Chapter

Management of Diabetic Eye Disease Using Carotenoids and Nutrients

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Abstract

Diabetic retinopathy is the leading cause of blindness and visual disability globally among working-age adults. Until recently, diabetic eye disease is primarily regarded by its microvasculature complications largely characterized by progressive retinopathy and macular edema. However, a growing body of evidence suggests that hyperglycemia-induced oxidative stress and inflammation play an integral role in the early pathogenesis of diabetic retinopathy by potentiating retinal neurodegeneration. The onset of type 2 diabetes mellitus starts with insulin resistance leading to insulin deficiency, hyperglycemia, and dyslipidemia. Which in turn enhances the pro-oxidant and pro-inflammatory pathways. Additionally, various poor dietary behaviors along with obesity worsen physiological state in diabetics. However, decreased levels and depletion of the endogenous antioxidant defense system in the retina can be sufficiently augmented via carotenoid vitamin therapy. Therefore, dietary supplementation of antioxidant micronutrients particularly macular carotenoids lutein, zeaxanthin and *meso*-zeaxanthin that promote retinal health and optimal visual performance, may serve as an adjunctive therapy in the management of diabetic eye disease.

Keywords: carotenoids, macular pigment, macular pigment optical density, MPOD, lutein, zeaxanthin and meso-zeaxanthin, diabetes, diabetic eye disease, diabetic retinopathy

1. Introduction

The prevalence of diabetes is endemic in the United States and developed countries. According to the 2018 reports it is estimated that the United States has more than 31 million adults diagnosed with diabetes [1]. Diabetes prevalence remains underestimated with approximately one in four individuals that have diabetes are undiagnosed [1]. There are various forms of diabetes and individuals with Type 2 diabetes (T2DM) account for 90–95% of all cases of diabetes within the US [1]. The incidence of diabetes is also likely to increase with 88 million individuals are diagnosed to be pre-diabetic who have potential ongoing subclinical damage [1]. The prevalence of diabetes mellitus in the US is predicted to reach 36 million by the year 2045 and will continue to pose a significant global health problem [2].

Nearly half a billion people are currently living with diabetes globally, and the total number of cases is projected to surge by 25% (578 million) in 2030 [3, 4] and to 700 million by the year 2045 [3–5]. Diabetes is severely underdiagnosed condition with one in two people (50.1%) currently living with the condition are unaware [3, 4] . The International Diabetes Foundation estimates the socioeconomic burden of diabetes to be USD 760 billion and potentially increase to USD 845 billion by 2045 [2]. The global estimates of socioeconomic burden are predicted to rise in response to the increasing prevalence of diabetes, improved survival rates (longer life expectancy with the condition), and consequently prolonged duration of diabetes mellitus [3, 4, 6–8].

Diabetic retinopathy (DR) is characterized by the hallmark feature of retinal capillary degeneration that could lead to, significant visual impairment. The natural history of unmanaged or poorly managed diabetic retinopathy leads to proliferative retinopathy (PDR) and/or macular edema [9, 10]; contingent upon the disease-severity, these complications may arise individually or simultaneously. DR affects roughly one in three individuals with diabetes and its severity is closely linked to both the duration of diabetes and the glycemic load [5, 6, 11, 12]. It is estimated that 4.1 million individuals in the US are afflicted with DR, and 899,000 of which are affected by vision-threatening retinopathy [1]. The global prevalence of DR is estimated to affect 146 million adults and projected to reach 191 million by 2030 [3, 4, 8]. Currently, DR remains a leading cause of irreversible, yet preventable, vision loss among adults and is associated with a poorer quality of life, increased susceptibility for developing further complications, and considerable rise in healthcare expenditures [5, 12].

1.1 Diabetic retinopathy

Clinically, retinopathy is routinely graded upon its presenting clinical features during ophthalmic examination in accordance with the International Clinical Disease Severity Scale for DR [7, 10, 13–16]. The five-stage disease severity classification system (**Table 1**) was created using prior clinical trials: the Early Treatment Diabetic Retinopathy Study (ETDRS) and the Wisconsin Epidemiological Study of DR (WESDR) [14–17]. The stages of non-proliferative diabetic retinopathy (NPDR) are based on the severity of microvascular abnormalities limited to the surface of the retina; in addition to reflecting the patients' risk of developing more

Disease Severity Scale	Clinical Features
No apparent retinopathy	No fundus abnormalities
Mild NPDR	Microaneurysms only
Moderate NPDR	More than just MAs, but less than severe NPDR
Severe NPDR	Any of the following: (with no signs of PDR) extensive DBH in each of 4 quadrants (+20/quadrant), venous beading in at least 2 quadrants, and/or IRMA in at least 1 quadrant
PDR	One or more of the following: neovascularization, tractional retinal detachment, or vitreous/preretinal hemorrhage

NPDR = non-proliferative diabetic retinopathy; MA = microaneurysms; PDR = proliferative diabetic retinopathy; DBH = dot blot hemorrhages; IRMA = intraretinal microvascular abnormalities.

