Published in final edited form as:

Curr Eye Res. 2018 July ; 43(7): 913–920. doi:10.1080/02713683.2018.1460380.

Rod-mediated Dark Adaptation and Macular Pigment Optical Density in Older Adults with Normal Maculas

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Abstract

Purpose: To examine the association between macular pigment optical density (MPOD) and rod-mediated dark adaptation (RMDA) in persons 60 years old with normal maculas as determined by an accepted color fundus photography grading system.

Methods: This cross-sectional analysis used baseline data from eyes in the Alabama Study on Early Age-Related Macular Degeneration. Eyes at step 1 in the AREDS 9-step grading system were considered normal. Eyes were additionally assessed by spectral domain optical coherence tomography (SD-OCT). Foveal MPOD was estimated via heterochromatic flicker photometry, and RMDA was assessed with a computerized dark adaptometer. The association between RMDA and MPOD was examined via Spearman correlation coefficients adjusted for age.

Results: In 306 eyes from 306 persons (mean age 68.2 years) in normal macular health, MPOD was not associated with RMDA (age-adjusted rank correlation = 0.043, $p = 0.45$). After 81 eyes with incidental macular findings by SD-OCT evaluation were excluded, the association between MPOD and RMDA remained null ($N=225$, age-adjusted $r=0.015$, $p=0.82$).

Conclusion: In a large sample of normal aged eyes, RMDA, a visual function that is rate-limited by retinoid availability to photoreceptors across the complex of retinal pigment epithelium, Bruch's membrane, and choriocapillaris, is not related to MPOD in the neurosensory retina.

Keywords

macular pigment; dark adaptation; aging; Müller cells

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Declaration of Interest

Cynthia Owsley is a patent holder on the device used to measure dark adaptation in this study. She has previously received research support from Genentech. Christine A. Curcio receives research support from Hoffman LaRoche and Heidelberg Engineering.

Introduction

Macular pigment (MP) is comprised of the xanthophylls lutein, zeaxanthin, and meso-zeaxanthin.¹ Pigments especially zeaxanthin are highly concentrated in the fovea, decline by an order of magnitude within 2° of fixation,² and remain at low levels throughout the retina.^{3,4} The role of MP in vision has been actively investigated under several mechanistic hypotheses. The protection hypothesis suggests that MP protects the retina from cumulative damage of ambient blue light via anti-oxidant properties.⁵ The optical hypothesis suggests that MP improves visual performance and visual comfort by attenuating chromatic aberration and light scatter, via properties of short wavelength light-filtering and dichroism.^{6,7} The neural efficiency hypothesis suggests that MP improves vision by a direct interaction with neurons⁸ that perhaps conferred an evolutionary advantage.⁶ MP biology has expanded beyond the macula to the brain, where xanthophylls have been detected and studied in reference to cognition and aging.^{9,10} MP is of clinical interest because dietary supplements containing lutein and zeaxanthin are recommended for patients with intermediate age-related macular degeneration (AMD).¹¹

The relationship between macular pigment optical density (MPOD) and visual function has been studied in cross-sectional association studies and in longitudinal studies of dietary supplements containing xanthophylls. In cross-sectional studies of younger adult eyes in normal retinal health, higher levels of MPOD are associated with better cone-mediated visual function, including better acuity and contrast sensitivity, reduced glare disability, faster photo-stress recovery, and critical flicker fusion.^{12–14} In older eyes in normal retinal health, MPOD is associated with better foveal light sensitivity.¹⁵ Most^{7,13,14,16–19} but not all²⁰ studies of normal eyes with MP experimentally augmented by dietary supplements report improved photopic and mesopic visual function. Associations between rod-mediated vision and MPOD have also been reported for both younger and older normal eyes,^{6,15} although one study reported no association.²¹

