Lutein: More than just a filter for blue light

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Lutein: More than just a filter for blue light
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Abstract
Lutein is concentrated in the primate retina, where together with zeaxanthin it forms the macular pigment. Traditionally lutein is characterized by its blue light filtering and anti-oxidant properties. Eliminating lutein from the diet of experimental animals results in early degenerative signs in the retina while patients with an acquired condition of macular pigment loss (Macular Telangiectasia) show serious visual handicap indicating the importance of macular pigment. Whether lutein intake reduces the risk of age related macular degeneration (AMD) or cataract formation is currently a strong matter of debate and abundant research is carried out to unravel the biological properties of the lutein molecule. SR-B1 has recently been identified as a lutein binding protein in the retina and this same receptor plays a role in the selective uptake in the gut. In the blood lutein is transported via high-density lipoproteins (HDL). Genes controlling SR-B1 and HDL levels predispose to AMD which supports the involvement of cholesterol/lutein transport pathways. Apart from beneficial effects of lutein intake on various visual function tests, recent findings show that lutein can affect immune responses and inflammation. Lutein diminishes the expression of various ocular inflammation models including endotoxin induced uveitis, laser induced choroidal neovascularization, streptozotocin induced diabetes and experimental retinal ischemia and reperfusion. In vitro studies show that lutein suppresses NF kappα-B activation as well as the expression of iNOS and COX-2. Since AMD has features of a chronic low-grade systemic inflammatory response, attention to the exact role of lutein in this disease has shifted from a local effect in the eye towards a possible systemic anti-inflammatory function.
1. Introduction

The primate retina is unique by the fact that it contains a yellow coloured area located in its optical centre called the macula lutea (Fig. 1). This yellow colouration is caused by a local accumulation of pigments such as lutein, zeaxanthin and meso-zeaxanthin. Both lutein and zeaxanthin are common xanthophylls considered to play an important role in maintaining eye health. Besides the eye, the skin is also a site where xanthophylls may play a role in the protection against sun light (Roberts et al., 2009). Xanthophyll biosynthesis only takes place in plants, algae, bacteria and certain fungi. Humans cannot synthesize these xanthophylls and uptake is therefore dependent on the consumption of certain fruits, vegetables or animal products such as eggs (Calvo, 2005). In view of a possible role of xanthophylls in promoting eye health, including the prevention of age related macular degeneration, many individuals throughout the world are now taking supplements containing xanthophylls. This has resulted in a booming business now considered to represent a billion US dollar market and an explosion of studies pointing at providing scientific evidence for the health claims of these molecules (Fernandez-Sevilla et al., 2010). Although lutein and zeaxanthin have a large range of overlapping properties we will focus our discussion in this review on lutein and will extend the traditionally held blue filter and anti-oxidant properties of this molecule to newer insights dealing with its direct role in controlling inflammation. The effect of lutein on inflammation is moreover relevant since evidence is accumulating that systemic inflammation, as shown by the presence of circulating complement activation products, is involved in the pathogenesis of AMD (Hecker et al., 2010; Scholl et al., 2008; Smallhodzic et al., 2012). Furthermore the group of Nussenblatt from the NIH have recently shown higher levels of the TH17 related interleukins IL-17 and IL-22 in the circulation of AMD patients (Liu et al., 2011). They also showed that one of the complement activation products, C5a was able to stimulate monocytes to induce TH17 cell activation, thereby providing a link between the innate and the adaptive immune response in the pathogenesis of AMD.

2. Lutein structure

The structure of lutein can be described as a long carbon chain with alternating single and double carbon—carbon bonds with attached methyl side groups. At both ends of the carbon backbone the molecule contains a cyclic hexenyl structure with an attached hydroxyl group. They belong to a group of carotenoids whereby the differences in structure between lutein and zeaxanthin is due to the position of the double bonds in the hexenyl ring and the position of methyl groups on the long carbon chain (Fig. 2). The presence of a hydroxyl group at both ends of the molecule distinguishes lutein and zeaxanthin from other carotenoids. The characteristic structure with nine double bonds is responsible for the absorbance of certain wavelengths of light and the emission of other wavelengths leading to the characteristic colour properties of these molecules. In view of its blue light absorption, lutein has a yellow or orange like appearance depending on its concentration. Lutein has a number of different isomers such as trans, cis and epoxy lutein, whereby it is not yet known if properties such as bioavailability or other physiological functions are affected (Calvo, 2005). The main isomer of lutein in plants and vegetables is the trans form, but processing may result in significant isomer changes (Khoo et al., 2011). In nature, lutein is often present as a fatty ester whereby either one or two of the hydroxyl residues are bound to a fatty acid (Chung et al., 2004). So called saponification of crude lutein from for instance marigold plants yields the free non esterified lutein as shown in Fig. 2.