Table 1.
International clinical disease severity scale for diabetic retinopathy [14].

advanced, vision-threatening retinopathy. Examination of NPDR by ophthalmoscopy may reveal the presence of microaneurysms, hard exudates, intraretinal hemorrhages ("dot and blot" shaped), and intraretinal microvascular abnormalities (such as tortuous sinus shunt vessels) [14, 15, 17].

Progressive oxidative injury in NPDR is evident within the vasculature, by the presence of acellular capillaries and endothelial apoptosis. This injury is further exaggerated within local tissue by the onset of capillary nonperfusion and vascular occlusion that may develop in the disease [13, 18, 19]. The resultant retinal injury due to ischemia further exacerbate pro-oxidant and pro-inflammatory mechanisms by compromising oxygenation of the metabolically demanding retinal neurons. This in turn promotes angiogenesis through the release of vascular endothelial growth factor (VEGF) [13, 18–20]. The retinal neurodegeneration induced by hypoxia can be observed by the presence of abnormal fluffy white patches known as cotton wool spots, upon fundoscopic examination [19]. The ensuing retinal neovascularization indicates the clinical progression of NPDR into the advanced-stages of PDR. These aberrant new blood vessels are fragile and ineffective in restoring tissue perfusion because they grow from the retinal surface and towards the posterior pole of the vitreous cavity [7, 10, 13, 16]. Thus, subsequent risk of acute vision loss is evaluated on the extent of neovascular proliferations, particularly on/near the optic disc, which perniciously emanate from the steady vasculature of the retina.

Diabetic macular edema (DME) develops when fragile or damaged capillary beds leak and cause thickening of macula due to fluid accumulation. Alterations in the microvasculature such as endothelial cell proliferation and retinal pericyte necrobiosis gradually enhance the vascular permeability, which ultimately causes the breakdown of the blood-retinal barrier [13, 18]. The progressive deposition of fluid and proteins can amass on or under the macula which can be clinically examined by optical coherence tomography (OCT), identifying areas of diffuse retinal thickening or hard exudates, indicating the extent of focal leakage [21, 22]. Alternatively, onset of macular edema can occur during any stage of retinopathy (NPDR or PDR) leading to vision loss [6, 13].

Traditionally, diabetic eye disease has largely been considered to be a microvascular end-organ complication of diabetes mellitus. The severity of retinopathy correlates with the susceptibility of further complications such a peripheral neuropathy, nephropathy and cardiovascular disease [6, 11, 12, 23–25]. It is established that chronic hyperglycemia promotes oxidative injury in the highly-susceptible retina; in part due to high metabolic demands and constant light exposure [26, 27]. However, a growing body of evidence strongly implicates retinal neurodegeneration is potentiated by pro-oxidant and pro-inflammatory processes during the early pathogenesis of DR. Indications of retinal dysfunction that can be detected in diabetic patients even prior to manifestation of clinical signs of retinopathy [6, 12, 19, 25, 27–31]. Appropriately, the American Diabetes Association defines DR as a highly tissue-specific neurovascular complication and has identified several modalities of management of disease and its progression [7, 11, 28].

The body's inherent defense against oxidative damage, involving the neutralization of reactive oxygen species (ROS), relies upon the interplay between both endogenous and exogenous antioxidants to maintain redox homeostasis [27, 32]. The interdependence between hyperglycemia, oxidative stress, and changes in redox homeostasis are essential facets in the pathology of diabetic retinopathy [26, 32]. In particular, exogenous antioxidants such as vitamin C, vitamin E and xanthophyll carotenoids (including lutein, zeaxanthin and *meso*-zeaxanthin) possess significant antioxidant and anti-inflammatory effects on the retina [32]. Studies have demonstrated the clinical benefits in visual performance associated with dietary supplementation of carotenoids and antioxidants, such as the Age-Related Eye

Disease Study 2 (AREDS-2) and various other studies [33–36]. Despite similarities in pathogenesis of macular degeneration and diabetic retinopathy involving oxidative damage in the retina, only a limited number of studies have concentrated on the relationship between dietary carotenoids intake to influence pathophysiology in diabetes mellitus.

