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Ocular Carotenoid Status in Health and Disease

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Keywords

macular pigment, macular carotenoids, carotenoid supplementation, ocular nutrition, retinal imaging

Abstract

Retinal carotenoids are dietary nutrients that uniquely protect the eye from light damage and various retinal pathologies. Their antioxidative properties protect the eye from many retinal diseases, such as age-related macular degeneration. As many retinal diseases are accompanied by low carotenoid levels, accurate noninvasive assessment of carotenoid status can help ophthalmologists identify the patients most likely to benefit from carotenoid supplementation. This review focuses on the different methods available to assess carotenoid status and highlights disease-related changes and potential nutritional interventions.

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INTRODUCTION

Carotenoids are organic pigments produced by plants, algae, and bacteria. They are unique substances that work as antioxidants and thereby protect different tissues in the body, especially in the eye, from light damage. This is of great importance as delicate sensory cells in the eye are continually exposed to potentially damaging light.

The inside of the human eye is lined with a sensory nerve layer called the retina. Within the retina, two significant regions are found: the optic nerve and the macula. At the optic nerve, all retinal axons are bundled, and the light signals are transferred to the brain. The macula is often referred to as the macula lutea: The term lutea stems from the funduscopically yellowish appearance of this spot in the macula, which is the area of best visual acuity. At this spot, retinal carotenoids accumulate and protect the macula from potentially phototoxic light influences. In a healthy eye, blood vessels do not cross directly over the macula lutea, resulting in the formation of a small dip, called the fovea centralis. In the center of the fovea, no other layers are present above the photoreceptor layer, which further improves the visual contrast at this area. The fovea centralis has a diameter of approximately 1.5 mm. **Figure 1** shows the funduscopic image of a healthy eye, highlighting both the macula and the optic nerve.

Good central vision is an essential function that allows people to clearly see fine details, objects, and text, yet a variety of diseases can affect the macula and cause visual disturbances or even blindness. Many of these diseases can be acquired or inherited and are discussed thoroughly in

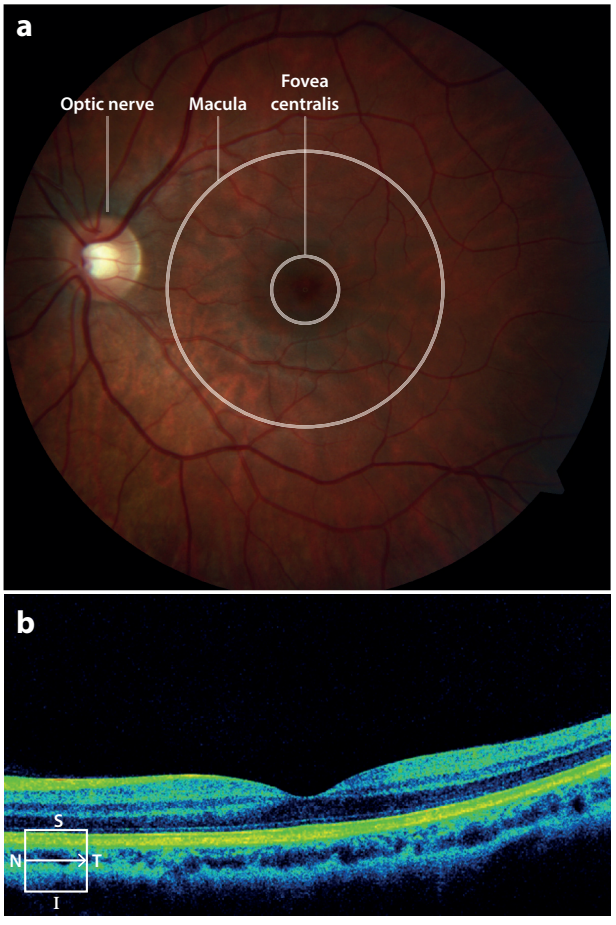

Figure 1

Anatomy of a healthy eye. Images from (*a*) fundus photography and (*b*) optical coherence tomography. The line in the lower left corner of panel *b* indicates the position of the scan within the macula. Abbreviations: I, inferior; N, nasal; S, superior; T, temporal.

this review. These diseases include age-related macular degeneration (AMD), a prevalent disease in the Western world that causes vision loss due to changes in the macula lutea. Any distortion to the retinal layers in this area can lead to decreased central vision. In addition, inherited diseases, such as retinal dystrophies or even albinism, can cause visual impairment. Therefore, many different techniques have been developed to image and investigate the macula. It is imperative to characterize the early stages of disease-related changes in the eye as early disease detection may lead to better treatment.

The breadth and scope of ophthalmic imaging in the posterior segment of the eye has greatly advanced, from rudimentary fundus photographs to progressively specialized techniques. Fundus autofluorescence (FAF) imaging detects altered retinal fluorescence signals, while fluorescein angiography can detect changes in the vasculature, such as leakage. The broadly used optical coherence tomography (OCT) technique depicts the reflection of light at each individual retinal layer, providing high-contrast images of the retina. Other imaging techniques focus on investigating retinal carotenoids. Changes in carotenoid status are associated with many retinal diseases, and

AMD: age-related macular degeneration

FAF: fundus autofluorescence

OCT: optical coherence tomography

these changes provide further information about disease status. Therefore, assessing carotenoids is an extremely important and powerful tool for imaging retinal diseases.

This review describes the basic characteristics of carotenoids as well as different measurement modalities used to assess carotenoid status. The main focus, however, lies within their clinical applications in ophthalmology. Specifically, this article focuses on the differences between nutritional status in healthy eyes and how carotenoid status changes in different diseases. The aim is to provide a better understanding of the value of carotenoid imaging in ocular health and disease.

MP: macular pigment

L: lutein

Z: zeaxanthin

MZ: *meso*-zeaxanthin

CAROTENOIDS

Biochemical Background

Carotenoids are hydrophobic compounds that cannot be synthesized in animals or in the human body. Carotenoids can be divided into two groups. If they contain oxygen, they are categorized as xanthophylls; if not, they fall into the category of carotenes. At the fovea, the macular pigment (MP) carotenoids consist of a 1:1:1 mixture of three xanthophylls: lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ). L, Z, and MZ are constitutional isomers that share the same molecular formula of $C_{40}H_{56}O_2$ (14). **Figure 2** shows their biochemical structures. Like other carotenoids, the macular carotenoids are natural antioxidants with high efficacy. Extensive research during the past decades demonstrates that higher carotenoid status in the human retina is inversely associated with retinal diseases, such as AMD. Therefore, carotenoid supplementation is thought to help reduce the risk of developing AMD or to delay disease progression.

Through extensive years of research, macular carotenoids are now understood to have three distinct types of biochemical and biological specificities: chemical, distributional, and species (88).

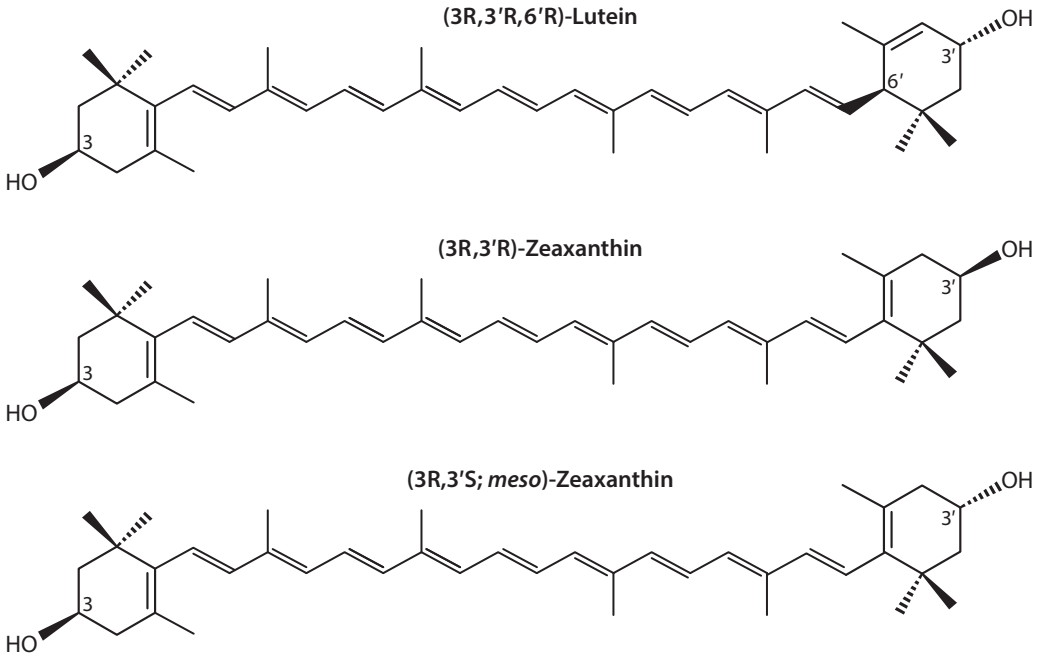

Figure 2

Chemical structure of the macular carotenoids lutein, zeaxanthin, and *meso*-zeaxanthin.

Chemically speaking, more than 600 carotenoids have been discovered in nature, approximately 50 of which are found in the human food chain and 15–20 of which are detected in the human bloodstream. Only L and Z can selectively accumulate in the human retina. The highly specific uptake mechanisms of such carotenoids are mediated by a series of proteins, especially high-density lipoprotein (HDL) cholesterol receptors and transporters, such as SR-BI and ABCA1; and HDL apolipoprotein A1 and apolipoprotein E (93). After being released from food and absorbed in the gut, carotenoids pass through intestinal transporter SR-BI to then be packed into chylomicrons in the liver. From there, L and Z are delivered to the eye via HDL lipoproteins. L and Z are preferentially presented in HDL complexes, which may partially explain the selective uptake of macular carotenoids. In addition, it was recently demonstrated that L can be converted to MZ by RPE65, confirming that MZ is synthesized in the human eye and not ingested by diet (131).

HDL: high-density lipoprotein

The second carotenoid specificity is distribution specificity. Macular carotenoids are highly concentrated in the foveal region of the macula, where the concentration can reach 1 mM, which is the highest concentration found in the human body. We have previously demonstrated that two carotenoid-binding proteins are responsible for the specific distribution of these xanthophyll carotenoids in the human retina: The Z-binding protein and L-binding proteins have been identified as, respectively, GSTP1 and StARD3 (20, 89). More recently, it was shown that transgenic overexpression of GSTP1 significantly increased the contents of Z in the MP within the retinas of mice (87).

The third carotenoid specificity concerns species specificity. Among mammals, MP carotenoids are uniquely accumulated within the retinas only of primates. In 2014, it was discovered that the xanthophyll cleavage enzyme BCO2 is relatively inactive in the human retina, while mouse BCO2 efficiently cleaves xanthophyll carotenoids (90). After knockout of BCO2, L and Z begin to accumulate in the retinas of mice fed carotenoids (91). Therefore, the inactivity of the human BCO2 coupled with the strong cleavage activity of BCO1 toward β-carotene appear to underlie the unique accumulation of carotenoids in the primate retina.

Carotenoids in the Healthy Eye

The carotenoids in the eye are collectively called MP (27). These yellow pigments are located anterior to the photoreceptors and are mainly localized in the outer plexiform layers, especially within the Henle fibers, and in the inner plexiform layer. The Henle fibers connect the individual photoreceptors of the macula to the bipolar and horizontal cells, which can be found more laterally (80, 133). The retinal carotenoids protect the sensory cells of the macula from light damage by absorbing and quenching light. Absorption of blue light results in central hypofluorescence in blue light FAF intensity images (71).

