, which were then purified by hydrophobic interaction, ion exchange, and gel filtration chromatography. Photo-affinity labelling and protein micro-sequencing revealed that tubulin was the major soluble carotenoid-binding protein. Furthermore, similar experiments on human macular tissue were performed, which also demonstrated that lutein and zeaxanthin were found to co-purify with tubulin. The investigators suggested that, as tubulin is found in abundance in the receptor-axon layer of the fovea, the binding interaction of carotenoids and this protein could explain the peak concentration of MP in
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Henle’s fibre layer, as previously described by Snodderly et al in 1991. More recently, Yemelyanov et al prepared a carotenoid-rich membrane fraction derived from human macula or peripheral retina by homogenisation, differential centrifugation, and detergent solubilisation. Then, further purification was achieved using ion exchange chromatography and gel filtration chromatography coupled with continuous photo-diode array monitoring for endogenously associated xanthophyll carotenoids. It was found that the most highly purified preparations contained two major protein bands that consistently co-eluted with endogenous lutein and zeaxanthin, and that the visible absorbance spectrum of the binding protein(s) preparation closely matched the spectral absorbance of MP. Furthermore, binding of exogenously added lutein and zeaxanthin was found to be saturable. Importantly, other carotenoids such as canthaxanthin and β-carotene exhibited no significant binding activity to solubilized retinal membrane proteins when assayed under identical conditions. The investigators also demonstrated only weak non-specific binding affinity for other known and potential mammalian XBP such as albumin, tubulin, lactoglobulin, and serum lipoproteins. Interestingly, the lipoproteins responsible for the transport of lutein and zeaxanthin in the serum, namely HDL and LDL, did not exhibit strong binding affinity for these carotenoids, suggesting that XBP, rather than the circulating lipoprotein profile, may determine MP accumulation. In other words, this important work provided the first direct evidence for the existence of specific XBP in the vertebrate retina and macula. These XBP are believed to have at least one of several physiological functions, including: mediation of the specific uptake of lutein and zeaxanthin from the bloodstream; stabilization of the highly insoluble carotenoids within the cell membrane, cytosol, or cytoskeleton; enzymatic roles mediating the interconversion of lutein, zeaxanthin and their various metabolites within the retina; facilitation of the antioxidant activity of the macular carotenoids.

In 2004, Bhosale et al purified and identified the Pi isoform of glutathione S-transferase (GSTP1) as a specific XBP in the human macula, with a high affinity for dietary (3R,3′R,6′R)-lutein, and, consistent with previous reports, it demonstrated no specific interaction with HDL, LDL, tubulin, albumin or β-lactoglobulin. Further work by these researchers examining the effects of GSTP1 has shown synergistic antioxidant effects of this binding protein on both dietary (3R,3′R)-zeaxanthin and non-dietary (3R,3′S-S-meso)-zeaxanthin in vitro. They suggest that this synergistic action results from the protection GSTP1 provides against irreversible oxidative degradation. Such a protective effect has previously been suggested to be due to stabilizing changes in the structure of meso-zeaxanthin when associated with specific binding proteins. Recently, it has been suggested that certain gene polymorphisms of glutathione S-transferase (GST) may be associated with the subsequent development of neovascular AMD. The precise mechanisms underlying this possible association require further research.

In an earlier study by Bernstein et al, carotenoids in ocular tissue were identified and quantified by comparing their chromatographic and spectral profiles with those of authentic standards. It was found that lutein and zeaxanthin were present in nearly all ocular tissues, with the exception of vitreous, cornea, and sclera. Uveal structures (iris, ciliary body, and RPE/choroid) account for approximately 50% of the eye’s total carotenoids, and 30% of the eye’s lutein and zeaxanthin. The presence of these carotenoids in the iris suggests a role in filtering out damaging short-wavelength visible light, whereas these carotenoids in the ciliary body are likely to have an antioxidant function only, and both mechanisms (light screening and antioxidation) may be operative in the RPE/choroid.

However, the RPE/choroid complex may represent an intermediate control and transfer point for lutein and zeaxanthin uptake by the neurosensory retina from circulating blood, as a similar transfer and control role for this tissue is well established for ocular retinoids. This hypothesis is supported by the observation that lutein and retinol are both found in substantial concentrations in sub-retinal fluid collected from humans with retinal detachments.

Beyond the mechanisms determining the retinal capture of lutein and zeaxanthin, MP may be determined by factors influencing the stabilization of its constituent carotenoids within the retina. For example, Siems et al investigated the degradation rates of lutein, zeaxanthin, lycopene, and β-carotene upon exposure to radical-initiated auto-oxidation in vitro. The free-radical generating sources they used were: azo-bis-isobutyronitrile (AIBN) as a source of a peroxyl radical initiator, hypochloric acid, UV light in the presence of Rose Bengal as...
a singlet oxygen generator, and natural sunlight exposure. Under each of these experimental conditions, they found that lycopene and β-carotene decompose more rapidly than either lutein or zeaxanthin. Of the two xanthophyll carotenoids, zeaxanthin decomposes more rapidly under these conditions. These observations prompted the investigators to suggest that the accumulation of lutein and zeaxanthin at the macula, to the exclusion of other carotenoids, may be attributable to this relative resistance to decomposition upon challenge with pro-oxidants.

It appears, therefore, that many factors may play a role in the retinal uptake of lutein and zeaxanthin, including the XBP profile, which is saturable. However, capture, accumulation, and stabilization of MP may also be subject to the oxidant/antioxidant balance in the retina, and the factors governing this balance.

**Conclusion**

Despite many recent advances in our understanding of the mechanisms involved in the absorption and metabolism of the xanthophyll carotenoids, there is still much to be discovered. Most of the research to date has focused on the metabolism of provitamin A carotenoids, particularly β-carotene. Nevertheless, it is likely that at least some of the metabolic pathways of the various carotenoids are either similar or shared, as evidenced by the competitive nature of the absorption of carotenoids from the gastrointestinal system and the use of the non-specific transporter protein, SR-BI, in the absorption process.

The transport of lutein and zeaxanthin in the serum seems to be dependent on the individual's lipoprotein, and apolipoprotein, profile. Current concepts would suggest a role for ApoE in this process, which seems to be in agreement with the finding that Apoε allele status has consequences for the risk of developing AMD, and is also consistent with the hypothesis that MP is protective against this condition.

Retinal capture of the xanthophyll carotenoids is mediated largely by XBP, in particular the Pi isofrom of GSTP1 in the case of zeaxanthin, as discovered by Bhosale et al. The specific XBP responsible for retinal uptake of lutein remains elusive. Interestingly, it has been found that the XBP system is saturable, which has implications for dietary supplementation with the xanthophyll carotenoids.

In order to fully explore the potential beneficial effects of lutein and zeaxanthin, in particular in the context of their putative role in the prevention of AMD, it is essential that we understand the mechanisms by which they are absorbed from the gastrointestinal system, transported in the serum, and taken up by the retina. The use of in vitro cell culture systems and the development of animal models for investigation of the multiple factors governing the metabolism of lutein and zeaxanthin will undoubtedly improve our understanding of these mechanisms in the future.

**Method of Literature Search**

References for this review were identified through a comprehensive English language literature search of the electronic Medline database (1966–2006) using the Pubmed search service. The following key words were used, alone or in combination, in compiling the search: age-related macular degeneration, age-related maculopathy, apolipoproteins, apolipoprotein E, carotenoids, carotenoid metabolism, lipoproteins, lutein, macular pigment, oxidative stress, retinal capture, transport of lutein and/or zeaxanthin and/or carotenoids, xanthophyll-binding proteins, zeaxanthin.

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