Recently, the onset of diabetes in experimental murine models consistently demonstrated a significant increase in pro-oxidant and pro-inflammatory molecules, such as malondialdehyde, oxidatively damaged DNA, and VEGF [37–45]. Importantly, administration of antioxidants including lutein and/or zeaxanthin was demonstrated to effectively prevent, and in some cases reverse, the hyperglycemia-induced changes in oxidative stress and inflammation [37–42, 45]. The beneficial effects of lutein and zeaxanthin were shown to augment the endogenous antioxidant defense system by improving retinal concentrations of glutathione (GSH) and glutathione peroxidase (GPx) [37–39, 42]. The antioxidant and anti-inflammatory effects of lutein and zeaxanthin were also shown to attenuate the microvascular abnormalities that characterize DR pathology, in addition to protecting the retina against accelerated vasoregression that may proceed alterations in the vasculature [37–44, 46].

To date, only a limited number of observational [26, 32, 47–53], and randomized-controlled trials [35, 54] have investigated the association of macular pigment optical density (MPOD) levels in diabetic eye disease. Generally, the evidence suggests that MPOD levels are lower in individuals with diabetes when compared to healthy controls [48, 51], and some studies indicate MPOD status may differ between types of diabetes (type 1 and type 2) [26, 32, 50]. MPOD depletion was also negatively correlated with the presence of retinopathy in T2DM [26, 32, 50] and may be attributed in part due to oxidative stress [49]. These findings are promising and begs for additional investigation to substantiate the beneficial role of carotenoid vitamin therapy in the management of diabetic eye disease.

2. Biomarkers and its importance in clinical care

Sensible, relatively inexpensive techniques to evaluate the status of macular carotenoids can serve as important biomarkers in monitoring retinal health in individuals with diabetes and increased risk of retinal neurodegeneration. Biomarkers serve as important tools with significant potential for innovating novel drugs and substantiating the safety and efficacy of available therapies [55, 56]; however, their application is not limited to clinical research and extends into improving clinical practice and establishing public health guidelines. The concept of biomarkers is delusively simple, with which a single biomarker may satisfy the criteria for several different purposes; therefore, it is critical to establish scientific justification how a particular biomarker will be defined according to its situation-specific application. Thus, several categories of biomarkers have been established by the FDA-NIH's "Biomarkers, EndpointS, and other Tools (BEST)" resource, described in more detail elsewhere [55, 57]. Moreover, by establishing the context of use, this directly expounds the nature, objective and methodology intended for utilizing a biomarker within a particular setting [55, 57, 58].

Advancements in retinal imaging modalities have allowed MPOD status to serve as a biomarker in multiple settings for diabetic retinal disease, including: (1) prognostic biomarker for screening individuals with sub-clinical disease with no overt retinopathy; (2) identification of surrogate biomarkers for the prediction of low MPOD in T2DM; and (3) monitoring biomarker for evaluating the efficacy of carotenoid supplementation on DR. The depletion of MPOD in diabetes has been

consistently reported in a number of cross-sectional studies, and some suggest that low MPOD may be a potential clinical feature of T2DM [26, 32, 48–51]. Studies have demonstrated significant correlations between MPOD and central subfield thickness, retinal volume, and photoreceptor outer segment length in diabetic and healthy controls [59–61]; thus, clinical measurements of MPOD levels may serve an important role in early-detection of retinal neurodegeneration and prognosticating treatment outcomes. Furthermore, one study identified possible surrogate biomarkers including smoking status, hypertension, and vitamin D insufficiency, that may predict low MPOD in T2DM [32]. Alternatively, serial MPOD measurements have been used as a monitoring biomarker to assess the benefits of the antioxidant micronutrients on visual performance and features of NPDR in Type 1 (T1DM) and T2DM [35]. Based on the systematic review conducted MPOD is found to be a prognostic, surrogate and monitoring biomarker as defined by the FDA-NIH [55].

3. Role of MPOD in the management of diabetic eye disease

3.1 MPOD basics

The macular pigment is comprised of three lipid-soluble carotenoids: including lutein, zeaxanthin, and *meso*-zeaxanthin [62, 63]. They are responsible for the fovea's yellow pigmentation and are densely concentrated within the photoreceptor axons, the inner plexiform layer and the outer plexiform layer at the center of the macula [62–66]. The carotenoids, lutein and zeaxanthin, cannot be synthesized *de novo* within the eye, and can only be acquired through dietary intake; found primarily in leafy green vegetables, like spinach and kale, and egg yolks [63, 67–69]. A biochemical isomer of zeaxanthin called *meso*-zeaxanthin is found in the macula. *Meso*-zeaxanthin in the eye is a byproduct of conversion of lutein in the retinal pigment epithelium (RPE). Several studies have demonstrated that oral supplementation of these carotenoids can greatly improve their levels within the serum [63, 67, 70] and can be retained in the human retina for a sustained period of time [71].