L, Z, and MZ are xanthophylls commonly found in plants as part of the photosynthetic proteins that absorb sunlight (50). They are also found in fruits, vegetables, and eggs (30). As L and Z cannot be produced by the human body, they are essential in the human diet. L is isomerized in the retina to form MZ (85). Therefore, carotenoid intake from food is indispensable.

The uniqueness of L, Z, and MZ lies in the hydroxyl groups at both ends of each molecule, which gives them a dipole character. This allows for discrete orientations in lipid membranes (27). Based on results from Sharifzadeh and coworkers (130), it appears that all MP is distributed within a circular area of 1 mm in diameter, centered at the fovea. This distribution is due to the presence of carotenoid-binding proteins localized within this area of the retina (19). Such binding proteins were found for Z and MZ as well as L (18, 20, 21). In the center of the fovea (0–0.25 mm from the center) one may find more than twice the amount of Z and MZ compared with the amount of L (the ratio is approximately 2.4:1). In the periphery, however, an inverse distribution can be

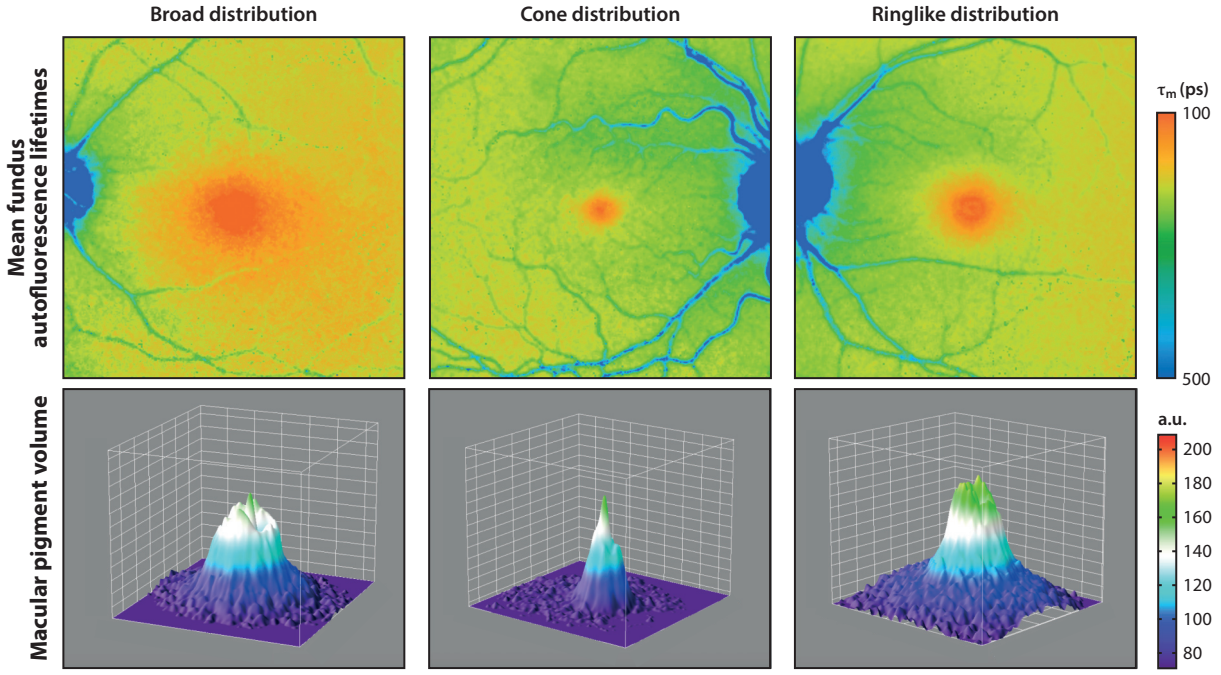

Figure 3

Distribution of macular pigment in healthy individuals. Abbreviations: a.u., arbitrary units; τ_m, amplitude-weighted mean fluorescence lifetime.

found, with L as the dominant carotenoid. Furthermore, the distribution of L and Z shows a linear correlation with the distribution of rods and cones (24). MP in healthy eyes shows a mountain-like distribution, with the peak at the center of the fovea. This mountain of MP can have different forms. The mountain may be either slim or broad, and some healthy individuals have a broad MP with a central dip (**Figure 3**).

The Importance of Retinal Carotenoids

MP protects the macula from oxidative and peroxidative light damage (142). According to Bone and coworkers (25), two different mechanisms for protection are possible: MP quenches free radicals, especially free oxygen radicals, or carotenoids absorb blue light before it reaches the photoreceptor layer, as this light is especially damaging to the retina. This phototoxic effect is due to the short wavelength of blue light that corresponds to high energy levels (25, 64, 84).

Because light-induced oxidative damage can be reduced with higher amounts of MP, low carotenoid levels are likely to be connected to a higher risk of retinal diseases caused by light damage. AMD is one example of these diseases. It has been shown that patients with AMD have decreased amounts of MP (10, 26, 100). A connection between visual function and the amount of MP in the early stages of AMD has been described (5). Therefore, retinal carotenoids are highly important to preventing retinal diseases, and their assessment is a necessary step in the evaluation of the fundus.

Retinal Nutrition and Supplementation

Retinal carotenoids can be supplemented in cases of low MP levels or if diseases of the retina are present. However, this supplementation has been controversial, especially with respect to its

unknown long-term effects (7, 11, 107). Previous trials investigating the impact of carotenoids on the retina include the Age-Related Eye Disease Study 2 (AREDS2) and the LUTEGA trial (2–4, 44). The LUTEGA trial concluded that supplementation increases MP levels to a saturation point and advised that it might be helpful to provide supplements to patients in order to prevent the development and subsequent progression of eye diseases (44). The first AREDS trial investigated using β-carotene and zinc supplementation, but a high incidence of lung cancer in patients who were smokers and received supplementation was reported. The subsequent AREDS2 trial examined L and Z substitution for β-carotene. Although current smoking was an exclusion criterion for β-carotene use, the incidence of lung tumors was still elevated in former smokers who received β-carotene. Therefore, when evaluating the recommendation to provide supplements to patients, smoking status should be evaluated carefully (2, 3). After 5 years of follow-up, the AREDS2 study reported a significantly reduced risk of developing AMD for the L and Z group compared with the no L and Z group and superior safety relative to β-carotene supplementation (136). Thus, supplementation with the L and Z levels used in AREDS2 appears to be a safe and cost-effective defense against AMD development and progression (5).

MacTel: macular telangiectasia type 2

HFP: heterochromatic flicker photometry

MPOD: macular pigment optical density

ASSESSMENT OF CAROTENOIDS

Impact of Carotenoid Assessment

Because carotenoids appear to play a key role in retinal diseases, intensive research has resulted in a variety of innovative carotenoid assessment techniques. The breadth of possibilities for assessing retinal carotenoids is often confusing because methodologies, units of measurement, and the presentation of results vary widely. Accurate readings of carotenoid status are important in order to correctly advise individuals with regards to supplementation. Furthermore, in diseases such as macular telangiectasia type 2 (MacTel), the assessment of carotenoids may be crucial to the diagnosis, as reduced MP levels as well as abnormal distributions are among the first signs of the disease. Therefore, the measurement of carotenoids can impact clinical practice, and the evaluation of MP may eventually become an integral part of comprehensive ophthalmological care. The following sections describe and aim to give an organized overview of different MP assessment techniques.

A large variety of methods are used to assess carotenoid status in humans, most of which are focused on the eye, but carotenoids can also be measured in tissue outside of the eye, such as the skin, blood, and the brain. Measurements of ocular carotenoids can be distinguished between subjective (psychophysical) and objective (optical) methods used to assess the amount of MP. In subjective methods, a direct answer from the patient is required, whereas objective measurement methods typically require just enough cooperation to generate an image (73).

Heterochromatic Flicker Photometry

Heterochromatic flicker photometry (HFP) is the most frequently used method for evaluating MP amounts in research and clinical settings (9, 134). This subjective method uses a flickering stimulus that rapidly alternates between an MP-absorbing wavelength (blue light of approximately 460 nm) and a nonabsorbing wavelength (typically green light at approximately 540 nm). To the patient, this stimulus appears as a flickering ring or spot, and the patient is asked to adjust the blue light in a way that minimizes or diminishes the flickering in the patient's perception. Using different fixation points, both centrally (areas with MP) as well as peripherally (areas without MP), ratios can be determined that allow for conclusions about the MP optical density (MPOD) of carotenoids at a limited number of eccentricities in the eye. Patients with high amounts of MP will need stronger

intensities of blue light to eliminate the flickering compared with patients who have only small amounts of MP (13). Although this test can be conducted without pupil dilation, high degrees of concentration and compliance are required from subjects in order to achieve reproducible and comparable measurements (98). Furthermore, the measurements are typically reported as MPOD at a single parafoveal eccentricity of 0.5–1.0°, where MP distributions may decline steeply. It is possible to use HFP to measure MPOD at multiple eccentricities, but this is a time-consuming option.

RPE: retinal pigment epithelium

AFI: autofluorescence imaging (dual-wavelength)

RRS: resonance Raman spectroscopy

Dual-Wavelength Autofluorescence Imaging

To estimate retinal carotenoids based on autofluorescence, the intrinsic fluorescence of lipofuscin is used (47). Lipofuscin is localized in the retinal pigment epithelium (RPE) and shows a strong fluorescence at the human fundus, but blue light autofluorescence imaging (AFI) of the human retina shows a dark central spot at the fovea due to the presence of yellow MP, which absorbs the incoming blue light. To quantify this effect, two autofluorescence measurements at different excitation wavelengths are compared. One measurement is acquired at the 488 nm excitation wavelength, at which carotenoids show strong absorption. The second measurement is done in a range at which retinal carotenoids show no absorption, either at 514 nm excitation with an argon laser or at 532 nm with a frequency-doubled diode laser (138). Digitally subtracting the green from the blue image with proper correction factors allows for a calculation of MP as well as conclusions about its distribution (48). Limitations of this technique include its cost, high light intensities, and need for pupil dilation. Low or irregular lipofuscin levels, as in infants, albinism, or AMD, can make the use of this technique challenging (49, 117).

Single- or Dual-Wavelength Reflectometry

This method is based on the assumption that MP is localized only in a certain region of the fovea, and, thus, it changes the reflection spectra of light in this area (29). Dual-wavelength reflectometry uses two different wavelengths (480–488 nm and 515–540 nm), while in single-wavelength reflectometry the fundus is homogeneously illuminated at one wavelength that is close to the absorption maximum of retinal carotenoids. Simplistically, it is assumed that the reflection is homogeneously distributed across the entire fundus and that absorption by the MP occurs solely in the fovea. To quantify this effect, reference areas from regions without MP (areas outside of a papillary diameter around the fovea) are used. Based on this reflection image, both the distribution as well as the optical density of MP can be captured (45, 126). Using fundus reflectometry requires dilated pupils, and results may be disturbed by reflections from the juvenile inner limiting membrane; therefore, it is typically recommended for use in patients who are 40 years or older (122). However, this modality has been used to image infant MP with a digital video fundus camera (RetCam, Natus, Pleasanton, California) (15). In fact, reflectometry is the only way to measure MP levels and distributions in young children because they cannot perform HFP, and they have minimal lipofuscin. Reflectometry requires pupil dilation and bright light levels and will not provide usable results if significant ocular pathology is present.

