Chapter 10

Lutein and the Aging Eye

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Additional information is available at the end of the chapter

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Abstract

Lutein is a carotenoid highly concentrated in the macula of the retina. Lutein cannot be synthesized and must be supplied in the diet, for example, dark green leafy vegetable and egg yolk. Lutein is believed to absorb blue light, leading to the protection of retina from light-related damage. It can also protect the retina against oxidative stress and inflammation. In fact, dietary and supplementary lutein have been shown to be associated with possible reduced risk of age-related macular degeneration, a leading cause of elderly blindness, attributed largely to lutein's antioxidant properties. Lutein is also beneficial as a nutritional supplement in preventing diabetic retinopathy. Moreover, lutein is very safe and widely used. In this chapter, we will discuss the basic chemistry of lutein; its uptake, transport, distribution, and functions in the normal eye. Lastly, the effects of lutein in age-related eye diseases will be summarized.

Keywords: macula, macular degeneration, vision, retinopathy, blindness

1. Introduction

Decades of research have indicated that abundant intake of carotenoid-rich food is correlated with the reduced risk of several age-related ocular diseases, for example, age-related macular degeneration (AMD) and diabetic retinopathy (DR). To date, among more than 1000 carotenoids discovered in nature, about 50 have been identified in the human diet [1]. However, only 25 dietary carotenoids and 9 of their metabolites have been found in human plasma, of which lutein, its stereoisomers zeaxanthin and meso-zeaxanthin are highly concentrated in the human retina [2].

Lutein is one of xanthophyll carotenoids (oxygen-containing carotenoids) which exist in the dark green leafy vegetables, yellow fruits and vegetables, and egg yolk [1]. Since animals are

not able to produce lutein, they need to depend on the dietary consumption. After absorption of lutein with fat, it is attached to the lipoprotein and then transported into the circulation; subsequently, with the serum concentration of 0.2 μm, lutein reached throughout the body and accumulated in the eye, especially in the retina, to serve certain biological functions [3]. In the human eye, the distribution of lutein varies. Lutein is found in higher quantities within the peripheral retina, retina pigment epithelium (RPE), choroid and ciliary body while exhibiting low concentrations in the iris and lens [2].

According to the most updated data from WHO, 253 million people suffer from vision impairment, and 81% people who are blind or have moderate or severe vision impairment are aged 50 or above [4]. A large number of studies have indicated that lutein plays an important role in decreasing the risk of AMD, the leading cause of blindness in the elderly people in the developed countries [5, 6]. Clinical trials have demonstrated that lower concentration of lutein in retina and serum was observed in DR patients when compared with patients without diabetes [7]. Moreover, DR patients receiving lutein and zeaxanthin supplements have shown improvement in visual acuity and contrast sensitivity, indicating a possible benefit in delaying the onset and development of DR [7]. In this chapter, we will introduce the background information of lutein, summarize its functions in the normal eye, and discuss the effects of lutein in age-related eye diseases.

2. Lutein

2.1. Chemistry and structure of lutein

Carotenoids are classified into two subgroups: carotenes, which are hydrophobic, consist of strictly hydrocarbons and xanthophylls, which are more hydrophilic, contain at least one oxygen atom in the polyene chain. The common characteristic of the carotenoid family is a $C_{40}H_{56}$ structure containing a long conjugated double-bound chain carrying the liner and cyclic alternatives. Lutein and zeaxanthin belong to the xanthophylls subgroup. They are characterized by the two hydroxyl groups attached to the end ionone rings in the nine conjugated carbon bounds polyene chain (**Figure 1**). The difference between lutein and its stereoisomer zeaxanthin is the position of the double bound in the terminal ring. In the human body, lutein and zeaxanthin could be transformed to each other via meso-zeaxanthin. Due to the presence of hydroxyl groups, lutein and zeaxanthin are more hydrophilic and polar in the serum and tissues, allowing them to react with oxygen produced in the liquid phase and scavenge reactive oxygen species (ROS) more efficiently. Due to the presence of chiral centers, lutein can exhibit eight stereoisomeric forms, of which (R,R,R) is mainly found in nature. On the other hand, zeaxanthin has three stereoisomeric forms, including (R,R), (S,S), and (R,S-meso).

2.2. Sources and safety of lutein

Lutein cannot be synthesized in human and lower animals, thus it must depend on the dietary supply in nature. Lutein, along with its structure isomer zeaxanthin is present in various natural foods, including kale, spinach, brussels sprout, broccoli, corn, lettuce, green peas,

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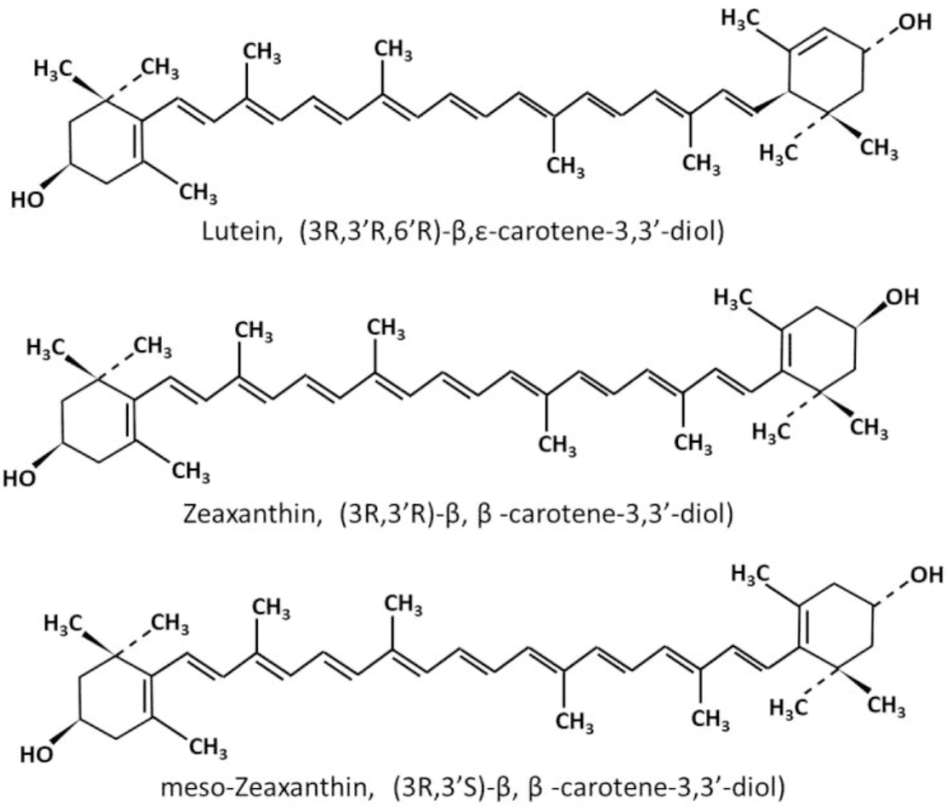