3. Lutein bioavailability and metabolism

Lutein (either free or esterified) in the diet is taken up in the gastrointestinal tract via uptake by enterocytes (Fig. 3). Lutein from plants is normally present in an unesterified form whereas lutein in eggs is present in its free alcohol form (Lai et al., 1996). Commercially available lutein (Floral®) extracted from plants (Tagetes erecta) is saponified to remove the esters. Until recently it was assumed that bioavailability was not so much influenced by lutein esterification but was mainly dependent on the solubilization from the food matrix and uptake into micelles (Bowen et al., 2002; Chung et al., 2004; Yonekura and Nagao, 2007). Lutein uptake was thus considered to be dependent on its formulation and the quantity of fat in the meal during which it is taken (Roodenburg et al., 2000). A recent random controlled study however showed that serum levels following the administration of free lutein were approximately 20% higher as compared to supplementation with esterified lutein (Norkus et al., 2010). The serum lutein response
following egg consumption was approximately two to three times higher as compared to lutein in a supplement or from spinach, because eggs provide a fat containing environment and due to the relatively simple food matrix (Chung et al., 2004). Furthermore the lutein in eggs is present in its free unesterified form (Lai et al., 1996). It should be noted that the lutein content in eggs may vary considerably since it is dependent on the type of feed the animals are given and the housing conditions (Hesterberg et al., 2012; Schlatterer and Breithaupt, 2006). Free roaming chickens had twice the amount of carotenoids in their egg yolk as compared to laying hens kept in battery cages (Hesterberg et al., 2012). Of interest is the observation made in chickens, that tissue uptake is dependent on carotenoid levels during embryonic development (Koutsos et al., 2003). Whether high carotenoid levels in ovo result in higher local tissue levels of lutein binding proteins was not investigated by these authors and the exact mechanisms responsible for this observation remain to be clarified.

Before uptake by enterocytes the lutein esters are hydrolysed by gastrointestinal enzymes such as cholesterol esterase. The presence of fat in the diet promotes solubilization of lutein esters in the fat phase and at the same time stimulates the release and activity of esterases and lipases, enzymes that are essential in the hydrolysis of lutein esters (Hof et al., 2000). The bioavailability of lutein is much more sensitive to a fat containing diet than other carotenoids such as beta-carotene. This was shown in experiments whereby the plasma response to a lutein-ester supplement in combination with a full-fat spread was twice as high as compared to a low fat spread (Hof et al., 2000). Dietary fibres also play an important role in lutein bioavailability due to trapping of carotenoids, loss of dietary lipids and inhibition of lipase activity (Yeum and Russell, 2002).

The uptake of non esterified lutein by enterocytes was at first considered to only take place via simple diffusion but evidence is...
now accumulating suggesting an additional receptor mediated process (Li et al., 2010; Yonekura and Nagao, 2007). The scavenger receptor class B type 1 (SR-B1) has been implicated as one of the possible receptors that is involved in this process (Kiefer et al., 2002; Li et al., 2010). This receptor has low substrate specificity implying competition with other lipophilic substances in the diet (Kostic et al., 1995; Wang et al., 2010). Studies with aged rats have for instance shown that β-carotene may interfere with lutein absorption (Mamatha and Baskaran, 2011). Taken together these findings suggest that optimal lutein absorption can be achieved with a diet containing sufficient fats but with relatively low dietary fibres and β-carotene.

From the enterocytes lutein is packed into chylomicrons and is transported via the lymphatic system to hepatocytes (Furr and Clark, 1997). It is subsequently bound to lipoproteins and further transported throughout the body via the blood circulation (Clevidence and Bieri, 1993). In blood, lutein is only present in its free unesterified form. Especially HDL is thought to play an important role in the transport and retinal uptake of lutein (Loane et al., 2010; Mutungi et al., 2009; Wang et al., 2007). The recent finding that the HDL raising allele of the hepatic lipase (LIPC) gene is associated with a reduced risk of AMD supports a role for lutein in the prevention of AMD (Neale et al., 2010; Reynolds et al., 2010). Lutein supplementation studies have shown that high circulating HDL levels correspond to a stronger increase in the macular pigment (manuscript in preparation, van der Made et al). The LIPC polymorphism in these individuals was not available at the time this study was performed.

The retina is the tissue with the highest uptake of lutein, with concentrations exceeding 1 mM in some humans (Table 1), which suggests a receptor (SR-B1) mediated uptake process (Li et al., 2010).

As already mentioned above, a competition may occur between dietary uptake of carotenoids and a single carotenoid may alter the assimilation of other carotenoids (Mamatha and Baskaran, 2011; Wang et al., 2010). The retina appears to have the capacity to preserve accumulation of lutein and zeaxanthin, but this capacity is diminished when intake of beta-carotene is high (van den Berg and van Vliet, 1998; Wang et al., 2010).

Epidemiological studies indicate that lifestyle factors such as smoking may affect bioavailability of lutein as evidenced by lower lutein plasma concentrations and macular pigment values in smokers (Gruber et al., 2004; Hammond et al., 1996). The exact mechanisms responsible for this effect are not yet known. Smoking can induce a higher state of inflammation (Goncalves et al., 2011) resulting in higher intra and extracellular levels of H2O2 which in turn may lead to oxidation of lutein. Human and mouse studies have shown that supplemented lutein is readily oxidized (Fig. 4) resulting in the formation of keto-carotenoids (Khachik et al., 2006; Yonekura et al., 2010). The main keto-carotenoid found in the mouse liver was 3′-Hydroxy-β,β-caroten-3-one (and its cis isomer), where it accounted for more than 50% of the carotenoids (Yonekura et al., 2010). The exact role and biological properties of these lutein metabolites are not yet known, nor is it known to which degree the oxidation can be attributed to chemical or enzymatic processes. The chemical oxidation can be envisioned during the process of its properties as an anti-oxidant. Dietary lutein can also be (enzymatically) converted to meso-zeaxanthin. This metabolite is not found in human plasma or liver but is present in ocular tissues suggesting specific metabolic pathways in the eye (Khachik et al., 2002). Reduction of keto-carotenoids can revert the metabolite back to the parent lutein (Khachik et al., 2006).