Two recent studies suggested that lower MPOD levels, psychophysically estimated by heterochromatic flicker photometry (HFP), are associated with greater delays in rod-mediated dark adaptation (RMDA);^{6,22} however, the results were based on small samples. In addition, the biological basis for expecting a relationship between MPOD and RMDA is not clear. Evidence gathered over 25 years supports a vascular-metabolic theory^{23,24} attributing slowed RMDA in aging and AMD to impaired translocation of retinoids and other essentials from the choroid across the retinal pigment epithelium (RPE)-Bruch's membrane-subretinal space, where specific AMD pathology such as drusen builds up over time. This model received strong recent confirmation with our finding that delayed RMDA, for which retinoid supply is rate-limiting,²⁵ is a functional biomarker of increased risk for incident early AMD after 3 years.²⁶ Also, RMDA slowing is exacerbated in intermediate AMD,²⁷ and particularly impaired by the presence of subretinal drusenoid deposit between RPE and photoreceptors.^{28,29} In the present study on a large sample of older adults using a rigorous definition of normal macular health, we asked whether the reported association between MPOD and RMDA can be confirmed.

Methods

This study was approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB) and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant after the nature and purpose of the study were explained. The sample was part of the baseline cohort of the Alabama Study on Early Age-Related Macular Degeneration (ALSTAR), a prospective study designed to examine whether delayed RMDA is a risk factor for incident early AMD.^{26,30} Participants were recruited from two primary care ophthalmology practices in the Callahan Eye Hospital at UAB between May 2009 and December 2011, based on the most recent dilated fundus examination. The eligibility criteria were (1) age ≥ 60 years old; and (2) no previous diagnoses of retinal diseases other than AMD, glaucoma or other optic nerve conditions, corneal disease, diabetes, Alzheimer's disease, Parkinson's disease, brain injury or other neurological or psychiatric conditions as revealed by the medical record or self-report. Three-field digital stereo-fundus photographs (450 Plus camera; Carl Zeiss Meditec, Dublin CA, USA) were taken to exclude eyes with AMD. Study eyes were in normal macular health defined as step 1 in the AREDS 9-step classification system (total drusen area less than the 125-μm-diameter sizing circle, and lacking increased pigment, depigmentation, and geographic atrophy).³¹ An experienced grader (M.E.C.) masked to all other participant characteristics evaluated photographs.

We also acquired spectral-domain optical coherence tomography (SD-OCT) volumes of the macula on all eyes (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany). B-scans were horizontally oriented and centered over the fovea across an area of 20° × 15° (5.7 × 4.2 mm). Automatic real-time averaging was set between 8 and 18. The purpose of capturing these images was to confirm that study eyes were in normal macular health and to exclude eyes with incidental retinal findings that might interfere with assessing RMDA and MPOD. A retina specialist (J.N.C), masked to participant characteristics, evaluated macular volumes for pathologies routinely seen by SD-OCT in a retina specialty clinic, including early and intermediate AMD, neovascular AMD, epiretinal membrane (ERM), lamellar macular holes, vitreomacular traction syndrome, and macular telangiectasia type 2 (MacTel2).

Information on demographics and use of all dietary supplements were obtained through interview. Dietary supplements were checked against product formulary information to identify those containing lutein and zeaxanthin. Best corrected visual acuity (BCVA) under photopic conditions (100 cd/m²) was assessed with the Electronic Visual Acuity tester³² (EVA; JAEB Center, Tampa FL) and expressed as the logarithm of the minimum angle resolvable (logMAR).

RMDA was measured in one eye only, i.e., the eye with better BCVA, because of time constraints in the study visit. Prior to testing, the eye was dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride to attain a pupil diameter of at least 6 mm. RMDA was measured psychophysically using the AdaptDx (MacuLogix, Hummelstown, PA), a computer-controlled dark adaptometer.^{30,33} Trial lenses corrected optical blur at the 30-cm viewing distance, if needed, and the fellow eye was occluded. The participant's head was