Figure 1. Chemical structures of macular pigments.

orange pepper, kiwi fruit, orange, zucchini, and squash. Dark-green leafy vegetables are the major source of lutein, especially in kale and spinach, containing 15,800–39,550 μg/100 g and 7040–11,940 μg/100 g, respectively [1]. There are 44 and 26 mg of lutein per cup of cooked kale and spinach, respectively [8]. However, the dietary origin of lutein varies in different countries, depending on the preference for specific foods. In Canada, lutein mostly comes from spinach, broccoli, lettuce, corn, and oranges; while in Germany, spinach and green leafy salads contribute almost 50% of the total lutein supply [1]. Egg yolks, although does not contain lutein as high as kale and spinach, are treated as a great source of xanthophylls due to the high fat content in eggs, resulting in increased bioavailability. The concentrations of lutein and zeaxanthin are 292 ± 117 μg/yolk and 213 ± 85 μg/yolk (average weight of yolk is 17–19 g), respectively [9]. It has been demonstrated that consumption of 6 eggs/week increased the macular pigment optical density (MPOD) significantly, while the serum concentration of total cholesterol, triacylglycerols, high density lipoprotein cholesterol, and low density lipoprotein cholesterol stayed unaffected [10]. Because of the limitation in separating and quantifying lutein and zeaxanthin, most researches and databases frequently report the combined data of these two compounds in food. Thus, it may result in the inappropriate estimation of lutein content in several xanthophyll-rich foods (e.g. oranges and grapes). The microalgae, especially the genus Chlorella, are also an important natural source of lutein. Compared to the marigold flower, the conventional source of lutein in market, microalgae have faster growth rate and can be obtained throughout the year. Therefore, they can be used as a potential source for commercial lutein products.

According to the National Health and Nutrition Examination Survey, the intake of lutein and zeaxanthin combined is approximately 1–2 mg/day in USA [11]. In addition, German adults consume 1.9 mg/day in average and 1.4 mg/day of lutein consumption was reported for Canadians [1]. No adverse effects were reported after the supplementation of dietary lutein up to 20 mg/day for 48 weeks, 30 mg/day for 120 days, and 40 mg/day for more than 8 weeks [12–14]. Animal studies have demonstrated similar results. For rat, uptake of lutein up to 35 mg/day for 8 weeks or 208 mg/kg/day for 13 weeks, or 639 mg/kg/day was not associated with any exposure-related toxicity and adverse events [15, 16]. Thus, lutein is recognized as Generally Recognized as Safe (GRAS) by FDA. Although there is no relationship between side effects and long term, high dose supplementation of lutein, the total intake should not exceed 20 mg/day according to the report from Council for Responsible Nutrition (CRN) in 2006 [17]. Generally, the recommendation dose of lutein supplements is 10 mg/day. A recent case report has demonstrated bilateral "foveal sparkles" in an Asian woman who has taken a 20 mg/day lutein supplements together with a high consumption of dietary lutein. After 7 months of discontinuous uptake of lutein supplements but insistence of her high-lutein diet, the crystal dissolved in the right eye, but still existed in the left eye [18]. However, it is worth noting that upon the population-based surveys, consumption of lutein has gradually declined in the USA and Europe. Therefore, actions should be taken to emphasize the importance of adequate intake of carotenoid-rich food, especially from dark-green leafy vegetables.

2.3. Absorption, metabolism, and transport of lutein

Since lutein and zeaxanthin are soluble in the fat, the absorption of these compounds follows a similar path like other lipophilic nutrients. After uptake of carotenoid-rich foods, xanthophylls are released from the food matrix with the aid of a variety of enzymes (e.g. esterase) and disperse in the stomach. The free xanthophylls then form micelles by incorporating with biliary phospholipids, bile salts, or dietary fats, which makes them more easily absorbed into the mucosal cells in the small intestine. Subsequently, they are transported from intestinal tract to the liver in the form of chylomicrons, where xanthophylls such as lutein and zeaxanthin are repackaged, carried by the relevant lipoproteins and released into the systemic circulation. In the circulation system, lipoproteins are responsible for transporting hydrophobic lipid including fat, plasma lipid, carotenoids, retinoids, etc. There are four types of lipoproteins: ultra-low density lipoproteins (ULDL), also known as chylomicrons; very low density lipoproteins (VLDL); low density lipoproteins (LDL); and high density lipoproteins (HDL). Compared to the non-polar carotenes such as lycopene and β-carotene, which are loaded onto VLDL and LDL, lutein and zeaxanthin are primarily transported by HDL. Both lutein and zeaxanthin are distributed in a variety of human tissues, but the distribution of them is not balanced among different tissues and organs. Retina, especially the macula, is regarded as the region where lutein, zeaxanthin, and its metabolite meso-zeaxanthin are concentrated, accounting for 25% of total carotenoids. Therefore, lutein, zeaxanthin, and meso-zeaxanthin are known as macular pigments (MPs), which play an important role in maintaining the normal functions of the eye. Although lutein is richest in the retina, it is also absorbed and distributed in other tissues such as adipose tissue in human body. It has been estimated that level of lutein in the retina was affected in obesity group, suggesting adipose tissue may compete with retina in terms of xanthophylls uptake [19].