Resonance Raman Spectroscopy

Resonance Raman spectroscopy (RRS) can also be used to assess MP. This method was first described by Bernstein and coworkers in 1998 (16). It was validated with monkey and human cadaver eyes and later was used in clinical studies (17, 56, 58, 147). RRS is based on Raman scattering at

conjugated double bonds of macular carotenoids. In the integral mode, participants are asked to fixate upon a 1-mm spot from an argon laser that excites the MP. The background fluorescence is then subtracted, and the level of light scattering intensity at the carbon–carbon double-bond stretch frequency can be calculated. This scattering is linearly associated with the amount of MP (16, 58). Pupil dilation is generally required to minimize the effect of light scattering from the lens, which may affect the measurement (55). This technique has been described as sensitive, specific, and repeatable in patients with abnormal MP levels (17). Nevertheless, the device is expensive, difficult to implement in an imaging mode, and not widely available. Furthermore, RRS needs high light levels to obtain information on carotenoids and, therefore, is not routinely used.

FLIO: fluorescence lifetime imaging ophthalmoscopy

HPLC: high-performance liquid chromatography

Fluorescence Lifetime Imaging Ophthalmoscopy

Fluorescence lifetime imaging ophthalmoscopy (FLIO) is a novel method used to assess MP. The method was developed by Schweitzer and coworkers in 2002 (127), and it has become a valuable modality for nutritional imaging after fluorescence of MP; the effect of the FLIO measurement was first described in 2015 (52, 121, 122, 127). FLIO offers a sensitive method to detect metabolic changes in the human retina, potentially leading to the earlier diagnosis of retinal diseases (53, 118, 128).

A dominant fluorophore (lipofuscin) as well as at least a second minor fluorophore exist in the macula, with the latter initially thought to be either flavin adenine dinucleotide or a different, unknown fluorophore (47). More than 10 years after this initial study, the fluorescence of carotenoids in vivo was first described using RRS (130). The kinetics of carotenoids strongly resemble those of the minor fluorophore. The spatial dominance of these fluorophores inside the fovea provides another indication of carotenoid fluorescence. Finally, FLIO has been used in clinical studies to confirm the fluorescence of carotenoids (117, 122). The principle that fluorescence lifetimes are independent of fluorescence intensity is the main factor that allows for the detection of MP with this method (86). Similar to RRS, FLIO signals originate from retinal carotenoids themselves rather than from the absorption of incoming light (as in AFI, HFP, and reflectometry). Additionally, since MP is anterior to the RPE, competing FLIO signals from lipofuscin are suppressed at the fovea.

The main parameter of interest in FLIO imaging is the amplitude-weighted mean fluorescence lifetime (or τ_m). Retinal carotenoids show short autofluorescence lifetimes, although the autofluorescence itself is weak in intensity. Ex vivo studies determined these lifetimes to be around 50–60 ps (117). Currently, this imaging is performed on FLIO prototypes that are based on the Heidelberg Engineering SPECTRALIS confocal imaging platform (Heidelberg Engineering, Heidelberg, Germany). For good image quality, patients' pupils should be dilated. The benefits of utilizing FLIO in studies include the minimal effort required by the patient, the noninvasiveness and short measurement times lasting approximately 2 min, as well as the ability to obtain an image that does not require healthy reference regions to calculate the amount of MP. Therefore, it can be used in patients with abnormal lipofuscin distributions as well as patients with albinism (117).

Carotenoids in Tissues Other Than the Eye

Carotenoids can be assessed noninvasively in the skin and by high-performance liquid chromatography (HPLC) of blood and tissue samples. It has been shown that RRS measurements of skin carotenoids show strong correlations ($r = 0.7$ to 0.9) with carotenoids in biopsies of human skin

(57, 96). Skin RRS and reflectometry are particularly useful to assess the carotenoid status of children, with skin carotenoid levels strongly associated with fruit and vegetable intake (123). Using HPLC, the carotenoid status in the plasma can also be assessed (77, 104), and higher L levels in the serum of patients have been associated with higher visual function. Similarly, carotenoid assessment in brain tissue suggests that higher carotenoid levels might be beneficial for overall cognitive performance (65, 74).

EVALUATION OF DIFFERENT ASSESSMENT METHODS

Of all the techniques used to investigate MP that we have presented, HFP is the only subjective one. It was the first method used to assess MP, and HFP is still used widely in psychophysical research to correlate MPOD with visual function or as a surrogate for brain levels, but a single parafoveal MPOD is usually reported. However, the MP continues well past this one eccentricity, and MP can have vastly different distribution patterns among healthy individuals and patients with eye diseases. The next part of this review focuses on eye diseases. Ophthalmologists, in particular, are used to evaluating images of the retina and prefer to look at MP distribution patterns over single MPOD measurements. Additionally, in order for HFP to be used correctly, patients must be highly committed and cooperative, as well as having fairly intact visual acuity. Patients have to be able to adjust the device according to their subjective perception. It is imperative that investigators are extremely careful when explaining the technique to patients, ensuring that they understand the tasks necessary for the measurement. The investigator is not able to validate the results externally, because the patient has control over the measurement outcome. In the realm of ophthalmology, many patients are relatively old, especially those with AMD; therefore, accurate HFP may be difficult to obtain in a clinical setting.

We think it is reasonable to say that compared with objective imaging methods, HFP is a less-expensive alternative for assessing carotenoid status in the eye. Additionally, it is similar to other psychophysical testing, and pupil dilation is not required. Bright light levels are used with all testing methods, but objective methods usually obtain results faster. Although the equipment used for objective MP imaging may be more expensive, ophthalmologists are able to obtain MP distributions and not just single MPOD numbers, thus facilitating insight into the pathology behind changes in carotenoid status. Many objective methods are based on existing ophthalmological modalities, such as the Heidelberg SPECTRALIS, making it easier to add carotenoid imaging to the ophthalmological clinical routine. Furthermore, objective methods are highly reproducible and can be used in longitudinal studies.

It is difficult to determine a preferred technique among the many objective methods for measuring MP because they all possess advantages and disadvantages. All objective measurement modalities except FLIO rely on healthy surrounding areas in order to calculate the amount of MP. FLIO, however, may be influenced by cataracts and the natural fluorescence of the lens. Therefore, we suggest that a combination of FLIO used with either RRS or AFI might be the best way to truly assess MP in patients with different eye diseases.

CAROTENOIDS IN VARIOUS EYE DISEASES

Abnormal Carotenoid Levels Due to Altered Intake, Diet, and Age

Retinal carotenoids are diet derived. Both extremely low and excessively high intakes can result in abnormalities of the fundus. Literature describing the amounts of MP in healthy populations often conclude that carotenoid profiles vary greatly even within a healthy population (39, 122).

A self-induced vitamin A deficiency was reported for one participant with an abnormally low carotenoid intake who ate essentially no fruits or vegetables (39). The serum retinol concentration in this person was 0.09 mg/L (normal values are approximately 0.30 to 1.20 mg/L). The authors mentioned that this participant showed the lowest carotenoid measures they had ever encountered, with serum carotenoid levels nearly undetectable (30.67 ng/mL). The mean serum carotenoid status for participants was greater than 500 ng/mL (39). Nevertheless, AFI showed a small peak of MP in the foveal center of this participant, possibly from the consumption of eggs. This likely indicates that despite poor dietary intake, carotenoids still bind avidly at the foveal center in otherwise healthy eyes. That finding is in contrast to abnormally low MP values that are disease related, which are also discussed in this review. An extremely low intake of carotenoids may lead to an increased risk of developing eye diseases (14, 109, 113).

MH: macular hole

However, the excessive intake of retinal carotenoids can also cause a rare crystalline maculopathy in which patients have no functional alteration or visual complaints. A case report of a 50-year-old Asian woman by Choi et al. (35) found that in addition to consuming a 20-mg L supplement and 4 g of fish oil daily, she also consumed an L-rich diet via a daily smoothie, which included spinach, avocado, broccoli, and kale. Her reported serum carotenoid levels were at an astounding 5,029 ng/mL (35). After discontinuing L supplementation, the crystals appeared to resolve in one eye at the 7-month follow-up visit (35). Her MP distribution was similar to that found in several other studies that noted a distinct MP distribution in patients with extremely high levels of macular carotenoids, which was described as a central dip or a ring-shaped distribution (39, 122, 130).

Age also seems to play a role in the amounts of MP in the retina. Premature infants, for example, do not have detectable MP at birth, while full-term infants do have MP, and it continues to increase during the first 7 years of life (15). Significant declines in macular carotenoid density have been reported to be related to increasing age in elderly people (10, 17, 66). It has been considered that the age-related decline in MP could be due to cumulative light exposure (66). However, other studies have argued against this finding, indicating that it must be a difference in dietary intake (that is, insufficient intake in elderly people) that accounts for age-related declines in carotenoid levels (10). This insufficient intake has been reported in other studies as well (22, 69). Other factors that lead to the depletion of carotenoids in patients could include the loss of photoreceptors and axons, which is associated with increased age (10, 41). Alternatively, increased oxidative load within aging eyes and in smokers could lead to excessive destruction of carotenoids, thereby diminishing MP (103, 109).

Overall, the amount of carotenoids found in the eye strongly depends on an individual's diet. Neither insufficient nor excessive intake of carotenoids seems to be beneficial. However, low levels of retinal carotenoids are likely worse than high levels, as low carotenoid content is associated with the development of different eye diseases. Therefore, dietary intake of a sufficient amount of carotenoids is likely helpful in preventing retinal diseases such as AMD.

Carotenoids in Macular Holes

A macular hole (MH) is a retinal defect that occurs in the center of the fovea, the area with the best visual acuity and highest abundance of MP. Therefore, MHs can function as good models to investigate the spatial distribution of retinal carotenoids, especially within different layers at the human fundus. These analyses are yielding important information about carotenoid properties. **Figure 4** shows the distribution of MP in a patient with an MH.

Patients with MHs often complain about visual disturbances, which are typically recognized when the unaffected eye is closed. The term MH is mostly used in reference to age-related,