comfortably positioned in the forehead–chinrest of the adaptometer. An infrared camera positioned behind the fixation light displayed the eye on a monitor viewed by the examiner, who helped align the test eye to the redfixation light using a reticule displayed on the eye's image. The procedure began with a photobleach exposure to a flash (0.25 ms duration, 58,000 scotopic cd/m² s intensity; equivalent ~83% bleach) while the participant was focused on the fixation light. The photobleach flash subtended 4° and was centered at 5° on the inferior vertical meridian (i.e. superior to the fovea on the retina) which was also the position of the test target. Threshold measurement for a 2° diameter circular target of 500 nm wavelength (green) light began 15 seconds after bleach offset. The participant was instructed to maintain fixation on the red fixation light and to press a response button when a flashing target first became visible. Threshold was estimated with a three- down/one-up modified staircase.³³ Log thresholds were expressed as sensitivity in dB units as a function of time after bleach offset. Dark adaptation speed is defined by the rod intercept time (RIT),³³ the duration in minutes required for sensitivity to recover to a criterion value of 5.0×10^{-3} scotopic cd/m², in the latter half of the second component of RMDA.²⁵

MPOD was psychophysically estimated for the eye tested for RMDA using HFP and a computer-controlled instrument (QuantifEye MPS I, ZeaVision, St Louis MO).³⁴ MPOD estimation with this device has been validated against both an optical method (retinal reflectometry)³⁴ and a widely-used instrument also based on HFP (Macula Densitometer, Macular Metrics II, Rehoboth, MA).³⁵ The QuantifEye device has good repeatability in large and small samples.^{34,36,37} Well-established for MPOD estimation, HFP is predicated on the fact that MPOD is greatest in the foveal center and negligible in the near periphery, thus differentially impacting the perceived luminance of colors. Flicker judgments for a 1° diameter target were made at fixation and at 6° on the horizontal meridian. Targets consisted of a series of pre-set ratios of two wavelengths of light -- a blue light (465 nm), which is absorbed by MP, and a green light (530 nm), which is not. Presentations began at 60 Hz, above the critical fusion frequency for cone photoreceptors, such that target luminance appeared steady. Flicker frequency was then gradually reduced in 6 Hz steps. Both the central and peripheral tests were preceded by a brief pre-test to determine the patient's flicker sensitivity to be used for subsequent tests. The luminance ratio of the two wavelengths was changed by 0.2 dB, incrementing blue and decrementing green, with mean target luminance held constant. The participant pressed a button when flicker became visible.³⁴ The instrument plots minimum flicker points against the luminance ratio of the two wavelengths. MPOD is estimated by calculating the difference between the minima of the central and peripheral measurement curves along the x-axis (blue–green ratio plotted on a log scale). HFP estimates of MPOD test of 0 and 1.0 were considered unreliable per the manufacturer and were not included in the analysis.

Statistical Analysis.

Spearman correlation coefficients were used to examine the association between RMDA and MPOD. Partial rank correlation coefficients (r) were used to examine the association adjusting for age. In addition, spectrophotometric and two-wavelength autofluorescence MP imaging have shown secondary rings and shoulders on the central MP peak with a broader distribution in women than in men.^{2,38–41} Therefore, the analyses were stratified by gender

to examine whether the relationship between RMDA and MPOD differed among men and women. The main analysis was repeated restricting the sample to only those eyes that did not have incidental retinal findings on SD-OCT, only those eyes of participants not consuming dietary supplements containing lutein and zeaxanthin, and only those eyes that were pseudophakic.

Results

A total of 306 eyes from 306 participants had both RMDA and MPOD data. Table 1 presents demographics, dietary supplement use, and ocular characteristics of the sample. Mean age was 68.2 years, ranging from 60 to 82 years. Women comprised 68.3% of the sample; 95.4% of participants were white of non-Hispanic origin. The vast majority of participants (97.7%) did not use dietary supplements containing lutein and zeaxanthin. The rate of RMDA (RIT) averaged 11.09 minutes (standard deviation (SD) 6.20). The distribution of MPOD in the cohort is depicted as a frequency histogram in Figure 1, with average MPOD of 0.35 (SD 0.19).