Macular carotenoids are quantified by the macular pigment optical density (MPOD) and are associated with maintaining retinal health and optimal visual performance; suggesting that MPOD levels may serve as an important biomarker in health and diseased states [63, 65, 69, 72]. Research suggests that carotenoids serve to protect the retina, specifically the macula, via two proposed methods: 1) they act as a filter against blue light, and 2) they reduce oxidative stress and inflammation in the retinal tissue [63, 69, 72–76]. The macular pigment attenuates the amount of blue light that reaches the photoreceptor cells, due to the peak wavelength of MPOD's absorption spectrum (peak ~460 nm) which lies within the range of blue light on the visible light spectrum (400-500 nm); and may provide some preservation and improvement in visual function [62, 76, 77]. Short wavelengths of blue light are of high energy, which can prompt the formation of ROS and induce oxidative injury; causing damage to the lipid bilayer of cell membranes, proteins and DNA, and cause mitochondrial dysfunction leading to cellular necrosis [63, 74–78]. Thus, the neuroprotective capabilities of the macular carotenoids in the retina, namely MPOD levels, have led researchers to further investigate the role of MPOD levels and its depletion in various ocular diseases.

3.2 Measuring MPOD

Techniques to quantify MPOD levels may also serve as susceptibility/risk biomarkers for diabetic eye disease, prior to indications of retinopathy that become

clinically evident. Meanwhile, more expensive and advanced imaging modalities such as OCT, can play a more significant role in prognosticating outcome or determining course of treatment [79]. Several methods have been described aiming to effectively quantify levels of MPOD non-invasively in clinical settings; categorized by either psychophysical (subjective) or objective techniques [65, 69, 80]. In brief, these techniques are differentiated by patient-response, or participation required from the individual being evaluated, and requiring minimal participant-involvement to collect measurements, respectively [65, 69, 80, 81].

The clinical measurements of MPOD levels are primarily heterochromatic flicker photometry (HFP) [65, 69, 80, 82–84]. HFP technology is based on a stimulus of light, alternating between two wavelengths, that differ according to the retinal absorption spectrum of macular pigments (400-540 nm); a short-wavelength blue light maximally absorbed by the pigments, and a longer-wavelength (green) stimuli with minimal absorption [65, 69, 81]. Briefly, current HFP devices collect measurements in the fovea by adjusting the intensity of the target-stimuli, which is perceived as flickering light, according to the participant's involvement indicating the appearance of flickering light; estimating the level of MPOD as the difference in responsive sensitivity (of blue- and green-wavelength flicker) required at the fovea [65, 69, 81, 83, 85–87]. Thus, individuals with higher MPOD would require greater intensity blue light (perceive less blue light) at foveal measurements as a result of higher concentrations of macular pigment in the fovea [65, 69, 81, 88].

Objective techniques such as reflectometry [89–94], fundus autofluorescence [95–97] and resonance Raman spectroscopy [66, 98, 99], are all non-invasive, *in vivo* imaging modalities that can quantitatively measure levels of macular pigment [69, 80]. Briefly, measurements collected by fundus reflectance can be performed with a digital fundus camera integrated with a reflection photometer or a spectrometer to quantify and analyze the light reflected from the retina and choroid [88, 90, 92, 93, 96, 100, 101]. Similarly, dual-wavelength confocal scanning laser ophthalmoscopy (cSLO) can collect measurements reliably by using the autofluorescence of lipofuscin deposits in the RPE as an indirect measure of MPOD, while concurrently generating a 3-D topographical map of the retina [22, 95–97, 101, 102]. The resonance Raman spectroscopy is an optical technique that elicits an extraordinarily, resonance-enhanced Raman spectra of the macular carotenoids, in a molecule-specific manner, upon excitation by blue (488 nm) argon laser [66, 99, 103, 104].

The topic of debate for more than three decades, each technique exhibits unique advantages along with clinical limitations that have been discussed in more detail elsewhere [80, 95, 96, 100, 102]. The heterochromatic flicker photometry is the current gold standard of MPOD measurement.

3.3 Procedure of systematic review on carotenoids and diabetic eye disease

A systematic review was performed and published articles on the topic were identified using database searches from PubMed and Web of Science indexes. We identified all relevant publications which reported on the association between diabetic retinopathy and MPOD/carotenoids (lutein and/or zeaxanthin and/or *meso*-zeaxanthin), from human and animal studies prior to 21 December 2020. The search query terms used include 'carotenoids', 'lutein', 'zeaxanthin', 'macular pigment', 'macular pigment optical density AND diabetic eye disease', 'macular pigment optical density AND diabetic retinopathy', and 'MPOD AND diabetes'. Initial entries were selected based on titles and abstracts available in English. Eligible full-text publications were scanned and retrieved in regard to carotenoid levels or supplementation and diabetic retinopathy. Clinical studies evaluating carotenoids levels in diabetes had to quantify either serum concentrations of lutein and/or

zeaxanthin, or measure levels of MPOD using validated, repeatable measurement techniques. Studies were considered eligible only on diabetic retinopathy and diabetic macular edema. Other types of diabetic eye diseases were excluded.