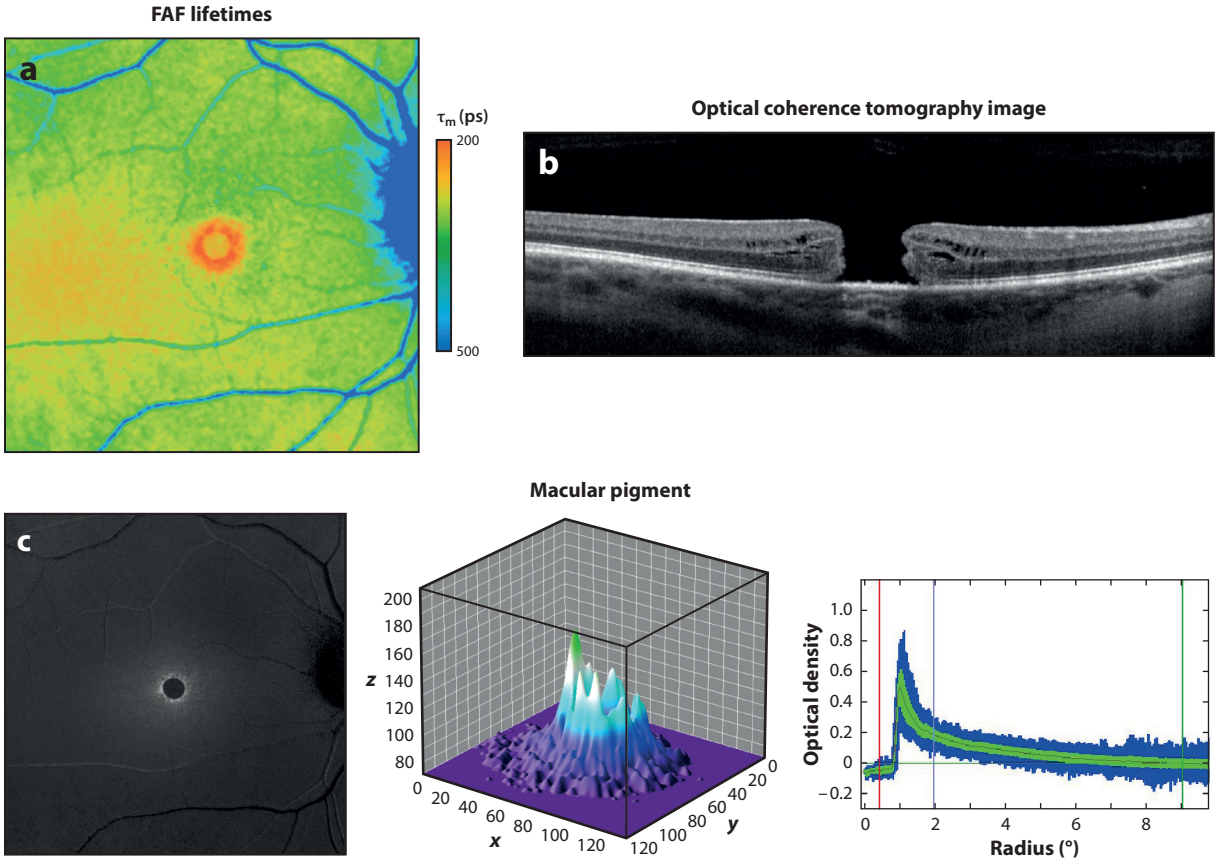

Figure 4

Macular pigment distribution in an eye with a macular hole. Images from (*a*) fluorescence lifetime imaging ophthalmoscopy, (*b*) optical coherence tomography, and (*c*) dual-wavelength autofluorescence assessment of macular pigment. Abbreviations: FAF, fundus autofluorescence; τ_m, amplitude-weighted mean fluorescence lifetime.

idiopathic MHs, which comprise the majority of holes in the macular area. Other holes within the fovea may be the result of trauma, diabetic or hypertensive retinopathy, microaneurysms, or other causes. In this section we focus on the idiopathic forms.

MHs were first described in 1869, and 3 in 1,000 people appear to be affected. The disease shows a higher prevalence in women in their sixth or seventh decade of life. Pathologically, tangential vitreofoveal traction interactions between the vitreous body and the retina cause the formation of MHs (61). For diagnostic purposes, clinical imaging, primarily the use of OCT, is the gold standard (51). While MHs were long thought to be untreatable, an effective treatment with vitrectomy and gas tamponade was published in 1991 and has been used since (79). Approximately 80–90% of treated eyes show improvement in visual acuity, and more than 65% of patients have a visual outcome of 20/40 or better (76).

Many MHs show the presence of an operculum, an opacity at the posterior vitreous that may be found above the foveal hole (81). It has been demonstrated that these opercula contain MP (59, 62). FLIO has been used to demonstrate the carotenoid status in MHs as well as in opercula (121). FLIO was compared with both fundus spectroscopy and the dual-wavelength AFI method

for assessing MP in patients with MHs (117, 121). In MHs, the MP can be found adjacent to the hole as well as in opercula above the holes. It was also found that MP is distributed around the defect, in the same area where the inner retinal layers are found to be distributed based on OCT images (75). One FLIO study also investigated macular pseudoholes, a condition in which the Henle fiber layer is disturbed, but the layers below are still present (121). The presence of short FAF lifetimes despite the disruption of the Henle fiber layer allows for the conclusion that MP is not restricted to this layer, and it may also be detected in layers below. This verifies previous studies (12, 59, 62, 133).

Imaging MP in MHs offers the possibility of understanding the basic distribution of carotenoids in the retina, but MP imaging may also be used to determine whether visual improvement has occurred as a result of surgery. It was found that MP in MHs tends to be distributed at the nasal side of the MH (121). With successful surgery, MP redistributes toward the center of the fovea, which seems to correlate with better visual outcomes (121).

VEGF: vascular endothelial growth factor

GA: geographic atrophy

Carotenoids in Age-Related Macular Degeneration

AMD is the leading cause of blindness in the Western world, with a global prevalence of 8.69% in those aged 45–85 years and with prevalence increasing with age (141). As the global population ages, the prevalence as well as the incidence of AMD are likely to rise. AMD can be categorized into two forms: exudative and nonexudative. Initially, exudative AMD was recognized as the most devastating disease manifestation because it leads to the sudden outgrowth of vessels (neovascularization), often in combination with leakage of fluid and rapid visual decline. Exudative AMD can be treated with intravitreal injections of vascular endothelial growth factor inhibitors (anti-VEGFs). There is no comparable or even sufficient treatment for nonexudative AMD (31, 112). Nonexudative AMD appears to progress more slowly than the exudative form, but it still leads to central vision loss and legal blindness in the late stages. The late form of nonexudative AMD is called geographic atrophy (GA) (135). Atrophic areas may initially spare the fovea, which results in relatively normal central visual acuity. Extensions of atrophic lesions are often inevitable and can lead to complete central visual loss (72, 95). Because there is no curative treatment, delaying disease progression is the most promising solution for AMD; thus, carotenoids are of great importance, as supplementing L and Z was reported to slow disease progression in AMD (3, 92).

Although the pathophysiologic processes leading to the development of AMD remain unknown, oxidative stress and subsequent retinal damage seem to play leading roles. Generally, drusenoid and basal linear deposits are initial signs of AMD, but the involvement of lipofuscin in AMD pathogenesis remains unclear (42, 63). MP levels seem to be altered in AMD (78). **Figure 5** shows significantly altered MP in a patient with AMD.

Bone and coworkers (26) investigated retinal tissue from 56 eyes with AMD and 56 healthy eyes. The authors examined three different regions of interest: the inner region (0–5°), the medial region (5–19°), and the outer region (19–38°). Tissues were cut from retinal samples and the amount of carotenoid was determined by HPLC (26). Levels of MP were lower in AMD eyes, likely attributable to the disease pathology. In living patients, there are detectable differences in carotenoid levels between healthy eyes and AMD eyes. Higher amounts of serum carotenoids inversely correlate with the risk of developing AMD (94, 129). In particular, one large study conducted by Seddon and coworkers (129) investigated 356 patients with AMD as well as 520 controls. After adjusting for other risk factors relating to the disease, a comparison of the highest with the lowest quintile of carotenoid intake showed a 43% decrease in the risk of AMD. Carotenoids from dark green, leafy vegetables were associated with the greatest risk reduction, with spinach and collard greens providing the most beneficial carotenoid sources. The consumption of vitamin A, E,

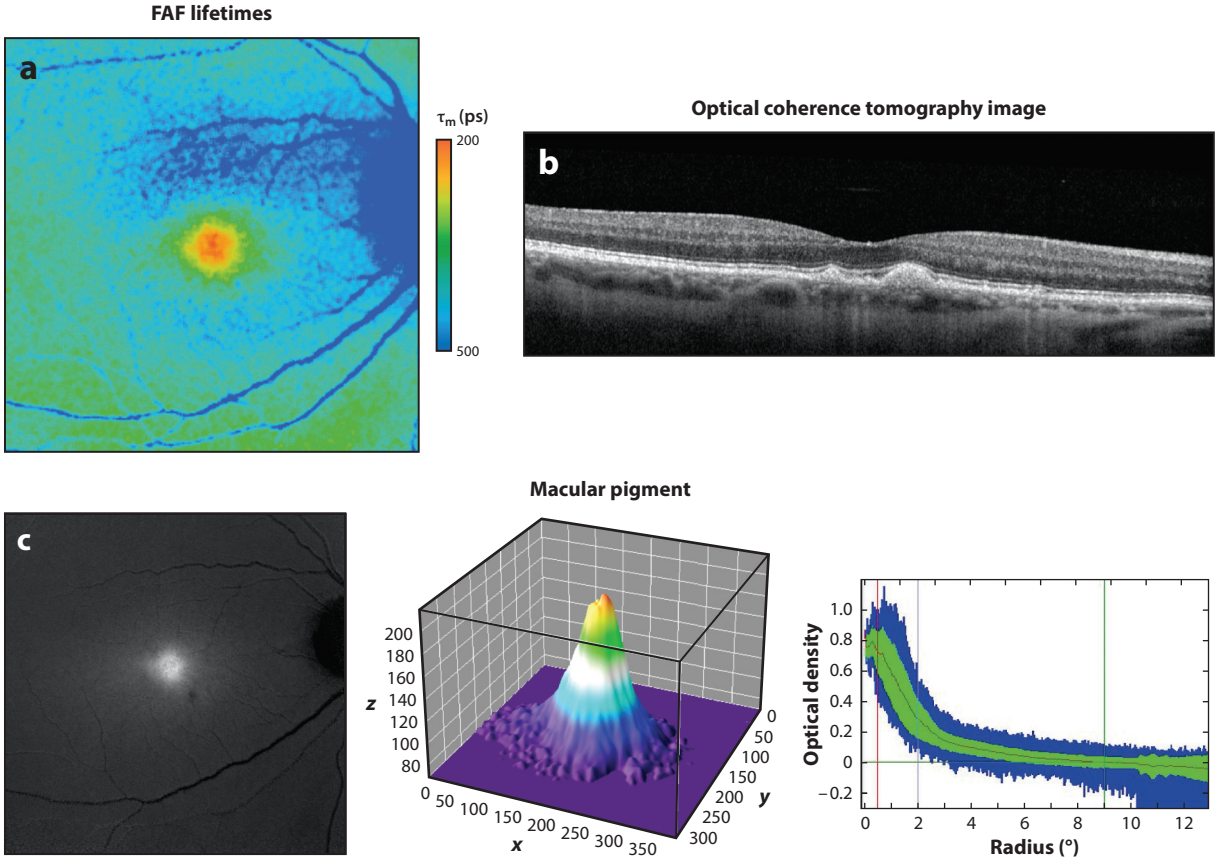

Figure 5

Macular pigment distribution in an eye with age-related macular degeneration. Images from (*a*) fluorescence lifetime imaging ophthalmoscopy, (*b*) optical coherence tomography, and (*c*) dual-wavelength autofluorescence assessment of macular pigment. Abbreviations: FAF, fundus autofluorescence; τ_m, amplitude-weighted mean fluorescence lifetime.

or C was not significantly associated with a lower risk of AMD. A different study, by Gale et al. (60), investigated carotenoid serum levels in 380 elderly participants. The authors of this study came to the same conclusion: Lower carotenoid intake is associated with a higher risk of AMD. Other studies imaging the levels of MP inside the eye similarly concluded that low levels of MP increase the risk of AMD development (10, 14, 17, 113). In 2002, a study investigated 93 eyes with AMD and 220 healthy control eyes using RRS to assess their carotenoid levels (17). On average, carotenoid levels were 32% lower in AMD eyes in patients who did not consume supplements. Interestingly, patients with AMD who started carotenoid supplementation with ≥4 mg of L each day at the time of diagnosis had retinal carotenoid levels that remained within the normal range (17). Furthermore, patients at risk of developing AMD (that is, with advanced disease in the other eye and increased age) showed reduced carotenoid levels (10). It has also been shown that lower amounts of MP are found in both forms of AMD, nonexudative as well as exudative (109). Beatty and coworkers (10) enrolled 121 participants and positively related higher MP levels to better visual performance. Other studies have also reported that in early disease stages, the amount of retinal carotenoids seems to be associated with visual function (5). Carotenoid supplementation

was identified as the best defense mechanism against AMD in terms of both preventing disease development and slowing progression (5).