A scatterplot illustrating the relationship between RMDA (RIT) and MPOD (Figure 2) does not reveal a relationship between these variables. Statistical evaluation of the data in Figure 2 indicates lack of any association between RIT and MPOD ($N=306$, age-adjusted $r=0.043$, $p\text{-value}=0.45$). This was the case for both men and women (Table 2). The association between RIT and MPOD remained null, even after the exclusion of 81 eyes with incidental macular findings per SD-OCT evaluation ($N=225$, age-adjusted $r=0.015$, $p\text{-value}=0.82$). Seven of our 306 participants reported taking dietary supplements containing lutein and zeaxanthin. The relationship between RMDA and MPOD was unchanged when these eyes were removed from the analysis (age-adjusted $r=0.036$, $p\text{-value}=0.53$). The association remained null even among the eyes that were pseudophakic ($N=92$, age-adjusted $r=0.044$, $p\text{-value}=0.68$).

Discussion

Our large sample ($N=306$) study of older adults in normal macular health as assessed by an accepted fundus grading classification system augmented with SD-OCT assessment found no association between RMDA and MPOD as assessed by HFP. This finding contrasts with two earlier reports that lower MPOD was associated with slower dark adaptation.^{6,22} A study by Patryas et al.²² was limited by a small sample ($N=33$), a wide age range (15–68 years) which did not include individuals ≥ 70 years old, and use of a non-standardized photobleach protocol (30–98% depending on the subject). Further, Patryas et al. based their conclusion on a subsample analysis of the lowest and highest deciles of MPOD ($n=8$ each); the association was not significant when the entire sample was analyzed. We further suggest that our inclusion of a very large sample as well as focusing on adults over 60 years old allows for a more rigorous test of the hypothesis of a MPOD/RMDA association. Our data can also be compared to those of Stringham et al.⁶ who examined rod- and cone-mediated vision in 27 subjects 21–51 years of age. They found that both time to mesopic sensitivity recovery after a bright light (i.e., mediated by both rods and cones) and steady-state absolute scotopic sensitivity correlated with MPOD averaged over its spatial profile. The mechanistic

bases of their two psychophysical tasks differ from the classic RMDA task²⁵ used in our study, which is an assay of efficient retinoid transfer across the RPE-Bruch's membrane-choriocapillaris interface. RMDA is also a functional biomarker for increased risk for incident AMD,²⁶ a disease with prominent pathology in the tissue layers comprising this interface.

We assessed HFP using a commercially available instrument (QuantifiEyes)³⁴ that tested participants' ability to detect alternating blue and green lights as flickering in the fovea and at 6° eccentricity. Early research on HFP tested subjects using Maxwellian view.^{2,15} More recent work including the present study uses table-top instruments with free viewing, flicker frequencies determined for each subject in a pre-test, and detection of flicker appearance rather than disappearance.^{34,35} These changes were introduced to facilitate use in clinical trials and clinics by making the task easier and faster for older adults. As summarized in **Table 3** the distributional properties of our MPOD data on eyes in normal macular health are consistent with those reported in many other observational studies of normal eyes and controlled trial placebo arms at baseline, mostly assessed with other free-viewing HFP-based devices but also with the instrument we used.

The underlying rationale of the hypothesis that MPOD is associated with rod function can be examined critically. Two studies on a small number of human retinas and using different preparative techniques and assay conditions reported that 25%⁴² and 10–15%⁴³ of total xanthophyll pigment localized to rod outer segments. Of relevance to MP's role is a presumption that effects are dose-dependent, and the limited available evidence suggests that MP levels in rod outer segments is low relative to the high levels in the foveal center, Henle fiber layer, and outer plexiform layer. Outer segment MP was not mentioned in the original Snodderly et al.³ description of monkey retina. In subsequent color depictions of these data, outer segment MP is undetectable. A definitive study by Bernstein et al.⁴⁴ assayed MP in many ocular tissues of approximately 100 different eyes, but did not check outer segments. Although existing data do not suggest that MP concentrations in outer segments are high enough to be physiologically meaningful, our knowledge of the presence, concentration, laminar distribution, and relative concentrations of xanthophylls in different retinal cells can be refined with new technologies that assay the composition of many tissue layers simultaneously, in individual eyes.^{45,46}