### 4. Lutein and the eye

The colour of the human macula is due to the presence of three different xanthophylls: lutein, zeaxanthin and meso-zeaxanthin (Landrum and Bone, 2001). The human lens is also known to contain lutein and zeaxanthin (Berendschot et al., 2002). As mentioned above, primates cannot synthesize the first two xanthophylls and localization in the eye is dependent on the uptake via the diet of the individual. The presence of meso-zeaxanthin is due to local conversion from lutein (Bone et al., 1993; Khachik et al., 2002).

The highest concentration of macular pigment is observed near the fovea and decreases rapidly with increasing eccentricity (van der Veen et al., 2009). In approximately half of the subjects

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Lutein concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Egg yolk</td>
<td>3.6 μmol/kg</td>
<td>(Koutsos et al., 2003)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Liver</td>
<td>1.2 μmol/kg</td>
<td>(Koutsos et al., 2003)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Skin</td>
<td>0.8 μmol/kg</td>
<td>(Koutsos et al., 2003)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Thymus</td>
<td>0.4 μmol/kg</td>
<td>(Koutsos et al., 2003)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Bursa</td>
<td>0.4 μmol/kg</td>
<td>(Koutsos et al., 2003)</td>
</tr>
<tr>
<td>Monkey</td>
<td>Serum</td>
<td>74 nmol/l</td>
<td>(Johnson et al., 2005)</td>
</tr>
<tr>
<td>Monkey</td>
<td>Adipose tissue</td>
<td>1.18 mmol/kg</td>
<td>(Johnson et al., 2005)</td>
</tr>
<tr>
<td>Human</td>
<td>Serum</td>
<td>0.37 μmol/l</td>
<td>(Johnson et al., 2000)</td>
</tr>
<tr>
<td>Human</td>
<td>Serum</td>
<td>0.2 μmol/l</td>
<td>(van Herpen-Broekmans et al., 2004)</td>
</tr>
<tr>
<td>Human</td>
<td>Plasma</td>
<td>0.22 μmol/l</td>
<td>(Peng et al., 1993)</td>
</tr>
<tr>
<td>Human</td>
<td>Buccal muc.</td>
<td>4.45 μmol/kg</td>
<td>(Johnson et al., 2000)</td>
</tr>
<tr>
<td>Human</td>
<td>Adipose tissue</td>
<td>0.23 mol/kg</td>
<td>(Johnson et al., 2000)</td>
</tr>
<tr>
<td>Human</td>
<td>Milk</td>
<td>0.037 μmol/l</td>
<td>(Bettler et al., 2010)</td>
</tr>
<tr>
<td>Human</td>
<td>Retina</td>
<td>1–3 mmol/kg</td>
<td>(Landrum et al., 1999)</td>
</tr>
<tr>
<td>Human</td>
<td>RPE</td>
<td>5.1 nmol/kg protein</td>
<td>(Rapp et al., 2000)</td>
</tr>
<tr>
<td>Human</td>
<td>Central disc</td>
<td>1.70 pmol/mm³</td>
<td>(Rapp et al., 2000)</td>
</tr>
<tr>
<td>Human</td>
<td>Lungs</td>
<td>0.1–2.3 μmol/kg</td>
<td>(Schmitz et al., 1991)</td>
</tr>
<tr>
<td>Human</td>
<td>Liver</td>
<td>0.1–12.2 μmol/kg</td>
<td>(Schmitz et al., 1991)</td>
</tr>
<tr>
<td>Human</td>
<td>Kidney</td>
<td>0.1–10.4 μmol/kg</td>
<td>(Schmitz et al., 1991)</td>
</tr>
<tr>
<td>Human</td>
<td>Skin</td>
<td>0.03 μmol/kg</td>
<td>(Peng et al., 1993)</td>
</tr>
<tr>
<td>Human</td>
<td>Lens</td>
<td>0.16 mmol/kg</td>
<td>(Yeum et al., 1995)</td>
</tr>
</tbody>
</table>

**Fig. 4.** Oxidation of lutein resulting in the formation of keto-carotenoids (Yonekura et al., 2010).
tested, the macular pigment concentration is distributed in a bimodal fashion and appears as a ringlike structure (Fig. 5) (Berendschot and van Norren, 2006; Delori et al., 2006; Dietzel et al., 2011b). Women are more likely to exhibit this bimodal distribution suggesting an association between gender and anatomical structure of the fovea (Delori et al., 2006). Macular pigment is considered to be mainly located along the fibres of Henle. These fibres connect the foveal cones with the laterally displaced bipolar and horizontal cells and local differences in their distribution may explain the ringlike macular pigment structures described above.

4.1. Transport to the macula

The selective uptake of lutein and zeaxanthin by the primate retina has puzzled scientists for a long time. Retinal uptake of both carotenoids probably involves different mechanisms, whereby each is captured by separate binding proteins (Loane et al., 2008). An isoform of GSTP1 has for instance been identified as a local retinal zeaxanthin binding protein, which was recently followed by the identification of several retinal lutein binding proteins (Li et al., 2010).

Lutein is deposited in many tissues (Fig. 3), but the relative amount of lutein in the retina is highest among the tissues studied so far (Table 1) and may reflect local density of lutein binding proteins. A lutein binding protein was recently isolated from peripheral human retina’s and shown to interact with antibodies against a protein belonging to the family of steroidalogenic acute regulatory domain (StARD) proteins (Bhosale et al., 2009; Li et al., 2010). Of the 15 known human StARD proteins evidence was recently provided that StARD3 (also known as MLN64) is the human retinal lutein binding protein (Li et al., 2011). Further studies by this group with monkey retinas showed that StARD3 could be localized in the cone inner segments and axons. Plasmon resonance studies furthermore showed that StARD3 binds lutein with a relatively high affinity (K(D) = 0.45 mu M) (Li et al., 2011).