Macular carotenoids seem to be protective against the development and expansion of retinal atrophy in nonexudative AMD (23). Understanding the mechanisms behind the disease and especially the phenomenon of foveal sparing is important, as the influence of MP on GA progression is of interest, but measuring MP in advanced GA is often difficult, and only a few studies have investigated the changes in MP during the course of disease. FLIO imaging has been used to show carotenoid distribution in patients with atrophic AMD (54), and short lifetimes were observed in spared foveas, consistent with a protective effect of MP (54, 120).

Carotenoids in Macular Telangiectasia Type 2

MacTel is an inherited retinal disease with reduced penetrance and relatively late onset, usually around age 40–60 years (33). It leads to vision changes that often cause metamorphopsia, as well as problems with reading, but in most cases it does not lead to legal blindness. Nevertheless, the gradually increasing neurodegenerative and vascular changes cause central vision loss in most patients. The prevalence of MacTel had initially been reported to be low, but it is now becoming clear that this is an underestimate. In many cases, patients with MacTel are initially diagnosed as having abnormal AMD, often resulting in a delay before correct diagnosis (82). Although the exact pathophysiology of MacTel remains unknown, a dominant inheritance with 38% penetrance was recently reported (110). The glycine/serine pathway has been identified as playing a role in the development of MacTel, but individual genes have yet to be identified (105, 124). Different imaging modalities may detect changes related to MacTel, with current diagnostic screening including color fundus imaging, blue light reflectance images, OCT imaging, FAF intensity imaging, and fluorescein and indocyanine green angiography (32, 115, 119). Although fluorescein angiography has been discussed as the gold standard, noninvasive imaging techniques to detect MacTel would be helpful, as well as beneficial for the patient (114, 137). FLIO has emerged as an excellent novel tool to diagnose MacTel in its early stages (119). Microperimetry testing seems to be a promising tool for monitoring the progression of vision loss in patients with MacTel (68). Previous studies using microperimetry in MacTel patients revealed that the dysfunction of rods seems to be more severe than that of cones (125). Ellipsoid zone loss is associated with a loss in central visual acuity as well as a loss of retinal sensitivity (106, 116). Many approaches to treating MacTel have failed. Treating the disease with anti-VEGF or intravitreal steroids has not been successful (83). However, very recently ciliary neurotrophic factor has been investigated as a potential treatment for MacTel. Clinical trials have already shown positive effects, so this treatment may be available for patients in the near future (34).

As no gene has been found for MacTel, the key to treating patients early is early clinical diagnosis. Another approach to early diagnosis is to investigate MP since MP levels in patients with MacTel are often extremely low. Although some patients with early disease may show nearly normal levels, patients with more advanced MacTel often exhibit an abnormal ring of MP at 5–9° eccentricity (14, 119). **Figure 6** depicts this finding. MP-binding proteins appear to be unaffected by MacTel; however, the typical MP distribution cannot be restored by supplementation with carotenoids (36, 145) and, instead, MP continues to accumulate in the ring-like area outside the MacTel region. Furthermore, supplementation may lead to the formation of MP deposits outside of the fovea (36). FLIO in MacTel has consistently shown low levels of MP and ring-like distributions (119). FLIO has also detected changes related to MacTel in the form of altered perifoveal FAF lifetime patterns, which makes FLIO an ideal tool to investigate this disease (118).

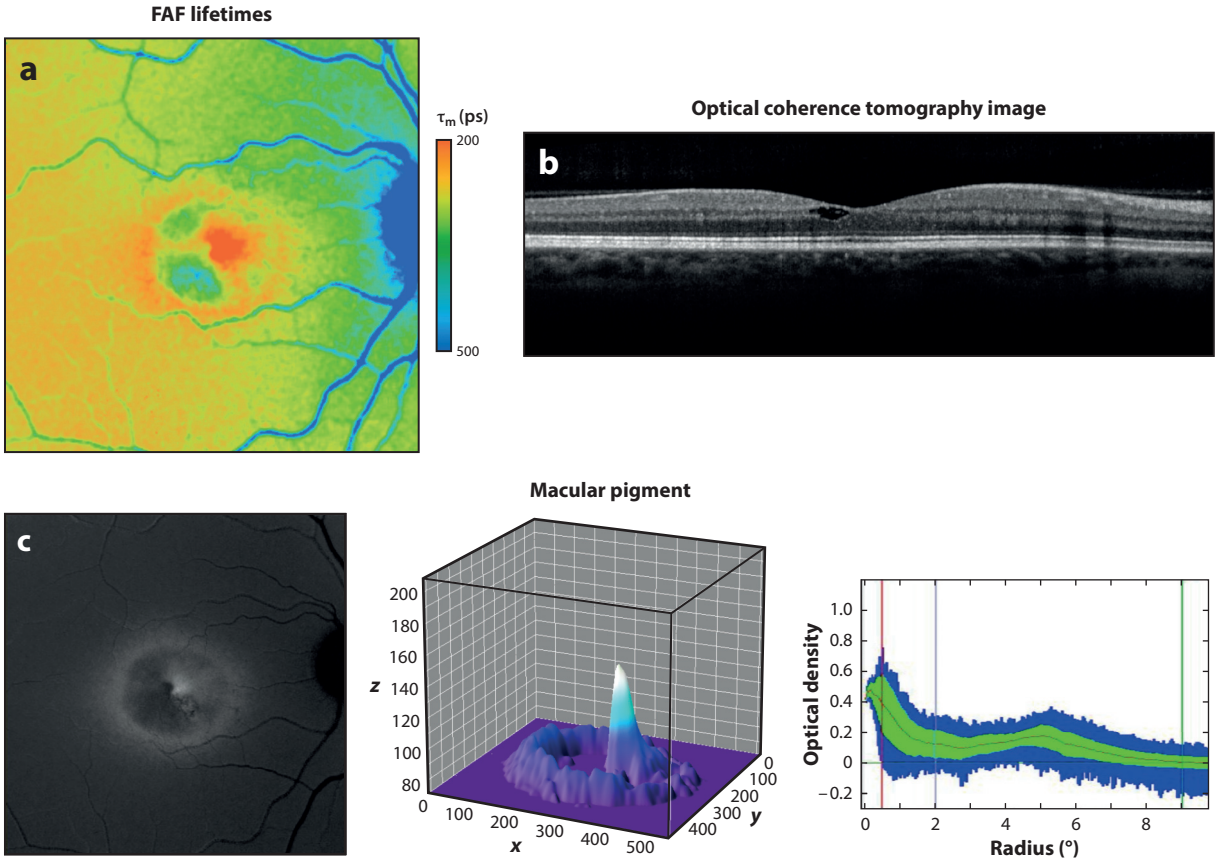

Figure 6

Macular pigment distribution in an eye with macular telangiectasia type 2. Images from (*a*) fluorescence lifetime imaging ophthalmoscopy, (*b*) optical coherence tomography, and (*c*) dual-wavelength autofluorescence assessment of macular pigment. Abbreviations: FAF, fundus autofluorescence; τ_m, amplitude-weighted mean fluorescence lifetime.

Carotenoids in Albinism

Patients with albinism often show foveal hypoplasia (67); photophobia, light skin tone, and vascular intrusion through the foveal avascular zone are also common. Electrophysiologically, indications of albinism can be found if the pattern onset of visual evoked potentials across the occipital scalp shows a reversal of the components of the visual evoked potential. Foveal hypoplasia, however, results in small or undetectable amounts of MP (117). **Figure 7** shows the MP assessment in a patient with albinism. Wolfson and coworkers (140) investigated MP in albinism using dual-wavelength AFI and described the possibility of finding evidence for an accumulation of MP in patients with albinism. Other reports found that MP is not measurable in patients with this disease (1, 108). However, MP might be miscalculated in AFI because the reference region may be affected by the absence of melanin. In AFI, this may result in a linearly sloping baseline toward the foveal center, which is not representative of MP (117). FLIO imaging may be of use in these patients as it does not need a reference region. A recent study found evidence of MP in one eye of a patient with a mild form of albinism and no evidence of MP in the other eye of the same patient or in either eye of another patient (117).

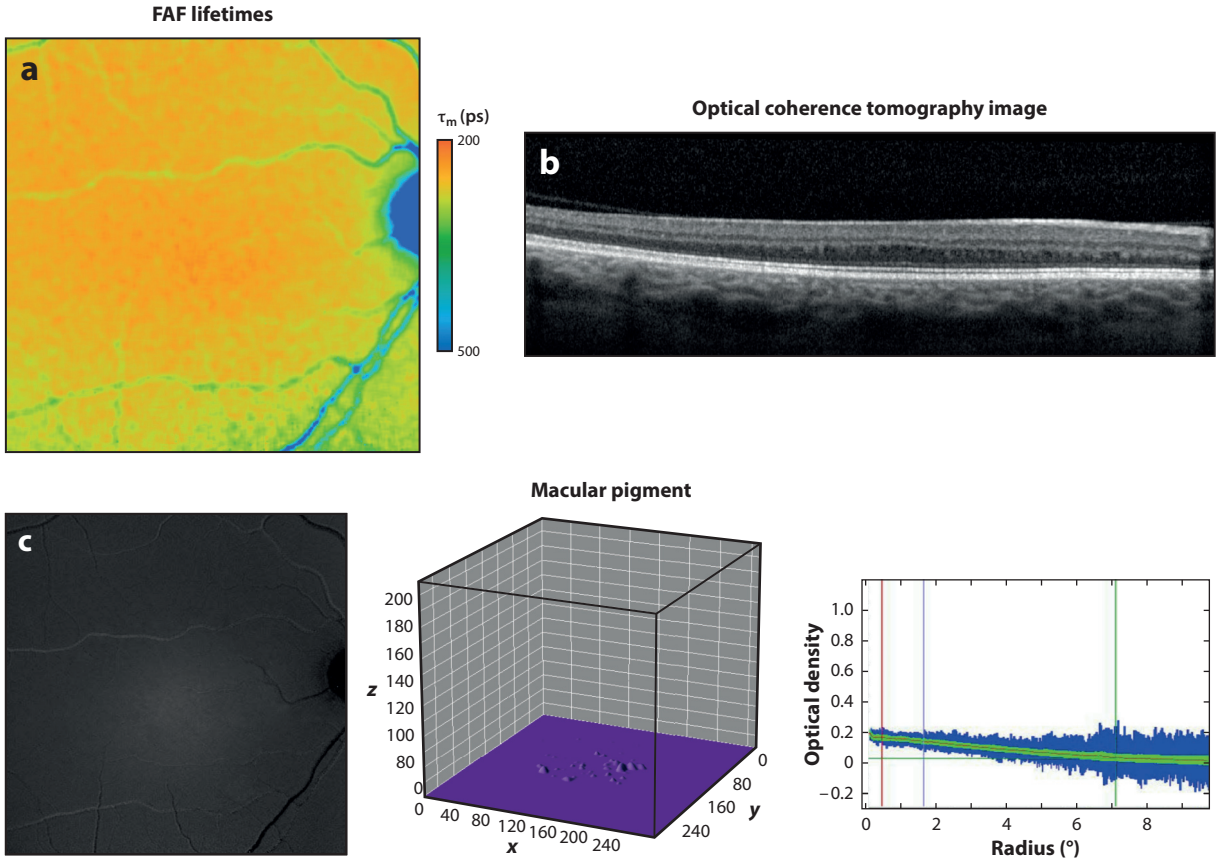

Figure 7

Macular pigment distribution in an eye with albinism. Images from (*a*) fluorescence lifetime imaging ophthalmoscopy, (*b*) optical coherence tomography, and (*c*) dual-wavelength autofluorescence assessment of macular pigment. Abbreviation: FAF, fundus autofluorescence; τ_m, amplitude-weighted mean fluorescence lifetime.