New data on MP biology and retinal imaging technologies will be useful for future studies addressing the impact of MP on vision, including our own, driven by recent evidence that the principal MP reservoir in human retina are the Müller glia. High MP concentration in the Henle fiber layer, foveal center,^{3,47–49} and inner plexiform layer is well explained by the morphology of individual Müller cells.^{50–52} In MacTel2, absence of MP is associated with histologically confirmed degeneration and loss of foveal Müller cells.^{53,54} Patients with Sjögren-Larsen syndrome exhibit both loss of clinically detectable MP and presence of inner retinal cysts suggestive of Müller cell degeneration.^{55–57} Surgically excised ERM are enriched both in Müller cell markers and xanthophyll pigment.^{58,59} Linking MP to Müller cells does not exclude xanthophylls or their binding proteins localizing to non-Müller cells,^{60,61} or the possibility of transfers between Müller cells and photoreceptors. Two wavelength autofluorescence imaging,^{41,62–64} now commercially available, uses the ratio of intensities

after blue and green excitation lights to localize MP; studies using these tools have revealed secondary rings and shoulders on the central MP peak, especially in women.^{38,41,49} Variations in MPOD topography have been attributed to structural differences in foveal shape,^{65,66} in turn attributable to Müller cells that span the retinal thickness as a structural framework for all cells.^{52,67,68} Answers to these structural questions are now accessible through SD-OCT^{40,49,69,70}

Strengths of this study are the use of a very large sample of older eyes with normal maculas as documented with a standardized classification system based on color fundus photography augmented with SD-OCT assessment, an accepted psychophysical method for MPOD estimation, and the largest number of eyes (N=306) tested to date for MPOD associations with RMDA. Limitations include lack of information about MP spatial distribution, the lack of spatial correlation between the MPOD and RMDA test areas, and the non-availability of an imaging-based method for MPOD estimation during the course of the study. To what extent our findings generalize to other ethnic/racial populations remains to be determined. Using sophisticated and comprehensive two wavelength autofluorescence imaging,^{71,72} our future work will newly address MPOD's relationship to rod- and cone-mediated vision and relation to foveal structure in older adults. This information is important for understanding the impact of dietary xanthophyll supplements recommended for patients with intermediate AMD.¹¹

Acknowledgements

None

Financial support: NIH R01AG04212, R01EY06109; EyeSight Foundation of Alabama; Dorsett Davis Discovery Fund; Alfreda J. Schueler Trust; Research to Prevent Blindness

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Figure 1.
Frequency histogram of the distribution of MPOD in our sample.

Figure 2. Scatterplot of MPOD and RMDA (RIT), N = 306. There was no association between MPOD and RIT (age-adjusted $r=0.043$, $p\text{-value}=0.45$).

Table 1.

Demographic, dietary supplement use, and ocular characteristics of the sample, N=306

	n (%)
Age, years	
60 – 69	207 (67.7)
70 – 79	91 (29.7)
80 – 89	8 (2.6)
Gender	
Men	97 (31.7)
Women	209 (68.3)
Race	
White, non-Hispanic	292 (95.4)
African American	12 (3.9)
Other	2 (0.7)
Use dietary supplements containing lutein and zeaxanthin	
Yes	7 (2.3)
No	299 (97.7)
Lens status	
Pseudophakic	92 (30.1%)
Phakic	214 (69.9%)
	Mean (standard deviation)
Rod-mediated dark adaptation, rod intercept time (RIT), minutes	11.09 (6.20)
Macular pigment optical density	0.35 (0.19)

Table 2.