3.4 Carotenoids in the management of diabetic eye disease (*Animal Studies*)

The therapeutic benefits of macular carotenoids have been documented in diabetic murine models, investigating the molecular mechanisms underlying the onset of hyperglycemia-linked retinopathy; in particular, the protective effects of lutein (L) and/or zeaxanthin (Z) on the progression of retinal neurodegeneration [37–45]. Data from these reports are consistent in providing further evidence that administration of L and Z may delay or prevent the onset of DR by counteracting the proposed causative factors including oxidative stress (by attenuating ROS production with a concomitant regeneration of endogenous antioxidants), in addition to ameliorating inflammation and augmenting neuroprotection of retinal tissue. Administration of the drug Alloxan or Streptozotocin (STZ), which are toxic glucose-analogs that preferentially amass within the pancreatic beta cells that produce insulin, are commonly used for inducing diabetes mellitus in mice and rats, which will later develop retinopathy [37–43, 105–107]. Genetic murine models, including the leptin-receptor deficient (db/db) mice, spontaneously develop hyperglycemia and obesity at 4–8 weeks of age [44, 45, 106, 107]. A summary of the experimental animal models evaluating the effects of carotenoids administration on diabetic eye disease is outlined in **Table 2**.

Hyperglycemia-induced oxidative damage has been strongly considered the causative factor in the onset and development of diabetic retinopathy; resulting from the proliferation of pro-oxidant stressors if left untreated. Following the onset of diabetes in mice and rats, there was a significant increase in retinal markers of oxidative stress including: malondialdehyde, lipid peroxide, oxidatively-modified DNA (8-hydroxy-2'deoxyguanosine, 8-OHdG), and nitrotyrosine [37–40, 42]. However, reports were consistent in demonstrating that administration of antioxidants (L and/or Z) ameliorated the diabetes-induced increase in these markers of oxidative stress, comparable to levels observed from control animals. Furthermore, one study evaluated the effects of an AREDS-based formula containing antioxidant micronutrients which were shown to attenuate the rise in expression of oxidative stress-related genes modulated by chronic hyperglycemia [38, 40, 108, 109].

Study	Design (DM induced by)	L and/or Z	Effect of L/Z on Outcomes
Arnal et al. [37]	Rats (STZ)	L	prevented loss of retinal thickness
Kowluru et al. [39]	Rats (STZ)	Z	ameliorated rise in 8-OHdG
Kowluru et al. [40]	Rats (STZ)	L and Z	significantly reduced total ROS levels
Muriach et al. [42]	Mice (A)	L	restored levels of GSH and GPx
Sasaki et al. [43]	Mice (STZ)	L	prevented cell loss in GCL & INL
Tang et al. [44]	Mice (db/db)	L and Z*	improved central retinal thickness
Yu et al. [45]	Mice (db/db)	L and Z*	enhanced mitochondrial biogenesis

DM = diabetes mellitus; L = lutein; Z = zeaxanthin; STZ = streptozotocin; A = Alloxan; db/db = leptin-receptor deficient; 8-OHdG = oxidatively modified DNA; ROS = reactive oxygen species; GSH = glutathione; GPx = glutathione peroxidase; GCL = ganglion cell layer; INL = inner nuclear layer.
** = Wolfberry.*

Table 2.
Effects of carotenoids lutein and/or zeaxanthin in experimental animal models for diabetic eye disease.

Similarly, two clinically distinct features of early-stage retinopathy, microvascular lesions and retinal capillary degeneration, were prevented following treatment with alpha-lipoic acid, a micronutrient with antioxidant properties commonly included in carotenoid supplements for clinical use, such as the EyePromise Diabetes Visual Function Supplement Study (DVS; DiVFuSS) formulation by ZeaVision (MO, USA) [33, 38, 110–112]. Supplementation treatment with L and Z prevented increase in total retinal ROS levels in rats, suggesting they may prevent the continuation of superoxide free radical production caused by hyperglycemia and subsequent progression of retinopathy [41, 108, 113].

The supplementation of L and Z also attenuates retinal expression of endoplasmic reticulum stress biomarkers like BiP (binding-immunoglobulin protein), PERK (protein kinase RNA-like ER kinase), ATF6 (activating transcription factor 6), and activate caspase-12, in diabetic mice [42, 44]. The administration of L and Z also prevented diabetes-induced dysfunction of the mitochondria and damage to mitochondrial DNA (mtDNA), which was confirmed by enhanced expression of mtDNA-encoded proteins of the electron transport chain [41]. Wolfberry, a traditional Asian fruit containing large amounts of diester forms of L and Z protected against mitochondrial stress and markedly enhanced retinal expression of proteins involved in mitochondrial biogenesis [44, 45, 114]. Thus, L and Z reduced oxidative injury on retinal mitochondria by possibly restoring the effective transfer of electrons during oxidative phosphorylation and attenuating mitochondrial dysfunction.