Carotenoids in Other Diseases

Carotenoids may be altered in many other diseases, possibly affecting vision as well as cognitive performance. These diseases include but are not limited to diabetes, Alzheimer's disease (AD), glaucoma, retinitis pigmentosa, and cataracts.

Carotenoids in diabetes have been extensively studied. The primary event in diabetes is hyperglycemia, which causes the formation of advanced glycation end products. This results in endothelial dysfunction as well as disturbances in the protein kinase C pathway (144, 146). A common complication of diabetes is retinal microangiopathy, which not only results in a disturbed blood–retina barrier but also causes inflammatory and degenerative processes at the human fundus (8, 38, 146). Macular edema is also a common finding. These retinal changes are known as diabetic retinopathy, and advanced glycation end products are thought to be important in terms of disease severity (46). Higher carotenoid intake may be beneficial in patients with diabetes, as carotenoid supplementation may enhance insulin sensitivity (111). Furthermore, an inverse correlation was found between serum carotenoid levels and metabolic syndrome, fasting serum insulin level, and

AD: Alzheimer's disease

HbA_{1c} level (40, 143). Overall, carotenoid supplementation seems to decrease not only the risk of diabetic retinopathy but also the risk of nonocular damage from diabetes (28, 132).

AD is the most common form of dementia, resulting in cognitive impairment that eventually leads to death. Age is the strongest risk factor for AD, and among people older than 65, AD has a prevalence of 1,275 new cases per 100,000 people (70). Since the retina is part of the nervous system, AD manifestations in the eye are of interest to many clinicians. A coexistence of AMD and AD has been found, with patients with AD also showing significantly lower amounts of MP (102). Supplementation with the carotenoids L and Z not only increases the amount of MP but it also significantly improves visual function and contrast sensitivity (99).

Retinitis pigmentosa is an inherited eye disease leading to peripheral retinal degeneration. Many different genotypes result in similar phenotypes, with the central macula usually being relatively spared. In a spared fovea, MP levels appear to be comparable to those in healthy controls. When assessed using FLIO and AFI, MP does not seem to be associated with the progression or severity of retinitis pigmentosa (6).

Similarly, the effects of retinal carotenoids on glaucoma have been extensively studied. In this condition, constantly increased intraocular pressure can lead to optic neuropathy. The increased pressure may have different origins, such as the impaired production, flow, or absorption of intraocular fluid. In contrast to some previous studies, a recent large study did not find a connection between retinal carotenoid status and glaucoma (43).

Other studies have investigated the influences of systemic diseases on carotenoid status. In malabsorption syndromes, such as celiac disease, inflammatory bowel disease, and cystic fibrosis, carotenoid levels are reduced (139). The effects of these systemic conditions on the risk of eye pathology need to be further evaluated.

Different studies have suggested that long-term multivitamin supplementation with antioxidants such as L and Z may be helpful in preventing cataracts (37, 97). This is reasonable, as sun exposure is a known risk factor for faster cataract progression. Although cataract surgery is a well-established treatment, delaying cataract progression will always be superior to surgical intervention.

Notably, a significant cataract of the lens may interfere with the measurement of MP as image-based MPOD measurements typically increase after cataract surgery relative to preoperative levels (101). Regression equations that correct for cataract density have been reported (101).

OUTLOOK AND CONCLUSIONS

Powerful

Retinal specialists are consulted daily by their patients with questions about recommendations for nutritional interventions to treat or prevent eye diseases. Because there are no fixed guidelines as to when and at what dosage patients should receive ocular supplements, the large variety of different products, and the costs not covered by insurance companies often lead to conservative views of carotenoid supplementation. Before advising patients to start supplementation, specialists often demand compelling evidence from large placebo-controlled studies that truly show a benefit of these interventions. Additionally, with the many different methodologies used to image MP, the limited availability of these instruments, and no universally established gold-standard methods for measuring and interpreting MP, even experienced ophthalmologists are often confused and unsure about making recommendations for carotenoid supplementation. Thus, there is a strong demand for a clear guideline on carotenoid supplementation that highlight not only the benefits and costs but also provide information about imaging modalities.

Powerful

Many retinal diseases, especially AMD, feature or are even preceded by altered carotenoid levels. Targeting this pathway with supplementation may be an elegant approach to prevent or delay

AMD progression. Furthermore, assessing retinal carotenoids may become essential to diagnose MacTel, as changes in carotenoid levels seem to be one of the earliest signs of the disease. With rapidly improving treatment options, a correct early diagnosis is indispensable.

We believe that the assessment of carotenoids in the clinical realm is still underrated, as many devices are research oriented and the benefits of assessing carotenoid status may be poorly understood by clinicians. Although psychophysical researchers prefer HFP, they have different goals for carotenoid assessment compared with ophthalmologists who rely on quick, reproducible, imaging-based methods that yield distribution patterns of MP. Techniques that can be combined with existing ophthalmological appliances, such as the SPECTRALIS system, are especially practical for clinical ophthalmologists. MP modules could be added to the systems that already are used in clinical routines. For example, carotenoid supplementation could be suggested not only to patients with intermediate or advanced AMD (as recommended by the AREDS2 guidelines) but also to younger people with low MP levels and a positive family history of AMD or other macular diseases, before they develop maculopathy.

For the future, we suggest that a standardized combination of AFI or reflectometry imaging should be supplemented with the novel FLIO technique, although the latter is not yet available at many different centers. We suggest that improving the presentation of MP data would be helpful, possibly in a three-dimensional plot that could easily be overlaid with other images of the eye. Similarly, establishing a normative database of MP levels and distributions would be helpful for clinicians. We strongly encourage the expanded assessment of carotenoids in clinical practice as this will likely result in improved diagnosis of retinal diseases and optimized recommendations regarding carotenoid supplementation.

DISCLOSURE STATEMENT

P.S.B. has been a consultant on the scientific advisory board of ScienceBased Health in the past. P.S.B. and the University of Utah hold patents on the use of resonance Raman spectroscopy to measure carotenoids in the human eye and other tissues. L.S. has been a medical consultant for Novartis. Both P.S.B. and L.S. have received reimbursement for travel to conferences from Heidelberg Engineering. The FLIO device was provided by Heidelberg Engineering at no cost for use in research at the Moran Eye Center. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abadi RV, Cox MJ. 1992. The distribution of macular pigment in human albinos. *Investig. Ophthalmol. Vis. Sci.* 33:494–97

2. Age-Relat. Eye Dis. Study Res. Group. 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* 119:1417–36
3. Age-Relat. Eye Dis. Study 2 Res. Group. 2013. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 309:2005–15
4. Age-Relat. Eye Dis. Study 2 Res. Group, Chew EY, Clemons TE, Sangiovanni JP, Danis RP, et al. 2014. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report no. 3. *JAMA Ophthalmol.* 132:142–49
5. Akuffo KO, Nolan JM, Peto T, Stack J, Leung I, et al. 2016. Relationship between macular pigment and visual function in subjects with early age-related macular degeneration. *Br. J. Ophthalmol.* 101:190–97
6. Andersen KM, Sauer L, Gensure RH, Hammer M, Bernstein PS. 2018. Characterization of retinitis pigmentosa using fluorescence lifetime imaging ophthalmoscopy (FLIO). *Transl. Vis. Sci. Technol.* 7:20
7. Andreatta W, El-Sherbiny S. 2014. Evidence-based nutritional advice for patients affected by age-related macular degeneration. *Ophthalmologica* 231:185–90
8. Barber AJ, Gardner TW, Abcouwer SF. 2011. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. *Investig. Ophthalmol. Vis. Sci.* 52:1156–63
9. Beatty S, Koh HH, Carden D, Murray IJ. 2000. Macular pigment optical density measurement: a novel compact instrument. *Ophthalmic Physiol. Opt.* 20:105–11
10. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. 2001. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Investig. Ophthalmol. Vis. Sci.* 42:439–46
11. Beatty S, van Kuijk FJ, Chakravarthy U. 2008. Macular pigment and age-related macular degeneration: longitudinal data and better techniques of measurement are needed. *Investig. Ophthalmol. Vis. Sci.* 49:843–45
12. Bernstein PS, Balashov NA, Tsong ED, Rando RR. 1997. Retinal tubulin binds macular carotenoids. *Investig. Ophthalmol. Vis. Sci.* 38:167–75
13. Bernstein PS, Delori FC, Richer S, van Kuijk FJ, Wenzel AJ. 2010. The value of measurement of macular carotenoid pigment optical densities and distributions in age-related macular degeneration and other retinal disorders. *Vis. Res.* 50:716–28
14. Bernstein PS, Li B, Vachali PP, Gorusupudi A, Shyam R, et al. 2016. Lutein, zeaxanthin, and *meso*-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog. Retinal Eye Res.* 50:34–66
15. Bernstein PS, Sharifzadeh M, Liu A, Ermakov I, Nelson K, et al. 2013. Blue-light reflectance imaging of macular pigment in infants and children. *Investig. Ophthalmol. Vis. Sci.* 54:4034–40
16. Bernstein PS, Yoshida MD, Katz NB, McClane RW, Gellermann W. 1998. Raman detection of macular carotenoid pigments in intact human retina. *Investig. Ophthalmol. Vis. Sci.* 39:2003–11
17. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. 2002. Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. *Ophthalmology* 109:1780–87
18. Bhosale P, Bernstein PS. 2005. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. *Biochim. Biophys. Acta* 1740:116–21
19. Bhosale P, Bernstein PS. 2007. Vertebrate and invertebrate carotenoid-binding proteins. *Arch. Biochem. Biophys.* 458:121–27
20. Bhosale P, Larson AJ, Frederick JM, Southwick K, Thulin CD, Bernstein PS. 2004. Identification and characterization of a Pi isoform of glutathione *S*-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye. *J. Biol. Chem.* 279:49447–54
21. Bhosale P, Li B, Sharifzadeh M, Gellermann W, Frederick JM, et al. 2009. Purification and partial characterization of a lutein-binding protein from human retina. *Biochemistry* 48:4798–807
22. Bogden JD, Oleske JM, Lavenhar MA, Munves EM, Kemp FW, et al. 1990. Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. *J. Am. Coll. Nutr.* 9:214–25