Crude and age-adjusted Spearman correlation between rod-mediated dark adaptation

	Overall			Men			Women		
	N	MPOD	P value	N	MPOD	P value	N	MPOD	P value
Unadjusted ^a									
Rod-mediated dark adaptation, rod intercept time (RIT), minutes	306	0.049	0.40	97	0.053	0.60	209	0.040	0.57
Age-adjusted ^b									
Rod-mediated dark adaptation, RIT, minutes	306	0.043	0.45	97	0.083	0.42	209	0.017	0.81

(rod intercept time) and MPOD, overall and stratified by gender

^aCorrelation estimated using Spearman rank^bCorrelation estimated using partial Spearman rank

Table 3.

MPOD estimates from heterochromatic flicker photometry (free viewing) reported previously for normal eyes of persons not consuming dietary supplements. Data reported are from cross-sectional analyses.^a

Year	First author and reference note	Mean $\pm$ standard deviation (n, age in years)	Study design
2000	Hammond ⁷³	0.22 $\pm$ 0.13 (n=217, 17–92)	Observational, cross-sectional
2001	Ciulla ⁷⁴	0.21 $\pm$ 0.13 (n=280, 18–50)	Observational, cross-sectional
2001	Beatty ⁷⁵	0.29 $\pm$ 0.16 (n=46, 21–81)	Observational, cross-sectional
2004	Ciulla ⁷⁶	0.26 $\pm$ 0.19 (n=110, 55–89)	Observational, cross-sectional
2004	Nolan ⁷⁷	0.32 $\pm$ 0.20 (n=100, 22–60)	Observational, cross-sectional
2006	Mares ⁷⁸	0.43 $\pm$ 0.23 (n=1648, 53–86)	Observational, longitudinal
2008	Stringham ¹³	0.54 $\pm$ 0.25 (n=40, 17–41)	Trial baseline data
2007	Iannaccone ⁷⁹	0.31 $\pm$ 0.21 (n=120, 72–86)	Observational, cross-sectional
2009	van der Veen ³⁴	0.33 $\pm$ 0.20 (n=5581, 20–90) ^b	Observational, cross-sectional
2010	Loughman ¹²	0.48 $\pm$ 0.19 (n=142, 18–41)	Observational, cross-sectional
2010	y ⁸⁰	$\pm$ 0.19 (n=484, 18–70)	Observational, longitudinal
2011	Nolan ⁷	0.51 $\pm$ 0.20 (n=60, 29 $\pm$ 6)	Trial baseline data
2012	Loughman ³⁶	0.31 $\pm$ 0.15 (n=50, 34 $\pm$ 10)	Observational, cross-sectional
2013	Beirne ⁸¹	0.42 $\pm$ 0.19 (n=73, 20–71)	Observational, cross-sectional
2013	Feeney ⁸²	0.20 $\pm$ 0.16 (n=4453, 62.5 $\pm$ 9.6)	Observational, longitudinal
2013	Yao ¹⁸	0.50 $\pm$ 0.19 (n=60, 25–47)	Trial baseline data
2014	Hammond ¹⁶	0.53 $\pm$ 0.21 (n=56, 18.4–30.6)	Trial baseline data
2014	Bovier ¹⁴	0.36 $\pm$ 0.16 (n=86, 18–32)	Trial baseline data
2016	van der Made ¹⁹	0.46 $\pm$ 0.16 (n=49, 63 $\pm$ 8) ^b	Trial baseline data
2017	Mewborn ⁸³	0.4258 $\pm$ 0.16 (n=48, 18–26) 0.4956 $\pm$ 0.17 (n=54, 64–86)	Trial baseline data
2018	Zarubina (current study)	0.33 $\pm$ 0.20 (n=581, 60–89) ^b	Observational, cross-sectional

^aCriteria for inclusion in this table: population that is not consuming xanthophyll-containing supplements, maculas considered normal (by varying criteria), and MPOD measured psychophysically via HFP in free-viewing mode. Trial data at baseline combined active and placebo groups if reported together and placebo only if reported separately. For longitudinal observational studies, only baseline data are shown.

^bStudy used a QuantifEyes instrument (used in the current study).

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