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There are several factors that affect the bioavailability of lutein and zeaxanthin, including Species of carotenoids, Linkage at molecular level, Amount of carotenoids ingested per meal, food Matrix, Effectors of carotenoid absorption and conversion, Nutrient status of the individual, Genetic factors, Host-related factors, and Interactions among these factors (short for SLAMENGHI) [20, 21]. Compared with β-carotene, the bioavailability of lutein supplied in a diet containing a large range of vegetables is much higher. The reason should be the presence of the hydroxyl groups in lutein, which makes it more polar and hydrophilic, leading to higher release of lutein into the aqueous medium. In addition, uptake of dietary fat together with lutein facilitated the formation of micelles and absorption of lutein in the gastrointestinal tract. It has been demonstrated that 3–5 g fat per meal is suitable to enhance the serum concentration of lutein [22]. However, lower bioavailability of lutein was observed when certain dietary fibers were present in foods. Sucrose polyester, a nonabsorbable fat substitute, impairs the ingestion of carotenoids such as lutein due to its preference for incorporation with nonabsorbable sucrose polyester rather than with micelles. The methods of food processing like heating, which improves release of lutein from food matrix, also influence the bioavailability of lutein. Furthermore, interactions between different types of carotenoids also affect the bioavailability. Studies have shown that lutein hampered the absorption of β-carotene, while β-carotene reduced the bioavailability of lutein [20].

3. Lutein and the eye

3.1. Lutein in the retina

The eye is made up of three separate layers, including the cornea and the sclera forming the outer fibrous layer; the uveal tract, which consists of the iris, ciliary body and choroid, forming the middle vascular layer; and the retina forming the inner neural tunic (**Figure 2**). In the central and posterior part of retina, there is an oval-shaped yellow area (approximately 5–6 mm in diameter) known as macula, which contains the highest concentration of photoreceptors. It is characterized by the yellow pigments that are entirely composed of lutein and zeaxanthin. The fovea, in the center of macula, is a small pit which is in charge of central vision and high-resolution visual acuity as a result of closely assembled cone cells. In addition, the retina consists of 10 layers from the outermost to the innermost, including RPE, photoreceptor cell layer, external limiting membrane (ELM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL), and internal limiting membrane (ILM).

Although MPs exhibit high concentration in the retina, the distribution varies in different regions of the retina. The highest concentration of MPs is observed in the fovea at about 0.1–1 mM, which is over 100-fold higher than the rest area of retina. Moreover, the ratio of lutein and zeaxanthin also differs in different parts of retina. In the peripheral retina, lutein is the major carotenoids and the ratio of lutein to zeaxanthin is 2:1, whereas the ratio is reversed to 1:2 in the fovea.

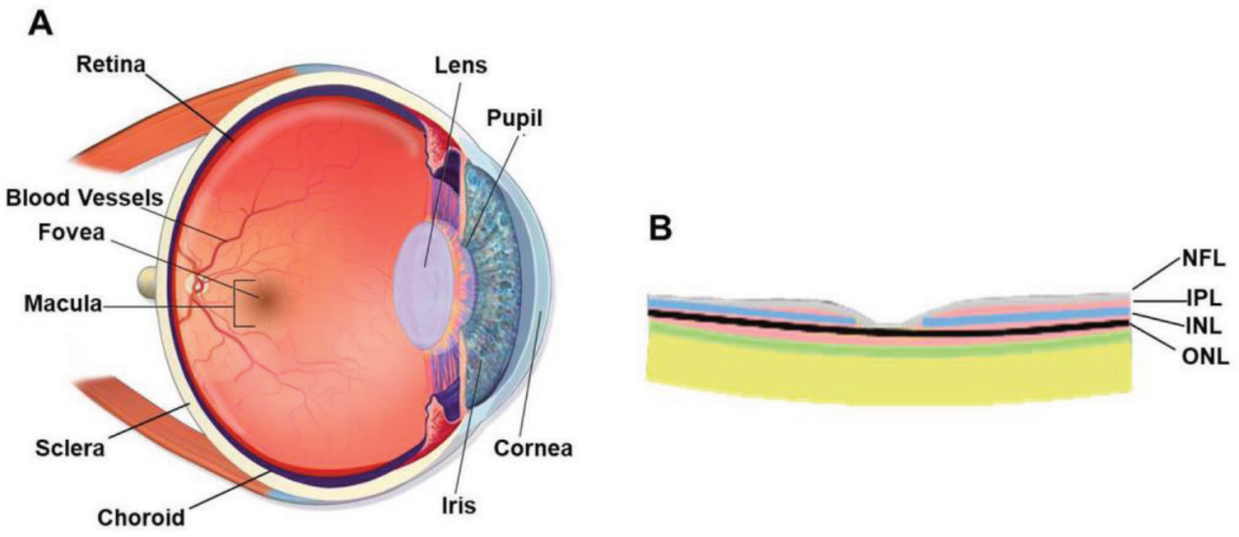

Figure 2. The human eye. (A) A schematic diagram demonstrating the anatomy of the human eye [23]. (B) A schematic image of optical coherence tomography (OCT) showing the vertical section of the center of the retina.

Of the 25 dietary carotenoids found in human tissues and blood, the selectively high rate of absorption and accumulation of lutein and zeaxanthin in human retina remained unclear until the discovery of specific macular carotenoid-binding proteins. Bernstein et al. have demonstrated that tubulin, a hydrosoluble protein, could bind to both lutein and zeaxanthin, and may be involved in the high distribution of MPs in the retina, but presented relatively low binding affinity and specificity. As a result, the research team continued to identify carotenoid-binding proteins with higher affinity and specificity. Subsequently, glutathione S-transferase P1 (GSTP1) was identified to bind zeaxanthin in the macula specifically compared to GSTM1 and GSTA1, the members of GST protein family [24]. GSTP1 was further confirmed to prevent lipid membrane from oxidation. In 2011, steroidogenic acute regulatory domain protein 3 (StARD3), one of lipid transfer-related protein family, was discovered as the lutein-binding protein [25]. Further studies need to be carried out to reveal more functions of StARD3. Generally, GSTP1 and StARD3 selectively bind zeaxanthin and lutein, respectively, leading to the high concentration and stabilization in human retina. In addition, the retinoid transporters including inter-photoreceptor retinoid-binding protein (IRBP) and retinol binding protein 4 (RBP4) are believed to be involved in the transport of MPs from circulation to retina [26].