23. Bone RA, Landrum JT, Dixon Z, Chen Y, Llerena CM. 2000. Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Exp. Eye Res.* 71:239–45
24. Bone RA, Landrum JT, Fernandez L, Tarsis SL. 1988. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Investig. Ophthalmol. Vis. Sci.* 29:843–49
25. Bone RA, Landrum JT, Friedes LM, Gomez CM, Kilburn MD, et al. 1997. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp. Eye Res.* 64:211–18
26. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. 2001. Macular pigment in donor eyes with and without AMD: a case-control study. *Investig. Ophthalmol. Vis. Sci.* 42:235–40
27. Bone RA, Landrum JT, Tarsis SL. 1985. Preliminary identification of the human macular pigment. *Vis. Res.* 25:1531–35
28. Brazionis L, Rowley K, Itsiopoulos C, O'Dea K. 2009. Plasma carotenoids and diabetic retinopathy. *Br. J. Nutr.* 101:270–77
29. Brindley GS, Willmer EN. 1952. The reflexion of light from the macular and peripheral fundus oculi in man. *J. Physiol.* 116:350–56
30. Calvo MM. 2005. Lutein: a valuable ingredient of fruit and vegetables. *Crit. Rev. Food Sci. Nutr.* 45:671–96
31. Chaikitmongkol V, Tadarati M, Bressler NM. 2016. Recent approaches to evaluating and monitoring geographic atrophy. *Curr. Opin. Ophthalmol.* 27:217–23
32. Charbel Issa P, Gillies MC, Chew EY, Bird AC, Heeren TF, et al. 2013. Macular telangiectasia type 2. *Prog. Retinal Eye Res.* 34:49–77
33. Charbel Issa P, Holz FG, Scholl HP. 2009. Metamorphopsia in patients with macular telangiectasia type 2. *Doc. Ophthalmol.* 119:133–40
34. Chew EY, Clemons TE, Peto T, Sallo FB, Ingerman A, et al. 2015. Ciliary neurotrophic factor for macular telangiectasia type 2: results from a phase 1 safety trial. *Am. J. Ophthalmol.* 159:659–66.e1
35. Choi RY, Chortkoff SC, Gorusupudi A, Bernstein PS. 2016. Crystalline maculopathy associated with high-dose lutein supplementation. *JAMA Ophthalmol.* 134:1445–48
36. Choi RY, Gorusupudi A, Wegner K, Sharifzadeh M, Gellermann W, Bernstein PS. 2017. Macular pigment distribution responses to high-dose zeaxanthin supplementation in patients with macular telangiectasia type 2. *Retina* 37:2238–47
37. Christen WG, Glynn RJ, Manson JE, MacFadyen J, Bubes V, et al. 2014. Effects of multivitamin supplement on cataract and age-related macular degeneration in a randomized trial of male physicians. *Ophthalmology* 121:525–34
38. Cogan DG, Kuwabara T. 1963. Capillary shunts in the pathogenesis of diabetic retinopathy. *Diabetes* 12:293–300
39. Conrady CD, Bell JP, Besch BM, Gorusupudi A, Farnsworth K, et al. 2017. Correlations between macular, skin, and serum carotenoids. *Investig. Ophthalmol. Vis. Sci.* 58:3616–27
40. Coyne T, Ibiebele TI, Baade PD, McClintock CS, Shaw JE. 2009. Metabolic syndrome and serum carotenoids: findings of a cross-sectional study in Queensland, Australia. *Br. J. Nutr.* 102:1668–77
41. Curcio CA, Medeiros NE, Millican CL. 1996. Photoreceptor loss in age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 37:1236–49
42. Curcio CA, Millican CL. 1999. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch. Ophthalmol.* 117:329–39
43. Daga FB, Ogata NG, Medeiros FA, Moran R, Morris J, et al. 2018. Macular pigment and visual function in patients with glaucoma: the San Diego Macular Pigment Study. *Investig. Ophthalmol. Vis. Sci.* 59:4471–76
44. Dawczynski J, Jentsch S, Schweitzer D, Hammer M, Lang GE, Strobel J. 2013. Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: the LUTEGA study. *Graefes Arch. Clin. Exp. Ophthalmol.* 251:2711–23
45. Dawczynski J, Schweitzer D, Lang GE. 2011. [Objective measurement of macular optical density]. *Klin. Monatsblätter fur Augenheilkd.* 228:57–61 (In German)
46. de la Maza MP, Garrido F, Escalante N, Leiva L, Barrera G, et al. 2012. Fluorescent advanced glycation end-products (AGEs) detected by spectro-photofluorimetry, as a screening tool to detect diabetic microvascular complications. *J. Diabetes Mellitus* 2:221–26

47. Delori FC. 1994. Spectrophotometer for noninvasive measurement of intrinsic fluorescence and reflectance of the ocular fundus. *Appl. Opt.* 33:7439–52
48. Delori FC. 2004. Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. *Arch. Biochem. Biophys.* 430:156–62
49. Delori FC, Goger DG, Dorey CK. 2001. Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Investig. Ophthalmol. Vis. Sci.* 42:1855–66
50. Demmig-Adams B. 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. *Biochim. Biophys. Acta* 1020:1–24
51. Dithmar S. 2005. [Macular hole: survey and relevant surgical concepts]. *Ophthalmologe* 102:191–206 (In German)
52. Dysli C, Quellec G, Abegg M, Menke MN, Wolf-Schnurrbusch U, et al. 2014. Quantitative analysis of fluorescence lifetime measurements of the macula using the fluorescence lifetime imaging ophthalmoscope in healthy subjects. *Investig. Ophthalmol. Vis. Sci.* 55:2106–13
53. Dysli C, Wolf S, Berezin MY, Sauer L, Hammer M, Zinkernagel MS. 2017. Fluorescence lifetime imaging ophthalmoscopy. *Prog. Retinal Eye Res.* 60:120–43
54. Dysli C, Wolf S, Zinkernagel MS. 2016. Autofluorescence lifetimes in geographic atrophy in patients with age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 57:2479–87
55. Ermakov IV, Ermakova MR, Gellermann W. 2005. Simple Raman instrument for in vivo detection of macular pigments. *Appl. Spectrosc.* 59:861–67
56. Ermakov IV, Ermakova MR, Gellermann W, Bernstein PS. 2004. Macular pigment Raman detector for clinical applications. *J. Biomed. Opt.* 9:139–48
57. Ermakov IV, Gellermann W. 2010. Validation model for Raman based skin carotenoid detection. *Arch. Biochem. Biophys.* 504:40–49
58. Ermakov IV, McClane RW, Gellermann W, Bernstein PS. 2001. Resonant Raman detection of macular pigment levels in the living human retina. *Opt. Lett.* 26:202–4
59. Ezra E, Munro PM, Charteris DG, Aylward WG, Luthert PJ, Gregor ZJ. 1997. Macular hole opercula: ultrastructural features and clinicopathological correlation. *Arch. Ophthalmol.* 115:1381–87
60. Gale CR, Hall NF, Phillips DI, Martyn CN. 2003. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 44:2461–65
61. Gass JDM. 1997. *Stereoscopic Atlas of Macular Diseases: Diagnosis and Treatment*. St. Louis, MO: Mosby
62. Gass JDM, Van Newkirk M. 1992. Xanthic scotoma and yellow foveolar shadow caused by a pseudooperculum after vitreofoveal separation. *Retina* 12:242–44
63. Gliem M, Muller PL, Finger RP, McGuinness MB, Holz FG, Charbel Issa P. 2016. Quantitative fundus autofluorescence in early and intermediate age-related macular degeneration. *JAMA Ophthalmol.* 134:817–24
64. Ham WT Jr., Ruffolo JJ Jr., Mueller HA, Clarke AM, Moon ME. 1978. Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. *Investig. Ophthalmol. Vis. Sci.* 17:1029–35
65. Hammond BR Jr. 2015. Dietary carotenoids and the nervous system. *Foods* 4:698–701
66. Hammond BR Jr., Caruso-Avery M. 2000. Macular pigment optical density in a Southwestern sample. *Investig. Ophthalmol. Vis. Sci.* 41:1492–97
67. Harvey PS, King RA, Summers CG. 2006. Spectrum of foveal development in albinism detected with optical coherence tomography. *J. AAPOS* 10:237–42
68. Heeren TF, Clemons T, Scholl HP, Bird AC, Holz FG, Charbel Issa P. 2015. Progression of vision loss in macular telangiectasia type 2. *Investig. Ophthalmol. Vis. Sci.* 56:3905–12
69. Hercberg S, Preziosi P, Galan P, Devanlay M, Keller H, et al. 1994. Vitamin status of a healthy French population: dietary intakes and biochemical markers. *Int. J. Vitam. Nutr. Res.* 64:220–32
70. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. 2007. How common are the "common" neurologic disorders? *Neurology* 68:326–37
71. Holz FG, Spaide RF. 2010. *Medical Retina: Focus on Retinal Imaging*. Berlin: Springer-Verlag
72. Holz FG, Strauss EC, Schmitz-Valckenberg S, van Lookeren Campagne M. 2014. Geographic atrophy: clinical features and potential therapeutic approaches. *Ophthalmology* 121:1079–91

73. Howells O, Eperjesi F, Bartlett H. 2011. Measuring macular pigment optical density in vivo: a review of techniques. *Graefes Arch. Clin. Exp. Ophthalmol.* 249:315–47
74. Johnson EJ, Vishwanathan R, Johnson MA, Hausman DB, Davey A, et al. 2013. Relationship between serum and brain carotenoids, α-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the Georgia Centenarian Study. *J. Aging Res.* 2013:951786
75. Jordan F, Jentsch S, Augsten R, Strobel J, Dawczynski J. 2012. [Study on the time course of macular pigment density measurement in patients with a macular hole—clinical course and impact of surgery]. *Klin. Monatsblätter fur Augenheilkd.* 229:1124–29 (In German)
76. Kanski JJ. 2008. *Klinische Ophthalmologie: Lehrbuch und Atlas*. Munich: Elsevier
77. Karppi J, Nurmi T, Olmedilla-Alonso B, Granado-Lorencio F, Nyyssonen K. 2008. Simultaneous measurement of retinol, α-tocopherol and six carotenoids in human plasma by using an isocratic reversed-phase HPLC method. *J. Chromatogr. B* 867:226–32
78. Kaya S, Weigert G, Pemp B, Sacu S, Werkmeister RM, et al. 2012. Comparison of macular pigment in patients with age-related macular degeneration and healthy control subjects—a study using spectral fundus reflectance. *Acta Ophthalmol.* 90:e399–403
79. Kelly NE, Wendel RT. 1991. Vitreous surgery for idiopathic macular holes: results of a pilot study. *Arch. Ophthalmol.* 109:654–59
80. Kijlstra A, Tian Y, Kelly ER, Berendschot TT. 2012. Lutein: more than just a filter for blue light. *Prog. Retinal Eye Res.* 31:303–15
81. Kishi S, Kamei Y, Shimizu K. 1995. Tractional elevation of Henle's fiber layer in idiopathic macular holes. *Am. J. Ophthalmol.* 120:486–96
82. Klein R, Blodi BA, Meuer SM, Myers CE, Chew EY, Klein BE. 2010. The prevalence of macular telangiectasia type 2 in the Beaver Dam eye study. *Am. J. Ophthalmol.* 150:55–62.e2
83. Kovach JL, Rosenfeld PJ. 2009. Bevacizumab (avastin) therapy for idiopathic macular telangiectasia type II. *Retina* 29:27–32
84. Krinsky NI. 1989. Antioxidant functions of carotenoids. *Free Radic. Biol. Med.* 7:617–35
85. Krinsky NI, Taylor RF, eds. 2011. *Carotenoids: Chemistry and Biology*. New York: Springer
86. Lakowicz JR. 2006. *Principles of Fluorescence Spectroscopy*. New York: Springer. 3rd ed.
87. Li B, Rognon GT, Mattinson T, Vachali PP, Gorusupudi A, et al. 2018. Supplementation with macular carotenoids improves visual performance of transgenic mice. *Arch. Biochem. Biophys.* 649:22–28
88. Li B, Vachali PP, Bernstein PS. 2010. Human ocular carotenoid-binding proteins. *Photochem. Photobiol. Sci.* 9:1418–25
89. Li B, Vachali PP, Frederick JM, Bernstein PS. 2011. Identification of StARD3 as a lutein-binding protein in the macula of the primate retina. *Biochemistry* 50:2541–49
90. Li B, Vachali PP, Gorusupudi A, Shen Z, Sharifzadeh H, et al. 2014. Inactivity of human β,β-carotene-9′,10′-dioxygenase (BCO2) underlies retinal accumulation of the human macular carotenoid pigment. *PNAS* 111:10173–78
91. Li B, Vachali PP, Shen Z, Gorusupudi A, Nelson K, et al. 2017. Retinal accumulation of zeaxanthin, lutein, and β-carotene in mice deficient in carotenoid cleavage enzymes. *Exp. Eye Res.* 159:123–31
92. Lima VC, Rosen RB, Farah M. 2016. Macular pigment in retinal health and disease. *Int. J. Retina Vitreous* 2:19
93. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S. 2008. Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration. *Surv. Ophthalmol.* 53:68–81
94. Loskutova E, Nolan J, Howard A, Beatty S. 2013. Macular pigment and its contribution to vision. *Nutrients* 5:1962–69
95. Maguire P, Vine AK. 1986. Geographic atrophy of the retinal pigment epithelium. *Am. J. Ophthalmol.* 102:621–25
96. Mayne ST, Cartmel B, Scarmo S, Lin H, Leffell DJ, et al. 2010. Noninvasive assessment of dermal carotenoids as a biomarker of fruit and vegetable intake. *Am. J. Clin. Nutr.* 92:794–800
97. Moeller SM, Voland R, Tinker L, Blodi BA, Klein ML, et al. 2008. Associations between age-related nuclear cataract and lutein and zeaxanthin in the diet and serum in the Carotenoids in the Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. *Arch. Ophthalmol.* 126:354–64