The metabolic correlates of diabetes, such as insulin resistance, insulin deficiency, hyperglycemia and hyperlipidemia have been linked with inhibition of the endogenous antioxidant defense system, caused by overwhelming generation of pro-oxidant stressors and compromised antioxidant capacity. Restoration of endogenous antioxidant levels, such as GSH, GPx and manganese superoxide-dismutase (MnSOD) are essential for nutrient metabolism, regulation of gene expression, free radical neutralization and inhibition of pro-inflammatory pathways [115–120]. In the diabetic retina, regeneration of GSH is compromised by reduced GPx activity and redox cycle [121, 122]; however, L and/or Z reversed the hyperglycemia-induced impairment in GSH and GPx activity in the retina [37–39, 42]. Similarly, diabetic impairment of total antioxidant capacity was sufficiently prevented with supplementation of L and Z [41] along with restoration of MnSOD activity and mRNA expression following administration of AREDS-based micronutrient formula [39, 40, 44].

Carotenoids may prevent the development of DR by suppressing pro-inflammatory pathways activated by overexpressed superoxide free radicals and oxidative injury which are significant contributors in this low-grade chronic inflammatory condition [115, 116, 118]. Metabolic and oxidative insults associated with hyperglycemia can promote induction of inflammation, and concurrently, inflammatory processes can induce oxidative stress. Administration of antioxidants (including L and Z) has been demonstrated to inhibit increased-activation of retinal redox-sensitive nuclear transcriptional factor-B (NF-kB), an important transcriptional regulator of cytokines and growth factors [38, 41, 42, 123–126]; in addition to suppression of pro-inflammatory cytokine, interleukin-1β [41, 124]. Increases in pro-angiogenic factors such as VEGF, which significantly contribute to the neovascularization of PDR, were effectively prevented by L and Z in both rats and mice [39, 41, 45, 126]. However, increased levels of VEGF also play a significant role in the early-stages of retinopathy, by enhancing cell permeability of vascular and non-vascular retinal cells [116, 118–120, 126, 127]. Impaired glutamate metabolism in glial cells, resulting from diabetes, may lead to vascular instability in adjacent blood vessels [128, 129]; these changes in glial cell permeability often occur rapidly

as a result of hyperglycemia, contributing to neural degeneration and may result in DME [127–129]. Thus, the protective effects of L and Z are effectual in attenuating multiple inflammatory response pathways and may preserve the retina from adaptive changes in microvasculature.

Clinical findings of early-stage retinopathy are currently characterized by pathogenic alterations in retinal vasculature, represented by microvascular abnormalities like vasoregression, along with choroidal occlusion and leakage [127, 130]. However, there is growing evidence in animal models that alterations in non-vascular cells (such as Mullers, bipolar, amacrine, and photoreceptor cells) are evident prior to the development of vascular abnormalities [131, 132]. The effects of L in retinal ischemic/reperfusion injury, a clinical feature of PDR, demonstrated improvements in cell viability and enhanced survival of Muller glial cells [133, 134]. Meanwhile, accelerated decline of total retinal thickness, including the inner nuclear layer (INL), outer nuclear layer (ONL), inner plexiform layer (IPL) and ganglion cell layer (GCL; thickness and cell number) were sufficiently prevented by L and/or Z in experimental murine models [37, 43, 44]. Significant thinning of the photoreceptor layer (inner segment and outer segment) and structural abnormalities (nuclear distribution) of the ONL were prevented by L and Z (wolfberry) in db/db mice [44]. The alterations in retinal histology, caused by diabetes mellitus, are closely linked with apoptotic oxidative injury in vascular cells; observed in humans and animals. Prevention of capillary cell apoptosis, determined by terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling (TUNEL)-staining, and increases in degenerative (acellular) capillaries, was achieved by L and/or Z; regarded as a surrogate endpoint for DR-therapeutic development and hallmark sign of early-stage NPDR, respectively [37–41, 43, 111, 112]. Thus, the neuroprotective potential of L and Z in maintaining the retina, an integral part of the central nervous system, is essential in preventing neural degeneration and irreversible vision loss.