3.2. Lutein and visual functions

3.2.1. Blue light filter

The peak value of MPs absorption is about 460 nm, which lies in the range of wave length of blue light (450–495 nm). Therefore, MPs can absorb 40–90% of incident high-energy, visible blue light depending on the concentration. The absorption offers protection from light-induced damages and reduction of light scatter in the retina. Junqhans A et al. [27] have investigated the efficacy of various carotenoids as the blue light filter using unilamellar liposomes with a

fluorescent dye which was excitable by blue light. Different carotenoids were incorporated with the lipophilic membrane, and fluorescence intensity was lower in carotenoid-containing liposomes than in control group when exposed to blue light, indicating a role of carotenoids as the blue light filter [27]. It is noted that lutein is more efficient in filtering blue light than zeaxanthin and meso-zeaxanthin because of the orientation in the biological membranes [27, 28].

3.2.2. Antioxidant function

A free radical is defined as a molecule, atom, or ion containing an unpaired electron. Because of the unpaired electron in the outer shell, the free radical is chemically highly reactive and unstable. Therefore, the free radical will react with other substances, even with themselves to reach steady state. Free radicals generated from oxygen are called reactive oxygen species (ROS), including superoxide anion (O_2^-), perhydroxyl radical (also known as hydroperoxyl radical, HO_2), and hydroxyl radical (OH) [29]. Superoxide can be inverted into only hydrogen peroxide (H_2O_2) and H_2O_2 together with singlet oxygen (non-radical compound) by enzymatic and non-enzymatic reactions, respectively [30]. Singlet oxygen, perhydroxyl radical, and hydroxyl radical are oxidants causing oxidation of protein, DNA as well as lipid peroxidation in cell membrane lipid bilayer, resulting in damages to the integrity of biological membrane and subsequently cell necrosis [29]. In physiological condition, production and detoxification of ROS are balanced in the body. However, when the balance is disrupted, no matter the increase in ROS generation or reduction of endogenous antioxidants, damages in the body occur. Thus it has been defined as "oxidative stress".

The retina is constantly exposed to ROS due to its high consumption of oxygen, conversion of light photons into electrochemical signals, and a number of mitochondria in rods. Massive blood supplies to the choroid in the retina make it the highest oxygen uptake tissue in the human body. Continuous exposure to the light photons, especially the blue light, triggers photo-oxidative reactions and damages DNA in RPE cells. Mitochondria, which are believed to be the major site for the generation of ROS, are rich in the inner segments of rod cells. It has been estimated that about 5% activated oxygen electrons in mitochondria could leak out as they go through the complicated electron transport chain, forming superoxide radicals [31]. Furthermore, a high content of polyunsaturated fatty acids in the outer segments of rods makes it more prone to peroxidation. In general, retina exhibits high susceptibility to ROS, resulting in irreversible oxidative damages.

Depending on the unique structure of MPs, one of the major biological functions of MPs in the retina is the prevention from oxidative damages via either physical quenching of singlet oxygen or chemical scavenging of free radicals. In the process of quenching of non-radical compound, such as singlet oxygen, the energy of singlet oxygen is transferred to the molecules of MPs, leading to excited triplet state of MPs and ground state of oxygen. Subsequently, the MPs in the triplet state dissipate the energy and return into the ground state. Since it is a physical mechanism, the structure of MPs is not changed, thus can be reused in the quenching cycles. It has been estimated that among carotenoids, lutein can react with singlet oxygen more strongly [32]. In contrast to physical mechanism, scavenging of ROS is achieved through chemical reactions in two ways. First, ROS accepts the missing electrons from MPs in which

electrons are available in the polyene chain, thus cannot induce oxidation of lipid, protein and DNA in cells. Second, lutein and zeaxanthin insert themselves into the cell membrane to pair the single electron in ROS, making the lipid bilayers more rigid. Lutein was found to insert into the biological membranes in perpendicular and parallel orientation, while zeaxanthin follows the perpendicular orientation in the lipid membrane [28]. It is the transmembrane alignment of MPs that reduce the susceptibility of lipid bilayers to oxidative injury and maintain the integrity and rigidity of biological membranes [33].

ROS is directly or indirectly involved in the most pathological processes observed in the retina, including inflammation, neuron degeneration, angiogenesis, or cell apoptosis. In the process of inflammation, excessive generation of ROS has been found to simulate many pro-inflammatory pathways. Moreover, oxidative injury is also associated with certain downstream signaling pathways in inflammation. Our research team has evaluated the anti-inflammatory effects of lutein in mouse model of ischemia/reperfusion and demonstrated that several pro-inflammatory factors, including nuclear factor-kappa B (NF-κB), interleukin 1β(IL-1β), and cyclooxygenase-2 (Cox-2), from Müller cells were significantly decreased in lutein-treated group when compared with control group, suggesting protection effects of lutein in retinal ischemia/reperfusion damage was achieved by its anti-inflammatory property [34]. Similarly, supplementation of lutein and zeaxanthin decreased NF-κB activity, while increased levels of erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1), which are the key factors to initiate phase II antioxidant protection to eliminate oxidative stress, in rats fed with high fat diet [35].

3.2.3. Other functions

In addition to the functions mentioned above, lutein also plays an important role in maintaining other visual performance. A huge number of studies have shown that lutein and/or zeaxanthin, or in combination with other antioxidants have improved visual acuity and contrast sensitivity in healthy, young adults, in subjects with AMD at early and/or advanced stage, and in people with diabetes. High levels of MPs have been reported to decrease the influence of bright lights via quick recover from bright lights and improvement of ability to see in glare conditions. Daily uptake of lutein (20 mg/day) for a year increased visual contrast and glare sensitivity in healthy Chinese drivers, thus benefiting driving or other vision-related tasks performed at night [17]. Furthermore, MPs are able to speed conversion of photic impulses into electrical impulses in retina as well as the transmission to the visual cortex in the brain by keeping neurons in healthy state [36].