Lutein also copurifies with tubulin in macular extracts (Bernstein et al., 1997; Li et al., 2010) and a lutein binding site was identified in β-tubulins (Crabtree et al., 2001). Tubulins are abundantly present in the photoreceptor cell axons (fibres of Henle) and macular pigment in the human retina is mainly located here (Trieschmann et al., 2008). Photoreceptors themselves also contain a substantial amount (approximately 10–25%) of the retinal carotenoids (Rapp et al., 2000; Sommerburg et al., 1999). Human RPE obtained from donor eyes was also shown to contain lutein (Rapp et al., 2000), which was confirmed by in vitro experiments showing uptake of lutein by RPE cells that was mediated via the scavenger receptor class B type 1 (SR-B1) (During et al., 2008). Lutein is transported in the blood as a complex with HDLs, which are a known ligand for SR-B1, suggesting a piggy back mechanism of uptake into the retina via RPE cells. Chickens with a genetic HDL defect show a deficiency of lutein in the retina, but not in other organs such as liver, heart or egg yolk, implying a major role for HDL in the transport of lutein to the eye (Connor et al., 2007). Further evidence for a role of HDL comes from recent epidemiological studies indicating that omega-3 long chain polyunsaturated fatty acids (LCPUFAs) enhance MPOD uptake of lutein and zeaxanthin (Delyfer et al., 2012). Other studies have shown that omega-3 LCPUFA supplementation may lead to an increase in HDL (Thomas et al., 2004) which as mentioned above, may promote lutein transfer to the retina. On the other hand it cannot be excluded that the omega-3 LCPUFAs can also affect the function and density of lutein binding proteins, affecting the transport within the retina itself.

Fig. 5. The highest concentration of macular pigment is observed near the fovea and decreases rapidly with increasing eccentricity. In approximately half of the subjects tested, the macular pigment concentration is distributed in a bimodal fashion and appears as a ringlike structure.
The recent finding that a polymorphism in the gene encoding for SR-B1 is associated with AMD supports the role of lutein and lipid pathways in the pathogenesis of this disease (Zerbib et al., 2009). Most of the lutein in the macula is found in the inner retina and the proteins involved in further transport have not yet been completely identified although a role for CD36 has been postulated (Li et al., 2010). Genetic variants of CD36 have been shown to modulate levels of lutein in plasma and the retina (Borel et al., 2011). Further insight in the transport of pigment in the macula may be obtained from patients with macular telangiectasia, an acquired disease whereby patients lose their macular pigment (Issa et al., 2009). Histological analysis of the retina in one patient showed pathological retinal capillaries and an absence of Muller cells, which suggests a role for Muller cells in the transport of xanthophylls to the retina (Powner et al., 2010) (Fig. 5). Patients with Sjogren-Larsson syndrome, an autosomal recessive hereditary disorder, also lack macular pigment and in these patients the visual symptoms are characterized by reduced visual acuity, photophobia and the presence of crystals in the retina (van der Veen et al., 2010). Degenerated Muller cells seen in the retinas of these patients also support a role for these cells in the retinal transport of xanthophylls (Fujikschot et al., 2008).

Lutein is also absorbed by other tissues in the body including adipose tissue (Table 1) and it has been suggested that these tissues may compete with retinal uptake (Thomson et al., 2002). This may explain why obesity can affect macular pigment levels (Burke et al., 2002). In vitro experiments with purified lutein show that it absorbs blue light at wavelengths around 450 nm with a peak absorption at 446 nm (Alves-Rodrigues and Shao, 2004). The spectral properties in the eye are shifted towards the right and show a peak absorption at 460 nm (Sharpe et al., 1998). These wavelengths are known to induce light mediated damage to the retina. By acting as a blue light filter, the lens and macular pigments can protect underlying retinal structures such as the photoreceptors from light induced damage. One of the targets of photoxidation in the retina is A2E. A2E accumulates with age and is not very toxic by itself, but following photoxidation may be extremely toxic for the retina (Wielgus et al., 2010).

Of the known carotenoids, lutein has been shown to have the highest blue light filtering properties (Junghans et al., 2001).

4.3. Antioxidant effects of lutein in the retina

The retina has a high demand for oxygen and due to the aerobic metabolism it has been proposed that various reactive oxygen intermediates such as hydrogen peroxide, singlet oxygen and hydroxyl radicals will be formed (Carpentier et al., 2009). These reactive intermediates may cause damage to the lipids in the retina, which are highly susceptible to oxygen mediated damage in view of the fact that they are mainly composed of polyunsaturated fatty acids such as docosahexaenoic acid (DHA). DHA comprises 60% of the polyunsaturated fatty acids in the retina. Peroxidation of these fatty acids in the lipid membranes of for instance photoreceptor cells may impair their function. Reactive oxygen species have been shown to induce apoptosis of photoreceptors and lutein has been shown to be able to block paraquat or H2O2-induced apoptosis of cultured retina photoreceptors (Chucair et al., 2007). Membrane bound lutein is considered to scavenge the oxygen intermediates (Woodall et al., 1997), whereby the oxygen scavenging property of lutein is caused by quenching of reactive oxygen intermediates via the numerous unconjugated double bonds in the lutein molecule. Dipolar carotenoids such as lutein are considered to be mainly oriented in a perpendicular fashion in the lipid membrane of cells but can also be distributed in a different fashion to retinal cells when bound to proteins (Subczynski et al., 2010). A transfer of protein bound lutein into the lipid membrane of retinal cells is likely and the close proximity of transmembrane lutein with polyunsaturated phospholipids allows optimal protection against reactive oxygen species.