Visual performance dysfunction caused by retinal degeneration, observed by electroretinogram (ERG) in the inner retinal layers, show a decrease in oscillatory potentials (OPs; OP3 and total OPs) in diabetic mice [43]; similar functional impairment observed clinically in early-stage retinopathy [135–137]. Similarly, the neuroprotective effects of L and Z were observed in ERG recordings which indicated the preservation of b-wave latency and a-wave/b-wave amplitudes, restoring retinal dysfunction induced by diabetes [37, 41–43]. Synaptophysin, a synaptic vesicle protein that plays an important role in neuronal synaptic network activity, is also reduced in diabetic retina [43]; which is caused by chronic activation of pro-oxidant extracellular signal-regulated kinase (ERK) [138, 139]. In the retina of hyperglycemia-induced mice, administration of L preserved synaptophysin protein and suppressed ERK activation. This provides evidence of neuroprotective potential of L to help maintain synaptic activity [43, 137]. Furthermore, supplementation of L demonstrated enhanced preservation of neural activity by restoring expression levels of retinal neurotrophic factor, BDNF (brain-derived neuronal trophic factor) [43]; an important mediator of synaptic network activity and cell survival in the inner retinal and ganglion cell layers [140–143]. The neuroprotective benefits of L and Z observed in animal models may be explained by supporting cell survival and increased viability and thus, enhancing overall visual function.

There are some limitation to the findings from animal models. Briefly, lack of studies on the effects of L and Z in non-murine models, restricts the translative potential for clinical use due to species differences between humans and rodents; namely, absence of the macula in these animals [144, 145]. Retinal preservation and neuroprotection with L and/or Z were observed in some [38, 42–45] but not all [37, 39–41] studies, independent of any change in hyperglycemic status and

thus, interpretation of these findings must be exercised with prudence. Moreover, the dosage of L and Z tested in experimental models is typically inflated to significantly higher amounts than those observed in clinical application to achieve a dose-dependent effect, which may prompt the necessity for renewed clinical trials to determine safety and toxicity of these carotenoids in larger amounts. It is not an exaggeration to conclude that these animal model experiments of diabetes provide substantial evidence in support of the putative anti-angiogenic and anti-inflammatory benefits of carotenoids lutein and zeaxanthin in protecting against retinal neurodegeneration.

3.5 Carotenoids in the management of diabetic eye disease (*Clinical Studies*)

To date, a limited number of studies have examined the complex association of macular carotenoids levels and diabetic eye disease in individuals with type 1 and type 2 diabetes [26, 32, 35, 47–54]. Studies evaluating the relationship between serum levels of L and Z and DR demonstrated that: (1) serum concentrations of L and Z were lower in patients with DR when compared to healthy controls; (2) higher plasma concentrations of non-pro-vitamin A carotenoids (including lycopene, L and Z) were associated with lower risk of developing or progression of retinopathy in T2DM, after adjusting for potential confounders; (3) supplementation with carotenoid vitamin therapy may improve visual function and features of macular edema in patients with DR [47, 54]. It is known that carotenoid levels in the plasma are positively correlated with concentrations in the macular pigment [71, 146]. However, there are limitations when measuring serum levels to evaluate the effects of L and Z on DR, namely that their concentrations are almost entirely dependent upon relatively-recent dietary-behaviors; fluctuations that can occur in response to dietary intake of high-glycemic index foods and/or sugar-sweetened beverages [147–150]. Moreover, these dietary habits, similar to those in the Western diet, have been attributed largely to the prevalence and onset of the metabolic syndrome [147–149, 151, 152].

Several studies investigated the putative role of L and Z in attenuating the pathogenesis of DR by evaluating levels of MPOD in cohorts that included both type 1 and type 2 diabetes [26, 32, 35, 48–53]. The findings from these reports suggest the following: (1) MPOD levels are lower in patients with diabetes, in particular T2DM, than healthy individuals; (2) in T2DM, MPOD was inversely associated with several behavioral, anthropometric, and novel serum biomarkers such as vitamin D insufficiency; (3) MPOD levels can be augmented with dietary supplementation in patients with diabetes (type 1 and 2) [26, 32, 47–53]. Generally, reports are consistent suggesting MPOD levels are significantly lower in individuals with diabetes, and a negative correlation has been indicated between severity of diabetic maculopathy and level of macular carotenoids [48, 49, 51]. The type of diabetes also had a statistically significant difference on MPOD when accounting for other covariates (including history of smoking, hypertension and bodyweight) [26, 32, 50]. Current smoking status and increased adiposity are potential predictors of low MPOD in diabetes [26, 32] and concomitantly, one study found low serum vitamin D ($\leq$50 nmol/L; $P = 0.006$) was significantly correlated with MPOD in T2DM after multivariate regression analysis [32]. The DiVFuSS study demonstrated that carotenoid supplementation, which included antioxidant micronutrients such as alpha-lipoic acid and vitamin D3, can significantly improve MPOD levels (mean increase of 27% in participants on active supplement) and measures of visual function in patients with diabetes (with no retinopathy) and those with mild to moderate NPDR [35, 109, 153].