Furthermore, lutein has been shown to have neuroprotective effects in the retina. We have reported that in mouse model of ischemia/reperfusion, lutein decreased the expression of nitrotyrosine, and nuclear poly(ADP-ribose) (PAR) in GCL and INL, which are the markers for oxidative stress; thus exhibited protection effects on cell loss and cell apoptosis in inner retinal neurons [37]. Similar results were observed in the cerebral ischemia/reperfusion injury [38]. We further used the in vitro model of oxidative stress and hypoxia to evaluate the neuroprotective function of lutein in retinal ganglion cells. Our data revealed that lutein could protect ganglion cells from either H_2O_2-induced oxidative stress or $CoCl_2$ (cobalt

chloride)-induced chemical hypoxia [39]. Moreover, in the treatment of $CoCl_2$ on Müller cells, lutein not only improved cell viability and enhanced cell survival but also inhibited the formation of autophagosome [40]. In the rat model of retinal detachment, lutein preserved cells in ONL and rhodopsin expression, indicating its neuroprotective and anti-apoptotic effects [41]. In general, lutein could protect retinal neurons from hypoxia-induced injury.

4. Lutein and AMD

4.1. AMD

AMD is the leading cause of visual impairment in people 65 years and above in developed countries. It is a slowly progressive disease that affects the central retina or macula. As estimated by the United Nations, approximately 20–25 million people are affected by AMD across the world, and the prevalence inevitably rises with the increasing of aged populations. By the end of 2020, it is expected that only in the USA, the number of AMD patients will reach to almost 3 million [42].

AMD is a complicated, multifactorial ocular disease, and the exact etiology still remains unclear. However, a number of risk factors are thought to be related to the pathogenesis of AMD. Of all those factors, age is the most obvious risk factor. Both the incidence and prevalence of AMD increase with age. Many investigators revealed that the family members of AMD patients were more prone to develop this disease, demonstrating the genetic factors in the genesis of AMD. Furthermore, the incidence in Caucasians is higher than that in other ethnic populations. There is no apparent sex preference in AMD patients, although some studies have indicated that women may be more susceptible [43]. In addition to the unmodified factors mentioned above, several other factors that can be modified are also involved in the pathogenesis of AMD. Smoking is considered as a frequent environmental risk factor, which is proved to double the AMD risk through increasing the oxidative stress in the macula [44]. Excessive exposure to the sunlight can lead to lipid peroxidation on cell membranes. Hypertension, overweight or obesity, poor nutrition status, and cardiovascular diseases are also correlated with AMD.

AMD is classified into a non-exudative or atrophic (dry) form, accounting for 90% of AMD, and an exudative (wet) form, accounting for only 10% of AMD. The atrophic form is characterized by the accumulation of drusen under the macula formed by photo-oxidation of lipids plus proteins, and progressive degeneration of RPE cells in the macula, affecting central vision to varying degrees. The exudative form is associated with choroidal neovascularization (CNV) in the submacular area and subsequent retinal hemorrhage, leading to severe central vision loss.

The most destructive type of AMD is the exudative or wet form because of the sudden loss of vision. Therapies for the wet form of AMD mainly focus on halting the progression of CNV, of which intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) drugs have been widely adopted by ophthalmologists as a standard treatment due to the up-regulation of

VEGF in the development of CNV. Although it has been proved that anti-VEGF compounds can restrict growth of abnormal blood vessels, therefore making vision stabilized or even improved, the cost of each injection is relatively high and monthly intravitreal injection may be required for some patients. In contrast, the current treatments for non-exudative AMD are very limited. Hitherto, no medicine has yet approved for dry AMD in the world. Hence, strategies to delay the onset of this severe visual loss have been focused on the decrease of modified risk factors. Among these modified risk factors, oxidative stress is recognized as one of the major contributing factors in AMD. Since lutein is a powerful antioxidant that is highly concentrated in the retina, the effects of lutein on AMD have been widely investigated.

4.2. Clinical trials

4.2.1. Observational studies (dietary intake and serum concentrations of lutein)

Initially, the relationship between dietary consumption of lutein plus zeaxanthin and AMD has attracted much attention from researchers. Although the results of these studies were not consistent, most of them have demonstrated that a high dietary intake of lutein and zeaxanthin is correlated with lower risk of AMD. A systematic review and meta-analysis was performed to analyze six longitudinal cohort studies and found that intake of lutein and zeaxanthin had different effects on early and late AMD [45]. Consumption of these dietary xanthophylls was strongly associated with the reduced risk of late AMD (relative risk [RR] 0.74; 95% confidence interval [CI] 0.57, 0.97) and neovascular AMD (RR 0.68; 95%CI 0.51, 0.92). However, an inverse relation was not observed between dietary intake of lutein plus zeaxanthin and the risk of early stage AMD. In the Age-Related Eye Disease Study (AREDS) report No. 22, 4519 subjects aged 60–80 years were included for the analysis of association between dietary lutein plus zeaxanthin and AMD status. Compared with the lowest quintiles of dietary lutein and zeaxanthin intake, there were a 55, 35, and 27% lower probability to develop geographic atrophy, neovascular AMD, and large or extensive intermediate drusen, respectively [6]. Similarly, in the Blue Mountains Eye Study, Tan and colleagues [46] evaluated dietary intake of different antioxidants in relation to the long-term risk of incident AMD in Australia, and indicated a 65% reduction in neovascular AMD between the individuals having highest and lowest uptake of lutein and zeaxanthin. The data from Rotterdam Study further revealed the influence of both genetic and environmental risk factors on AMD, demonstrating a protective role of high intake of dietary antioxidants including lutein and zeaxanthin, β carotene, omega-3 fatty acids, and zinc, in AMD at early stage [47].