Experiments with Rhesus monkeys undergoing long term dietary xanthophyll deprivation showed that lutein supplementation protected the fovea from blue light damage (Barker et al., 2011b). The amount of lutein at the retinal site of 0.5 mm eccentricity in these experiments was too low to have any blue filtering effect and the protection of the fovea was most likely due to quenching of radicals.

Xanthophyll free diets have been shown to affect the foveal density profile of RPE cells in Rhesus monkeys (Leung et al., 2004). In normal animals RPE cells show the highest density at the centre of the fovea. Animals on a xanthophyll free diet however showed a dip in the RPE cell density profile at the centre of the fovea. The authors have suggested that alterations in the RPE cell density profile may be a response to the fact that an absence of xanthophylls leads to an inability to cope with physiological stress in the macula (Leung et al., 2004).

Lutein has been shown to decrease lipofuscin accumulation in cultured RPE cells by possibly inhibiting peroxidation of membrane phospholipids of the photoreceptor outer segments (Sundelin and Nilsson, 2001). Peroxidation of phospholipids and polymerization is thought to affect degradation into simple molecules leading to a build up of lipofuscin in RPE cells.

4.4. Lutein and visual performance

A number of studies have reported positive effects between macular pigment optical density (MPOD) and visual performance (Stringham et al., 2010, 2011; Weigert et al., 2011), but only recently has the effect been studied using randomized, placebo controlled interventional trials (Nolan et al., 2011; Weigert et al., 2011). The results of such a lutein based supplementation trial, which was performed in young healthy subjects over a time span of 12 months, did not show an improvement of visual performance in the active arm, despite the fact that lutein serum levels more than doubled and central MPOD values also increased significantly. These findings may be explained by assuming that the pre-study MPOD values in the study group were already sufficient for good visual performance and that the MPOD increase achieved in the study was not large enough to affect the visual performance parameters tested.

We recently performed a randomized, placebo controlled, 12 months lutein supplementation in early-stage AMD patients and tested macular pigment optical density (MPOD) and best corrected LogMar visual acuity at baseline and at various intervals after the start of the study (Berendschot et al., manuscript in preparation). Lutein supplementation led to an increase in the MPOD levels in early-stage AMD patients, whereas no change was observed in the placebo group. Significant differences between the two groups were found concerning the change in VA over the supplementation period. Although not analysed in depth, many persons in the lutein...
treated group mentioned improvement in reading, driving and night vision. These findings are in agreement with an earlier double masked placebo controlled lutein supplementation study in AMD patients (Richer et al., 2004). The lutein receiving groups showed enhanced MPOD levels and had improved VA and a better contrast sensitivity function (Richer et al., 2004). More recent placebo controlled studies also showed a significant correlation between MPOD changes, VA and macular function (mean differential light threshold) in early AMD patients treated with an oral lutein supplement (Weigert et al., 2011).

Non placebo controlled supplementation studies by Stringham and Hammond showed that higher MPOD levels correlated with improved glare disability (Stringham et al., 2010; Stringham and Hammond, 2008). The discrepancy with the recent studies by the group of Nolan has been attributed to differences in technical analysis (Nolan et al., 2011). More recent studies using free-viewing conditions to account for iris pigmentation and pupil size confirmed the earlier studies and showed that higher MPOD levels correlated with faster photostress recovery times, lower disability glare contrast thresholds and lower visual discomfort (Stringham et al., 2011). Taken together the current findings suggest that individuals who can potentially increase their MPOD levels via a diet rich in lutein can also improve their visual function. Future studies should be undertaken to identify at which threshold MPOD level a lutein supplement would be advisable that could lead to either prevention of AMD or which could lead to improvement of visual function.

5. Lutein and ocular disease

5.1. Lutein and macular degeneration

Acting as a blue light filter absorbing between 390 and 540 nm, it seems plausible that lutein can protect the underlying photoreceptors in the centre of the macula from photochemical damage (van de Kraats et al., 2008). The anti-oxidant properties of lutein may also protect the macula from oxidative stress (Barker et al., 2011a). The role of lutein in the development of macular degeneration in the western world is however still a matter of debate (Davies and Morland, 2004). With the current diet it is well possible that a low threshold of macular pigment is already reached. On the other hand epidemiological studies have shown that an intake of 6 mg of lutein and zeaxanthin per day was associated with protection from macular disease (Seddon et al., 1994), whereas the daily intake in the Western world is approximately 1–2 mg per day, suggesting a marked deficit in the diet for these xanthophylls (Johnson et al., 2010). The currently available scientific data concerning the relation between the intake of dietary lutein and prevalence of AMD have not yet convinced regulatory bodies such as the EFSA or the FDA to accept proposed health claims of lutein (Trumbo and Ellwood, 2006). Interventional longitudinal studies are currently undertaken to study the effect of lutein on the development of macular degeneration (Coleman and Chew, 2007).

Whether lutein could play a role in the prevention of drusen formation is not clear. It is interesting to note that lutein was the major factor preventing amyloid fibre aggregation in an in vitro system using isolated carotenoid fractions from apricot (Katayama et al., 2011). This is in line with current concepts suggesting that lutein may improve cognitive function in the elderly and has been shown to be depleted in cases with Alzheimer’s disease (Johnson, 2010).