Evidence suggests that the MPOD depletion may be a clinical feature of T2DM, however, the proposed causal mechanisms may elucidate distinct contributing factors in the development of diabetic retinopathy; mechanistic associations with MPOD status that may differ between type 1 and type 2. Metabolic comorbidities observed in T2DM including increased adiposity and dyslipidemia, primarily characterized by reduced high-density lipoprotein (HDL) and hypertriglyceridemia, may substantially compromise the bioavailability of dietary carotenoids. Thus, diminished transport and assimilation of serum L and Z into the macular pigment may be directly represented by low MPOD levels [154–161]. Not surprisingly, L and Z are regularly deposited into visceral and subcutaneous adipose tissue, major body sites for carotenoids, which may make them less available to retinal tissue. In fact, reports have demonstrated higher percentages of body fat and body mass index (BMI) are inversely associated with MPOD levels [155, 158, 162–164]. Adipose concentrations of macular carotenoids vary according to the body site, coordinated by the hormonally-regulated deposition and mobilization of fatty acids, with demonstrably elevated levels in abdominal fat [164–166]; which may also explain sex-based differences observed in MPOD levels [154, 161]. Metabolic correlates like dyslipidemia may further contribute to low MPOD in T2DM by compromising the transport of plasma carotenoids to the retina in consequence of increased serum triglycerides to HDL (TG/HDL) ratio concurrent with worsening insulin resistance [166–168]. Furthermore, evidence suggests that serum carotenoids are predominantly transported by HDL particles and retinal absorption of L and Z is mediated by a 'piggy-back' mechanism involving scavenger receptor class B type-1 (SR-1B) in the RPE [157, 159, 169].

The depletion of MPOD in T2DM or poorly controlled T1DM is likely dependent upon the complex interplay between the development of metabolic perturbations including increased adiposity, dyslipidemia, insulin deficiency and hyperglycemia and the oxidative stress and inflammation induced by diabetes mellitus. Traditionally, adipose tissue has mainly been considered in the context of energy storage, however, they produce a variety adipocytokines and inflammatory mediators and has been suggested to function like a metabolically-active immune organ [170, 171]. In fact, increased intra-abdominal fat is a crucial determinant of the atherogenic lipid profile in T2DM with obesity, and research indicates visceral adipose tissue may be the principal mediator of inflammation associated with diabetes [172–174]. Therefore, this chronic low-grade inflammatory disease in turn exacerbates oxidative injury, causing a positive feedback loop between oxidative stress and inflammation, which may lead to compounding depletion of macular pigment concentrations [35, 115, 152, 175–177]. The elevated serum concentrations of a marker for total systemic oxidative stress *in-vivo*, 8-OHdG, have been positively correlated with BMI in T2DM [173, 177]. It is suggested that the metabolic correlates and comorbidities frequently associated with T2DM (or poorly controlled T1DM) contribute significantly to the onset and progression of retinopathy into PDR.

Results from these [26, 32, 35, 48–53] clinical studies that have investigated the implications of MPOD on diabetic eye disease are promising, but not without limitations: (1) with one exception [26], individuals with T1DM and T2DM were evaluated and analyzed homogeneously in comparison to controls; (2) only a limited number of studies evaluated cohorts based on status of DR; (3) relatively small and unequal sample sizes (of individuals with diabetes and controls) in multiple studies; (4) with one exception [35], studies were only observational in nature. Additional research is necessary to further elucidate the potentially different associations that may exist between MPOD status and T1DM and T2DM.

4. Conclusions

Diabetic retinopathy is the most common microvascular complication of diabetes mellitus and DR remains the leading cause of preventable blindness in developed countries among working-age adults. It appears chronic hyperglycemia has significant deleterious effects on the endogenous defense systems, resulting in the depletion of macular carotenoids lutein, zeaxanthin and *meso*-zeaxanthin, in addition to other potent antioxidants that are pertinent for maintaining retinal health. Additionally, the metabolic correlates of diabetes negatively impact concentrations of macular pigments, however, carotenoid vitamin therapy has shown promising results in augmenting MPOD levels and visual performance. To this accord, regularly measuring MPOD may be well suited for monitoring retinal neurodegeneration brought on by diabetes and screening at-risk patients before clinical features of retinopathy become apparent. Meanwhile, routine management of established risk factors such as poor glycemic control, obesity and hypertension are critical in preventing or delaying the progression of DR. However, there is tremendous need for both timely and functional prophylactic measures that can be implemented before irreversible loss of vision begins. Finally, carotenoid vitamin therapy shows great promise with increasing evidence both in animal and human studies, further clinical investigations must be performed to assess its full potential in the management of diabetic eye disease.

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Conflict of interest

Drake W. Lem none. Dr. Pinakin Davey is a consultant and has received research grants from ZeaVision and Guardion Health Sciences. Dr. Dennis L. Gierhart is an Employee, Chief Scientific Officer for ZeaVision manufacturer of various nutritional supplements including the DVS.

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