As early as 1993, Eye Disease Case-Control Study (EDCCS) has reported the direct correlation between serum levels of lutein plus zeaxanthin and AMD risk, demonstrating a distinct risk reduction of neovascular AMD to one-third in subjects with highest serum concentration of lutein and zeaxanthin when compared to those in the lowest group [48]. The research performed by Delcourt et al. [49] has further confirmed that AMD was significantly related with plasma lutein and zeaxanthin and tended to be associated with plasma lutein. A recent study carried out in an Irish population-based sample was in accord with the results discussed above, presenting a lower plasma concentration of lutein in AMD patients no matter whether they were aware of their suffering from AMD or not [50].

4.2.2. Observational studies (MPs levels in the retina)

In addition to dietary intake and serum concentration of lutein and zeaxanthin, MPs level in the retina was also inversely associated with the risk of AMD. In a case-control study, the actual amounts of lutein and zeaxanthin in donor retinas with and without AMD were measured. Levels of lutein and zeaxanthin in three concentric areas (inner, medial, and outer) centered on the fovea were markedly lower in AMD donor retinas than these in control donor retinas, especially in the outer area, where logistic regression analysis suggested that donors in highest quartile of MPs levels had an 82% lower risk for AMD when compared with those in the lowest quartile after adjustment of age and sex [51]. This is the first report showing the decreased retinal levels of lutein and zeaxanthin in AMD patients, which was consistent with above findings concluded from diet and serum xanthophylls concentrations. Subsequently, MPOD, an indicator for MPs levels in retina in vivo, has been widely studied between healthy individuals and AMD patients. There was a MPOD decline in healthy eyes as the individuals aged, and MOPD in healthy eyes at high risk of AMD was significantly lower than those at no such risk [52, 53]. Moreover, Bernstein and his co-workers [54] evaluated MPs levels in relation to the incidence of AMD using noninvasive resonance Raman spectroscopy, and found 32% reduction of retinal lutein and zeaxanthin levels in AMD versus normal participants. However, it was notable that lower MPOD has also been linked with other risk factors for AMD, such as smoking and family history of this disease [55]. This result further supported the hypothesis that lutein and zeaxanthin could prevent or delay the development of AMD by increasing MPOD.

4.2.3. Interventional studies (supplementation of lutein)

Observational results in relation to AMD have triggered a mass of interests in assessing effects of lutein supplementation on the risk of AMD. The supplementation trial was first reported in the Lutein Antioxidant Supplementation Trial (LAST) study [56]. This was a prospective, double-masked, placebo-controlled, randomized study to evaluate supplementation of lutein alone or lutein with other antioxidants, vitamins, and minerals in 90 atrophic AMD patients. After 12 months, higher MPOD, improved visual acuity and contrast sensitivity were observed in both of these groups than in placebo group. However, longer duration of the study, larger number of samples, and both genders are needed to examine the long-term effects of lutein or the combination of lutein with other nutrients in the treatment of dry AMD. Three years later, LASTII was performed to further analyze the specific factors that affected MPOD, including age, baseline levels of MPs, and combination of lutein and other antioxidants [57]. There was an increase in MPOD with supplementation, while a moderate reduction of MPOD was observed without supplementation. Patients with lowest baseline MPOD value were most likely to have a dramatic increase in MPOD than those with medium to high baseline MPOD during one-year supplementation of lutein or lutein with other nutrients. The reason might be the saturation mechanism that had an impact on the retinal transportation and stabilization of MPs.

In the Combination of Lutein Effects in the Aging Retina (CLEAR) study, Murray et al. [58] supplemented the patients at early stage AMD with 10 mg lutein esters per day for up

to 1 year. MPOD increased significantly after 8 months of supplementation, and plasma concentration of lutein increased by 1.8-fold to 7.6-fold compared to the baseline values. In addition, visual acuity in lutein group remained stable while the declined visual acuity was exhibited in the placebo group, indicating that stabilization of visual acuity was probably maintained by the elevated MPs level. These results were in accord with the study carried out by Ma et al. [13, 59], who demonstrated the significant improved responses of multifocal electroretinogram (mfERG) in lutein group and in lutein plus zeaxanthin group, and tended to be related to the increase of MPOD.

In a randomized controlled clinical trial (known as the Carotenoids with Coantioxidants in Age-Related Macular Degeneration [CARMA] study), 433 patients who were identified to be at highest risk of progression to advanced AMD received a daily supplementation of lutein, zeaxanthin, vitamin C, vitamin E, zinc, and copper at the duration of 12–36 months [60]. Visual acuity was increased in the intervention groups at 12 months, but not statistically significant until 24 months. Contrast sensitivity was slightly improved without significance. Level of MPs declined steadily in the placebo group, while MPs was increased in the supplemented groups throughout the whole trial. A rise of plasma concentration of all contents in the supplementation, especially lutein and zeaxanthin, was observed after 6 months. Although the increase of all antioxidants in blood was not associated with VA improvement, higher serum level of lutein slowed the progression of AMD. Fewer eyes progressed to severe state in the intervention group than in the placebo group (15.3 vs. 18%).

The Age-Related Eye Disease Study 2 (AREDS2) was a randomized, placebo-controlled, double-masked trial conducted in the USA from 2006 to 2012 [61]. The participants involved in AREDS2 were subjects aged 50–85 years at risk for progression to advanced AMD with bilateral large drusen or large drusen in one eye and advanced AMD in the fellow eye. The main objective of AREDS2 was to evaluate the effects of lutein, zeaxanthin, and omega-3 long-chain polyunsaturated fatty acids adding into the AREDS formulation, which was composed of vitamin C (500 mg), vitamin E (400IU), β-carotene (15 mg), and zinc (80 mg zinc oxide) with copper (2 mg cupric oxide). After the follow-up of 6.5 years on average, the AREDS supplements was proved to significantly decrease the development to advanced AMD, and an approximately 25% reduction in risk of progressing to late AMD was observed at 5 years [62]. Moreover, the beneficial effects of this AREDS formula were found to persist for 5 more years of follow-up after the end of this trial [63]. However, supplementation of β-carotene may lead to the increased risk of lung cancer in cigarette smokers [64, 65]. In addition, 80 mg/day zinc is out of tolerance for individuals and high amount of zinc was associated with increased genitourinary complications [62, 66]. Therefore, AREDS2 supplementation was changed as follows: the primary randomization was composed of AREDS formulation with (1) lutein (10 mg) + zeaxanthin (2 mg), (2) fish oil (350 mg DHA + 650 mg EPA), (3) lutein + zeaxanthin + EPA + DHA, and (4) placebo; the secondary randomization included (1) AREDS formulation, (2) AREDS formulation with low zinc (25 mg), (3) AREDS formulation without β-carotene, and (4) AREDS formulation with low zinc (25 mg) and without β-carotene. Former and current smokers are randomly assigned to the groups without β-carotene. In the primary analysis, no further reduced risk of developing advanced AMD was observed when comparing each of the treatment groups with placebo group [61]. Although the preconceived goal of 25% incremental