5.2. Lutein and cataract

The human lens is known to accumulate low levels of lutein (Table 1), although the mechanisms of uptake are not known (Yeum et al., 1995). The concentration in the lens is however much lower than in other tissues such as kidney and lungs (Table 1). In vitro studies have shown that lutein can inhibit UV-B induced lipid peroxidation of cultured human lens epithelial cells which was ascribed to its anti-oxidant and blue light filtering properties (Chitchumroonchokchai et al., 2004). The role of lutein in the development of cataract is however a controversial issue whereby observational studies support an association between lutein intake and a lower incidence and progression of cataracts (Berendschot et al., 2002; Fernandez and Afsahi, 2008). Prospective supplementation studies were however not able to confirm these studies and to date the FDA has not accepted health claims of lutein in relation to cataract prevention (Trumbo and Ellwood, 2006).

6. Lutein and disease mechanisms: immune response, inflammation, apoptosis

6.1. Lutein and the immune response

The effects of lutein on the immune response have been studied in various species of birds, mice, dogs and cats (Chew and Park, 2004). Studies in mice showed that lutein enhanced the antibody response to T dependent but not to T independent antigens (Yonouchi et al., 1994). Dietary lutein supplementation did not affect phytomagglutinin induced cutaneous hypersensitivity in laying hens, but did stimulate the secondary antibody response to live infectious bronchitis virus (IBV) vaccination at a feeding dose of 125 ppm but not at higher doses (Bedecarrats and Leeson, 2006). Dietary lutein stimulated both cellular and humoral immunity in cats, dogs and zebra finches (Kim et al., 2000a, 2000b; McGraw and Ardia, 2003). No studies have been reported concerning dietary lutein and immune response markers in humans. Most studies addressing the role of carotenoids and immunity have studied the effect of beta-carotene (Chew and Park, 2004). The mechanisms whereby lutein affects the immune response are not yet known and it is not yet clear whether the effects are due to its anti-oxidant properties. Excess anti-oxidant levels may even impair dendritic cell function (Verhasselt et al., 1999) and thereby inhibit immune responses, which suggests that the mechanism responsible for the immunostimulatory effect of lutein is different from its anti-inflammatory effects.

Adipose tissue has been considered a storage site for lutein and recent evidence from studies in birds show that it is mobilised from these sites during endoparasitic infection (Metzger and Bailrile, 2011). Plasma concentrations of carotenoids also decrease during parasite infection and return back to normal levels following antiparasitic treatment (Mougeot et al., 2007). Although this latter observation may be due to interference with carotenoid uptake, it may also be seen as evidence of carotenoid metabolism during the immune response and inflammation following infection. Enhanced carotenoid metabolism during immune activation leads to retinal carotenoid depletion in birds (Toomey et al., 2010) and these findings may provide further insight in the mechanisms explaining the link between chronic low grade systemic inflammation and AMD.

In many bird species, lutein plays an important role in the composition of sexual ornaments such as combs and a competition exists with the use of lutein for immune and inflammatory functions (Mougeot et al., 2007; Perez-Rodriguez, 2009).

6.2. Lutein and inflammation

The mechanisms whereby lutein affects the immune response may differ from its action on inflammation (Perez-Rodriguez, 2009). During inflammation lutein is thought to scavenge reactive
oxygen species generated during the inflammatory process. As will be mentioned below, the effect of lutein on H$_2$O$_2$ levels may affect the intracellular pathways leading to the expression of various proinflammatory molecules (Kim et al., 2008).

Since the eye and skin are continuously exposed to light, they are prone to light induced damage, a fact that has created a great deal of interest focussing on the protective role of lutein as a blue light filter and anti-oxidant in these tissues (Roberts et al., 2009). UV radiation models in mice have shown that feeding mice with a lutein diet leads to higher lutein skin levels and is associated with a reduction in the amount of reactive oxygen species in the skin (Lee et al., 2004). As shown in the following sections, much more work has been reported on the role of lutein in various models of ocular inflammation.

6.2.1. Endotoxin induced uveitis (EIU)

Endotoxin or lipopolysaccharide (LPS) induced uveitis (EIU) is a well known acute experimental model of uveitis that enables the study on the immunopathogenetic mechanisms involved in human intraocular inflammatory disease. LPS is given at a distant site such as the skin and within 24 h an intense intraocular inflammation is observed that is characterized by a breakdown of the blood aqueous barrier and influx of inflammatory cells in the anterior chamber of the eye. Japanese investigators have studied possible anti-inflammatory effects of lutein supplementation using this model (Jin et al., 2006). Rats were pre-treated with an intravenous injection of high doses of lutein up to 100 mg per kg bodyweight and subsequently received a subcutaneous injection of LPS. Rats developed severe ocular inflammation at 24 h and lutein at 100 mg/kg bodyweight almost completely blocked aqueous humour cell infiltration, a finding similar to that observed after pre-treating rats with 1 mg/kg bodyweight of dexamethasone. Lutein also blocked the increased protein concentration in the aqueous humour as observed during EIU. Both aqueous humour nitric oxide and PGE2 levels were markedly decreased in rats receiving lutein. Cytokine levels including IL-6, TNF alpha, CCL2 and CXCL2 were also decreased in the aqueous humour when rats received lutein prior to the induction of EIU. A dose of 100 mg/kg bodyweight almost completely blocked the expression of these mediators an effect that was similar to that seen with 1 mg dexamethasone. Small but significant effects on the expression of these mediators in the aqueous humour were already observed at a dose of 1 mg of lutein. The findings mentioned above were confirmed in an LPS-induced inflammation model in rats and evidence was obtained that this was mediated by a downregulation of NF-$\kappa$B activation (Kim et al., 2008). Reactive oxygen species (ROS) are thought to activate the NF-$\kappa$B pathway whereby lutein could play a crucial role in the clearance of intracellular ROS (Kim et al., 2008). Both in vitro and in vivo studies in an LPS-induced model of inflammation in chickens provided evidence for a lutein induced repression of NF-$\kappa$B signalling activation via peroxisome proliferators activated receptors (PPAR) and retinoic acid X receptor (RXR) pathways (Selvaraj and Klausing, 2006; Selvaraj et al., 2010). Repression of NF-$\kappa$B signalling by lutein thus results in the downregulated induction of a whole series of inflammatory genes.