98. Neelam K, O'Gorman N, Nolan J, O'Donovan O, Wong HB, et al. 2005. Measurement of macular pigment: Raman spectroscopy versus heterochromatic flicker photometry. *Investig. Ophthalmol. Vis. Sci.* 46:1023–32
99. Nolan JM, Loskutova E, Howard A, Mulcahy R, Moran R, et al. 2015. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. *J. Alzheimer's Dis.* 44:1157–69
100. Nolan JM, Stack J, O'Donovan O, Loane E, Beatty S. 2007. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp. Eye Res.* 84:61–74
101. Obana A, Gohto Y, Sasano H, Gellermann W, Sharifzadeh M, et al. 2018. Grade of cataract and its influence on measurement of macular pigment optical density using autofluorescence imaging. *Investig. Ophthalmol. Vis. Sci.* 59:3011–19
102. Ohno-Matsui K. 2011. Parallel findings in age-related macular degeneration and Alzheimer's disease. *Prog. Retinal Eye Res.* 30:217–38
103. Paolisso G, Tagliamonte MR, Rizzo MR, Manzella D, Gambardella A, Varricchio M. 1998. Oxidative stress and advancing age: results in healthy centenarians. *J. Am. Geriatr. Soc.* 46:833–8
104. Parker RS. 1993. Analysis of carotenoids in human plasma and tissues. *Methods Enzymol.* 214:86–93
105. Parmalee NL, Schubert C, Figueroa M, Bird AC, Peto T, et al. 2012. Identification of a potential susceptibility locus for macular telangiectasia type 2. *PLOS ONE* 7:e24268
106. Peto T, Heeren TFC, Clemons TE, Sallo FB, Leung I, et al. 2018. Correlation of clinical and structural progression with visual acuity loss in macular telangiectasia type 2: MacTel Project Report No. 6—The MacTel Research Group. *Retina* 38(Suppl. 1):S8–13
107. Pinazo-Duran MD, Gomez-Ulla F, Arias L, Araiz J, Casaroli-Marano R, et al. 2014. Do nutritional supplements have a role in age macular degeneration prevention? *J. Ophthalmol.* 2014:901686
108. Putnam CM, Bland PJ. 2014. Macular pigment optical density spatial distribution measured in a subject with oculocutaneous albinism. *J. Optom.* 7:241–5
109. Raman R, Biswas S, Vaitheeswaran K, Sharma T. 2012. Macular pigment optical density in wet age-related macular degeneration among Indians. *Eye* 26:1052–57
110. Ronquillo CC, Wegner K, Calvo CM, Bernstein PS. 2018. Genetic penetrance of macular telangiectasia type 2. *JAMA Ophthalmol.* 136:1158–63
111. Roohbakhsh A, Karimi G, Iranshahi M. 2017. Carotenoids in the treatment of diabetes mellitus and its complications: a mechanistic review. *Biomed. Pharmacother.* 91:31–42
112. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, et al. 2006. Ranibizumab for neovascular age-related macular degeneration. *N. Engl. J. Med.* 355:1419–31
113. Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. 2012. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. *Mol. Nutr. Food Res.* 56:270–86
114. Sallo FB, Leung I, Clemons TE, Peto T, Bird AC, Pauleikhoff D. 2015. Multimodal imaging in type 2 idiopathic macular telangiectasia. *Retina* 35:742–49
115. Sallo FB, Peto T, Egan C, Wolf-Schnurrbusch UE, Clemons TE, et al. 2012. "En face" OCT imaging of the IS/OS junction line in type 2 idiopathic macular telangiectasia. *Investig. Ophthalmol. Vis. Sci.* 53:6145–52
116. Sallo FB, Peto T, Egan C, Wolf-Schnurrbusch UE, Clemons TE, et al. 2012. The IS/OS junction layer in the natural history of type 2 idiopathic macular telangiectasia. *Investig. Ophthalmol. Vis. Sci.* 53:7889–95
117. Sauer L, Andersen KM, Binxing L, Gensure RH, Hammer M, Bernstein PS. 2018. Fluorescence lifetime imaging ophthalmoscopy (FLIO) of macular pigment. *Investig. Ophthalmol. Vis. Sci.* 59:3094–103
118. Sauer L, Andersen KM, Dysli C, Zinkernagel MS, Bernstein PS, Hammer M. 2018. Review of clinical approaches in fluorescence lifetime imaging ophthalmoscopy. *J. Biomed. Opt.* 23:1–20
119. Sauer L, Gensure RH, Hammer M, Bernstein PS. 2018. Fluorescence lifetime imaging ophthalmoscopy (FLIO)—a novel way to assess macular telangiectasia type 2 (MacTel). *Ophthalmol. Retina* 2:587–98
120. Sauer L, Klemm M, Peters S, Schweitzer D, Schmidt J, et al. 2018. Monitoring foveal sparing in geographic atrophy with fluorescence lifetime imaging—a novel approach. *Acta Ophthalmol.* 96:257–66
121. Sauer L, Peters S, Schmidt J, Schweitzer D, Klemm M, et al. 2017. Monitoring macular pigment changes in macular holes using fluorescence lifetime imaging ophthalmoscopy. *Acta Ophthalmol.* 95:481–92

122. Sauer L, Schweitzer D, Ramm L, Augsten R, Hammer M, Peters S. 2015. Impact of macular pigment on fundus autofluorescence lifetimes. *Investig. Ophthalmol. Vis. Sci.* 56:4668–79
123. Scarmo S, Henebery K, Peracchio H, Cartmel B, Lin H, et al. 2012. Skin carotenoid status measured by resonance Raman spectroscopy as a biomarker of fruit and vegetable intake in preschool children. *Eur. J. Clin. Nutr.* 66:555–60
124. Scerri TS, Quaglieri A, Cai C, Zernant J, Matsunami N, et al. 2017. Genome-wide analyses identify common variants associated with macular telangiectasia type 2. *Nat. Genet.* 49:559–67
125. Schmitz-Valckenberg S, Ong EE, Rubin GS, Peto T, Tufail A, et al. 2009. Structural and functional changes over time in MacTel patients. *Retina* 29:1314–20
126. Schweitzer D, Jentsch S, Dawczynski J, Hammer M, Wolf-Schnurrbusch UE, Wolf S. 2010. Simple and objective method for routine detection of the macular pigment xanthophyll. *J. Biomed. Opt.* 15:061714
127. Schweitzer D, Kolb A, Hammer M, Anders R. 2002. [Time-correlated measurement of autofluorescence: a method to detect metabolic changes in the fundus]. *Ophthalmologe* 99:774–79 (In German)
128. Schweitzer D, Schenke S, Hammer M, Schweitzer F, Jentsch S, et al. 2007. Towards metabolic mapping of the human retina. *Microsc. Res. Tech.* 70:410–19
129. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, et al. 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA* 272:1413–20
130. Sharifzadeh M, Bernstein PS, Gellermann W. 2006. Nonmydriatic fluorescence-based quantitative imaging of human macular pigment distributions. *J. Opt. Soc. Am. A* 23:2373–87
131. Shyam R, Gorusupudi A, Nelson K, Horvath MP, Bernstein PS. 2017. RPE65 has an additional function as the lutein to *meso*-zeaxanthin isomerase in the vertebrate eye. *PNAS* 114:10882–87
132. Sluijs I, Cadier E, Beulens JW, van der A DL, Spijkerman AM, van der Schouw YT. 2015. Dietary intake of carotenoids and risk of type 2 diabetes. *Nutr. Metab. Cardiovasc. Dis.* 25:376–81
133. Snodderly DM, Auran JD, Delori FC. 1984. The macular pigment. II. Spatial distribution in primate retinas. *Investig. Ophthalmol. Vis. Sci.* 25:674–85
134. Snodderly DM, Mares JA, Wooten BR, Oxton L, Gruber M, et al. 2004. Macular pigment measurement by heterochromatic flicker photometry in older subjects: the Carotenoids and Age-Related Eye Disease Study. *Investig. Ophthalmol. Vis. Sci.* 45:531–38
135. Sunness JS. 1999. The natural history of geographic atrophy, the advanced atrophic form of age-related macular degeneration. *Mol. Vis.* 5:25
136. Taskintuna I, Elsayed ME, Schatz P. 2016. Update on clinical trials in dry age-related macular degeneration. *Middle East Afr. J. Ophthalmol.* 23:13–26
137. Toto L, Di Antonio L, Mastropasqua R, Mattei PA, Carpineto P, et al. 2016. Multimodal imaging of macular telangiectasia type 2: focus on vascular changes using optical coherence tomography angiography. *Investig. Ophthalmol. Vis. Sci.* 57:268–76
138. Trieschmann M, Spital G, Lommatzsch A, van Kuijk E, Fitzke F, et al. 2003. Macular pigment: quantitative analysis on autofluorescence images. *Graefes Arch. Clin. Exp. Ophthalmol.* 241:1006–12
139. Ward MS, Zhao DY, Bernstein PS. 2008. Macular and serum carotenoid concentrations in patients with malabsorption syndromes. *J. Ocul. Biol. Dis. Inform.* 1:12–8
140. Wolfson Y, Fletcher E, Strauss RW, Scholl HPN. 2016. Evidence of macular pigment in the central macula in albinism. *Exp. Eye Res.* 145:468–71
141. Wong WL, Su X, Li X, Cheung CM, Klein R, et al. 2014. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob. Health* 2:e106–16
142. Woodall AA, Britton G, Jackson MJ. 1997. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability. *Biochim. Biophys. Acta* 1336:575–86
143. Ylonen K, Alfthan G, Groop L, Saloranta C, Aro A, Virtanen SM. 2003. Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. *Am. J. Clin. Nutr.* 77:1434–41
144. Yu DY, Cringle SJ, Su EN, Yu PK, Jerums G, Cooper ME. 2001. Pathogenesis and intervention strategies in diabetic retinopathy. *Clin. Exp. Ophthalmol.* 29:164–66

145. Zeimer MB, Padge B, Heimes B, Pauleikhoff D. 2010. Idiopathic macular telangiectasia type 2: distribution of macular pigment and functional investigations. *Retina* 30:586–95
146. Zhang XY, Zeng H, Bao S, Wang NL, Gillies MC. 2014. Diabetic macular edema: new concepts in patho-physiology and treatment. *Cell Biosci*. 4:27
147. Zhao DY, Wintch SW, Ermakov IV, Gellermann W, Bernstein PS. 2003. Resonance Raman measurement of macular carotenoids in retinal, choroidal, and macular dystrophies. *Arch. Ophthalmol.* 121:967–72

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