improvement over the original effective AREDS formulation was not achieved, analyses of patients with lutein and zeaxanthin supplements versus those without lutein and zeaxanthin supplements demonstrated a 10% decrease in the risk of progression to advanced AMD in the group with lutein and zeaxanthin [67]. Furthermore, the analyses of comparing participants receiving lutein and zeaxanthin with those receiving β-carotene were performed. The risk of developing advanced and neovascular AMD was significantly decreased in the group with lutein and zeaxanthin. In analyses restricted to eyes with bilateral large drusen at baseline, protective effects of lutein and zeaxanthin were more prominent. Therefore, considering beneficial effects of lutein and zeaxanthin as well as harmful effects of β-carotene on smokers, replacement of β-carotene with lutein and zeaxanthin in AREDS2 formula is preferred.

4.3. Basic research

Several animal models that mimic the pathological changes in AMD have been adapted to further study effects of lutein and zeaxanthin on AMD in human. Apolipoprotein E-deficient mice (apoE-/-), a well-established genetic mouse model of hypercholesterolemia, exhibited deposits on the basal laminar, vacuoles in RPE cells, and increased Bruch's membrane thickness, which are similar to the retinal changes in human AMD. These alterations were associated with the elevation of retinal lipid peroxidation and VEGF expression [68]. Administration of lutein alone could partially prevent the retinal alterations, and decrease expression level of VEGF but with no statistical significance was observed in comparison with controls. However, the combination of lutein and multivitamin and glutathione complex ameliorated all the morphological changes observed in retina and decreased VEGF levels significantly [68, 69]. In the mouse models that show similar retinal changes in human dry AMD, AREDS2 formulation prevented accumulation of liposomes and lipofuscin in RPE, loss of photoreceptors, and increased ONL thickness. In molecular level, mRNA expression levels of pro-inflammatory factors including inducible nitric oxide synthase (iNos), tumor necrosis factor-α(TNF-α), Cox-2, IL-1β, and angiogenic factors such as VEGF was significantly lower in AREDS2-treated group than control groups [70]. Furthermore, supplementation of lutein and zeaxanthin from grapes or marigold extract attenuated a reduction of a-wave amplitude in ERG, suggesting protective effects on photoreceptor functions [71]. Mouse model for the wet form of AMD is induced by laser photocoagulation, characterized by the formation of CNV. It has been reported that pretreatment of lutein significantly inhibited macrophage infiltration in CNV and expression of pro-inflammatory molecules such as NF-κB that subsequently resulted in significant suppression of CNV development [72].

Data from in vitro studies were also consistent with findings from animal experiments. Addition of lutein and other antioxidants (zeaxanthin, lycopene, or α-tocopherol) led to a significant decrease in formation of lipofuscin in RPE cells from bovine and rabbit under hypoxia condition [73]. Oxidative damages in ARPE-19 cells (a human RPE-derived cell line) were induced by the challenge of H_2O_2, leading to decreased cell viability, increased cell apoptosis, and ROS generation. Pretreatment of lutein protected ARPE-19 cells from these oxidative injuries and accumulation of Alu RNA, which is related to the pathogenesis of AMD [74, 75]. In addition, G2/M phase arrest triggered by oxidative damage was reversed by lutein in a dose-dependent manner [75].

5. Lutein and DR

5.1. DR

DR is the most common microvascular complication in diabetes. For individuals with diabetes aged 40 years and older, the estimated number of DR patients is 93 million around the world, of which 17 million are proliferative DR and 28 million are vision-threatening DR [76]. In the USA, approximately 2.8 million individuals may develop sight-threatening DR. DR used to be considered as high of prevalence in western countries, however, there is a rising prevalence of DR occurred in Asian countries (such as China and India) due to the changes in economics, diet habit, physical exercise, and so on.

According to the presence of microvascular lesions in the retina, DR is classified into early nonproliferative stage, featured with microaneurysms, vascular tortuosity, retinal hemorrhages, "hard" lipid exudates and microinfarcts in the NFL (known as the "cotton wool spots"), and late proliferative stage, characterized by the formation of new aberrant fragile blood vessels in the retina. Another important manifestation of DR is diabetic macular edema present at any stage, causing the abnormal thickening of retina and cystoid edema in the macula. Diabetic macular edema, together with retinal neovascularization, is the major cause of vision loss in patients with diabetes.

DR is considered to be a multifactorial disease with its exact pathogenesis being still uncertain. It has been proved that increased blood glucose concentration is the key factor in the onset and development of DR, leading to exacerbation of hypertension and dyslipidemia, overproduction of ROS that subsequently damages the retina. Oxidative stress disrupts retinal mitochondrial functions by inner membrane oxidation and mitochondrial DNA damage, which in turn lead to apoptosis of retinal capillary cells [77]. In addition, inflammation is also involved in the pathogenesis of DR. Increased retinal pro-inflammatory mediators such as intracellular adhesion molecule-1 (ICAM-1), TNF-α, and IL-1β are induced in diabetes. In clinical studies, presence and progression of DR were associated with the increased plasma levels of TNF-α, IL-1β and VEGF [78]. VEGF is also an important factor in the development of DR, which leads to the increased permeability of retinal blood vessel and angiogenesis.

Current major treatments for DR include laser photocoagulation and/or intravitreal injection of anti-VEGF drugs. However, these therapies are expensive, invasive, and need to visit ophthalmologists at certain intervals. Therefore, lutein, a powerful antioxidant, may be adopted as a natural, noninvasive, long-term medication for DR.