Most studies of EIU have addressed anterior segment inflammation although LPS is also known to induce retinal inflammation (Yang et al., 1996). The group of Tsubota examined retinal inflammation in EIU and showed that lutein decreased STAT3 activation in the retina (Sasaki et al., 2009). STAT3 is an important intracellular downstream signalling pathway following stimulation with inflammatory cytokines such as IL-6. They furthermore showed that lutein could prevent pathological changes of Muller cells during EIU.

6.2.2. Experimental age related macular degeneration (AMD)

Laser induced CNV is a widely used model to study neovascular AMD in humans (Kijlstra et al., 2005). A number of laser spots are given around the optic disc of the mouse eye and within a few days new vessels emerge from the choroid into the subretinal space. The CNV area is evaluated one week after applying the laser spots. Lutein was shown to inhibit choroidal neovascularization in a murine laser model of the choroid and details of the evidence are presented below (Izumi-Nagai et al., 2007). Pre-treatment of mice with 10 mg of oral lutein per kg of bodyweight resulted in a decrease in the CNV volume of 553 um$^3$ in controls as compared to 401 um$^3$ in lutein treated animals, respectively. This was associated with a marked reduction of infiltrating macrophages into the CNV area of the retina. Analysis of protein extracts from the RPE-choroid layer showed a significantly decreased expression of CCL2, VEGF and ICAM-1, following CNV induction in lutein treated animals as compared to controls.

6.2.3. Lutein and retinal ischemia

Retinal ischemia is a condition whereby the retinal circulation cannot fulfill its metabolic demands. It may be a feature of various ocular pathologies and can lead to loss of retinal ganglion cells and apoptosis of cells in the inner retina leading to irreversible blindness (Osborne et al., 2004). Reperfusion following ischemia may lead to oxidative stress. Various interventions aimed at reducing the detrimental effects of oxygen radicals following retinal ischemia and reperfusion have been reported (Osborne et al., 2004) but to date only few have addressed the role of lutein. In a mouse model of retinal ischemia and reperfusion, Li et al (Li et al., 2009) showed that lutein had a neuroprotective effect as evidenced by a lower cell loss in the retinal ganglion cell layer and less apoptotic cells. These effects were associated with a lower level of oxidative stress markers such as nitrotyrosine and poly-ADP-ribose (Li et al., 2009). Further in vitro study performed by this group showed that lutein could protect both the coxl (II) chloride induced hypoxia and the H$_2$O$_2$-induced oxidative damage to retinal ganglion cells (Li and Lo, 2010).

6.2.4. Lutein and diabetic retinopathy

Diabetic retinopathy is thought to be mediated by oxidative damage leading to the activation of the NF-$\kappa$B pathway. As mentioned already in the previous sections, abundant evidence is now becoming available that lutein can block this activation pathway due to its ability to quench oxygen radicals. Oral lutein was shown to reduce the biochemical and functional changes in experimental models of diabetes in mice without affecting blood glucose levels (Muriach et al., 2006; Sasaki et al., 2010). In these models, lutein administration was shown to markedly reduce the production of reactive oxygen species and the NF-$\kappa$B activity in the retina (Muriach et al., 2006; Sasaki et al., 2010). Lutein administration to rats with streptozotocin (STZ)-induced diabetes showed that it was able to inhibit the incidence of cataract formation in these animals (Arnal et al., 2009a). The same group showed that lutein was able to reduce the diabetic abnormalities in the cerebral cortex of rats with STZ induced diabetes (Arnal et al., 2010). Reactive oxygen species (ROS) are continuously induced during hyperglycaemia and antioxidants such as lutein may thus play an important role in decreasing oxidative stress in various organs and tissues of the diabetic patient. Although a large number of studies have addressed the role of carotenoids in the development of diabetes only few have examined their role in the development of diabetic retinopathy (Brazionis et al., 2009). The latter study showed evidence for a protective role of combined lutein/zeaxanthin and lycopene against diabetic retinopathy. To date no intervention studies have been reported concerning lutein in the prevention or treatment of diabetic retinopathy.
6.3. Anti-inflammatory effects of lutein in vitro

In vitro experiments showed that lutein (10 μg/ml) was able to inhibit TNF alpha induced ICAM-1 and CCL2 expression by cultured endothelial cells. High concentrations of lutein (100 μg/ml) were also able to decrease CCL2 and VEGF expression following TNF alpha stimulation of cultured ARPE-19 cells. VEGF expression by in vitro LPS stimulated macrophages was inhibited at both 10 and 100 μg/ml lutein. Expression of inflammatory cytokines is dependent on the activation of the NF-κB system. Appropriate stimuli lead to IκB phosphorylation and degradation in the cytosol followed by entrance of NF kappa B into the nucleus where it binds to the NF-κB motif of the iNOS promoter (Kim et al., 2008). This was proven by the fact that lutein inhibited LPS-induced IκB kinase (IKK) activation, IκB degradation, nuclear translocation of NF-κB and binding of NF-κB to the κB motif of the iNOS promoter (Kim et al., 2008). Similar findings were reported using an in vitro model of gastric epithelial cells whereby lutein was shown to inhibit the H2O2-induced increase of intracellular ROS levels and the subsequent activation of NF-κB and IL-8 expression in these cells (Kim et al., 2011). A scheme illustrating the inhibitory effect of lutein on NF-κB mediated proinflammatory effects is shown in Fig. 6.