5.2. Clinical trials

Although tremendous clinical studies have been performed to evaluate the relationship between carotenoids and diabetes, only a few have examined the effects of carotenoids including lutein and zeaxanthin on DR.

In a prospective study, Hu et al. [7] demonstrated that plasma levels of lutein and zeaxanthin was significantly lower in nonproliferative DR patients than in subjects without diabetes. Similarly, Brazionis et al. [79] assessed the serum concentration of different carotenoids in relation to the DR in type 2 diabetes and found significant lower blood levels of combined lutein and zeaxanthin and lycopene in diabetic patients with DR compared to these without DR. Moreover, a significant inverse correlation between odds of DR and plasma concentration of combined lutein and zeaxanthin and lycopene was shown in this study [79].

Retinal level of xanthophylls was examined via measurement of MPOD. Patients with DR had decreased MPOD in comparison with diabetic patients without DR [80]. Furthermore, MPOD in individuals with type 2 diabetes was significantly lower than in subjects with type 1 diabetes and normal control, despite similar dietary uptake of carotenoids among these groups [81]. The lower MPOD in patients with type 2 diabetes may probably be associated with obesity, where enhanced competition of lutein and zeaxanthin intake with higher body fat occurred (retina vs. adipose tissue). In type 2 diabetic patients with or without DR, MPOD was inversely associated with glycosylated hemoglobin, a more stable indicator for diabetes [82].

On basis of the above observational studies, influence of lutein and zeaxanthin supplementation on DR was evaluated. Administration of lutein (6 mg/day) plus zeaxanthin (0.5 mg/day) for 3 months led to a significant increase of serum lutein and zeaxanthin level, as well as the improvement in visual acuity, contrast sensitivity, and diabetic macular edema in nonproliferative DR [7]. This study was consistent with the results conclude from supplementation of 10 mg/day lutein for 36 weeks in patients with nonproliferative DR [83]. A recent study has shown an increased thickness in the central fovea and improved retinal response density after 2-year supplementation of combined lutein (10 mg/day), zeaxanthin (2 mg/day), and mesozeaxanthin (10 mg/day) in type 2 diabetic patients without DR, indicating beneficial effects of xanthophylls on visual functions in diabetes [84].

5.3. Basic research

In animal models of DR, lutein has been reported to have beneficial effects on affected retinal layers and visual functions by its antioxidant, anti-inflammation, and neuroprotection properties. The animal models used to study DR usually include mice or rats injected with alloxan or streptozotocin (STZ) that can directly destroy β cells in pancreas to halt insulin production and subsequently induce diabetes, and spontaneous diabetic mice (db/db mice), a type 2 diabetic animal model.

In alloxan-induced DR mice, oxidative makers (NF-κB and malondialdehyde) were increased, while antioxidants including glutathione (a powerful endogenous antioxidant) and glutathione peroxidase were decreased. Decreased b-wave amplitude in ERG was also observed. Supplementation of lutein (0.2 mg/kg) prevented all the diabetes-induced changes [85]. The same results were reported in STZ-induced diabetic rats after administration of lutein together with DHA. Moreover, prevention of histological changes including

decreased ONL, INL, and GCL thickness was also observed [86]. In STZ-induced murine models, Sasaki et al. [87] demonstrated changes in oxidative stress-related factors (increase of ROS, extracellular signal-regulated kinase activation, and depletion of brain-derived neurotrophic factor), retinal morphological changes (reduction of IPL, INL, and ganglion cells), and visual functions (decrease of oscillatory potentials in ERG, indicating dysfunction of neurons in inner retina). Likewise, lutein supplements (0.1 mg wt/wt) restored all the diabetes-induced damages in the retina [87]. Similarly, supplements containing lutein, zeaxanthin, omega-3 fatty acids, and other nutrients demonstrated protective effects on progression of DR in STZ-induced diabetic rats. Decreased ROS level, mitochondrial DNA damage, and inflammatory factors such as VEGF, IL-1β, and NF-κB, as well as reduction of retinal apoptosis, abnormal capillaries formation were demonstrated in treatment group compared with placebo control group. Furthermore, nutrient supplements ameliorated decreased amplitudes of a- and b-wave in ERG, suggesting prevention of retinal functions in diabetic rats with DR [88].

Wolfberry, a Chinese traditional fruit consumed for eye protection, is high in zeaxanthin (176 mg/100 g) and lutein (5 mg/100 g). In db/db mice, wolfberry elevated lutein and zeaxanthin levels in retina and liver, attenuated mitochondrial dysfunction and endoplasmic reticulum stress caused by hyperglycemia-induced oxidative stress, and restored retinal structure abnormalities [89, 90]. Furthermore, Lutein and zeaxanthin was able to protect cultured ARPE-19 cells from a high glucose challenge through the similar mechanisms, suggesting wolfberry's protection effects were at least partly due to high contents of lutein and zeaxanthin [89].

6. Conclusions

Lutein, synthesized in plants but not in mammals, is absorbed and highly accumulated in the macula. The uneven distribution of lutein is thought to afford a distinct function in the retina. Up to now, numerous epidemiological studies have demonstrated that higher levels of lutein in diet and plasma are correlated with lower risk of AMD, especially the late stage of AMD. Randomized and controlled clinical trials such as AREDS2 have reported that supplementation of lutein alone or with other nutrients leads to the increase of MPOD, improvement of visual functions, and decreased risk of progression to advanced AMD, especially the wet AMD. Laboratory experimental data also indicate that lutein can protect impaired retina by filtering blue light, attenuating oxidative stress and inflammation, and enhancing neuroprotection. However, the optimal dose of lutein, the best ratio of lutein and other antioxidants, therapeutic effects at different stages of AMD, adverse effects with even longer intake of lutein supplements in high dose, and the relationship between MPOD and AMD at different phases need to be further investigated in future studies. Although there are several studies assessing the effects of lutein on DR in clinical trials and laboratory experiments, further evaluations to fully understand its protective role in DR are necessary.

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Conflict of interest

The authors declare no conflict of interest.

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