Similar observations have been made showing a similar anti-inflammatory effect mediated via the NF-κB pathway by another carotenoid, astaxanthin (Lee et al., 2003). A marked interest exists in the cardiovascular field to employ this carotenoid (CardaxTM) to treat inflammatory events associated with atherosclerosis (Pashkow et al., 2008).

Lutein has also been shown to inhibit arachidonic acid release from a mouse macrophage cell line, which was attributed to a dose dependent inhibition of cytosolic phospholipase A2 (PLA2) (Song et al., 2010). PLA2 activation is important in the generation of inflammatory mediators such as prostaglandins, thromboxanes and leukotrienes. The effect of lutein on PLA2 has not yet been confirmed by other groups.

6.4. Lutein and circulating markers of inflammation and immunity

Epidemiological studies have shown an inverse relationship between serum lutein concentration and a circulating markers of inflammation such as soluble ICAM-1 (van Herpen-Broekmans et al., 2004) and CRP (Seddon et al., 2006). A study analyzing the non-smoking subset of the NHANES III population, showed that serum lutein/zeaxanthin levels, were significantly lower in participants with increased CRP levels, suggesting that inflammation results in lutein catabolism (Kritchevsky et al., 2000). This study has been criticized since the sensitivity of the used assay was low and a large part of the study population had CRP levels below the detection level (Calder et al., 2011).

![Fig. 6](image-url) Intracellular lutein can decrease the level of intracellular H2O2 accumulation by scavenging H2O2 thereby inhibiting the final pathway of NF-κB activation induced by various triggers such as the TNF receptor (TNFR), the IL-1 receptor (IL-1R), Toll ligand receptors (TLRs) and the CD40 molecule (Kim et al., 2008). Triggering these receptors leads to the activation of NADPH oxidase which in turn induces higher intracellular levels of H2O2. Activation of the IκB kinase (IKK) complex is mediated via H2O2 and scavenging via lutein will prevent IκB degradation, nuclear translocation of NF-κB and DNA binding of NF-κB thereby blocking the proinflammatory cytokine response.
To date many supplement studies have been undertaken but few have addressed the effect of lutein supplementation on inflammatory or immune function markers (Chew and Park, 2004).

No change was found in serum CRP, ICAM-1 or VCAM-1 levels in a recently conducted placebo controlled trial in healthy volunteers on lutein- and zeaxanthin-rich foods or on lutein/zeaxanthin supplements (Graydon et al., 2012). This may be due to the fact that the initial level of inflammatory biomarkers was too low in this group of healthy volunteers and supplementation may only reveal an effect in individuals with higher baseline levels of these markers.

These findings are in agreement with a recent placebo controlled study from our group that did not show significant changes in serum CRP values following a twelve month supplementation of healthy volunteers with a daily dose of 10 mg lutein (Berendschot et al., manuscript in preparation). As mentioned above, this may be due to the fact that CRP levels are already quite low in healthy volunteers and that modest CRP changes are not readily detected. A lutein supplementation study in individuals with inflammation and having high circulating CRP levels may shed more light on this issue. On the other hand it would also be interesting to study the effects of lutein supplementation on the levels of other circulating biomarkers of inflammation (Liu et al., 2011; Scholl et al., 2008).

6.5. Lutein and apoptosis

In experimental models of diabetes, apoptosis is one of the hallmarks in the retinas of these animals. Apoptosis evaluated by the TUNEL assay or by the expression of cleaved caspase-3, is elevated in cells from the ganglion cell layer in experimental models of diabetes. Independent groups have now shown that lutein feeding of animals with experimental diabetes resulted in a significant reduction in the amount of apoptotic cells in the ganglion cell layer (Arnal et al., 2009b; Sasaki et al., 2010). Apoptosis as seen in an experimental model of retinal ischemia was also inhibited by lutein administration (Li et al., 2009).

In vitro cultures with rat photoreceptor cells showed that the addition of lutein to these cultures was able to inhibit oxidative stress (paracquat or H2O2) induced apoptosis (Chucair et al., 2007). Dietary lutein has been shown to be incorporated into mitochondria and this localization may preclude oxygen radical mediated apoptosis induced via these organelles (Chew and Park, 2004).

Dietary lutein has furthermore been shown to decrease apoptosis in blood leukocytes of tumour-bearing mice as compared to un-supplemented animals (Chew et al., 2003).

7. Conclusions and future directions

Lutein is not only important as a blue light filter and antioxidant in the retina, but can also influence the immunological and inflammatory responses elsewhere in the body. This latter observation is of importance since both biological and genetic factors are involved in the development of age-related macular degeneration.

Whether the effect of lutein on the immune response, versus its effect on inflammation is mediated by a similar mechanism is not yet clear. The anti-inflammatory effects are most likely due to its anti-oxidant properties. By reducing intracellular H2O2 lutein has been shown to diminish the effect of proinflammatory pathways leading to the activation of NF-κB. Current studies addressing the effect of lutein supplementation on the development of macular degeneration should therefore not only address local effects on the retina but should also incorporate systemic markers of inflammation. Recent studies that have identified SR-B1 as a lutein binding protein in the retina and the fact that genes encoding this receptor and HDL metabolism predispose to AMD support further investigations in the role of cholesterol and lutein pathways in the pathogenesis of this disease.

References


