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CLINICAL REPORT

A Color Perimetric Test to Evaluate Macular Pigment Density in Age-Related Macular Degeneration

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ABSTRACT

Purpose. To evaluate differences in measurements of macular pigment optical density (MPOD) in patients with dry age-related macular degeneration (AMD) and a group of healthy patients (control group). Short-term repeatability of MPOD measures was also assessed in the control group.

Methods. This cross-sectional study included 31 eyes from 31 patients with bilateral dry AMD, 21 eyes from 21 cases with dry AMD in the study eye and exudative AMD in the fellow eye. The control group included 17 eyes from 17 healthy patients of similar age and sex. The MPOD values were measured using a commercially available color perimetry technique (CP). Short-term repeatability of MPOD measurements by the CP technique was assessed in 20 eyes of 20 healthy subjects who were measured 3 times on 3 consecutive days.

Results. The mean values for MPOD were 5.59 ± 2.06 dB in cases in which both eyes had dry AMD, 5.25 ± 2.72 dB in cases in which one eye had wet AMD and the studied eye had dry AMD, and 5.97 ± 2.14 dB in the eyes of the healthy control group. The mean value was lower in cases in which the fellow eye had wet AMD; however, no significant difference in MPOD was found between the three groups ($p = 0.659$) or between the group with dry AMD in both eyes and the healthy control group ($p = 0.977$). The intraclass correlation coefficient (ICC) value was 0.664 between day 1 and day 2, and 0.822 between day 2 and day 3.

Conclusions. Our results do not show a direct relation between MPOD and dry AMD. Color perimetry does not provide acceptable short-term repeatability for measuring MPOD. Learning effects may contribute to the measured test-retest variability. Other studies are needed to determine if CP is suitable for repeated measurements during the long term follow-up with the same patient.

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Key Words: age-related macular degeneration, color perimetry, macular pigment optic density, short-term repeatability

Age-related macular degeneration (AMD) is one of the leading causes of irreversible vision loss among people aged 65 years and older in the Western world. Visual impairment from late-onset AMD can also lead to significant functional loss, reduced quality of life, and depression, which are often underestimated. In contrast, early AMD has less impact, emphasizing the need for early detection and prevention. In recent years, in addition to treatment for wet-type AMD, major advances have been made in understanding the epidemiology, risk factors, and genetics of AMD.[1,2]

Macular pigment (MP) is thought to play a protective role in AMD by reducing the oxidative stress and deleterious effects of short-wavelength light on the retina.[3] Lutein and zeaxanthin, the main constituents of MP, extend throughout the macula. Macular pigment is most concentrated in the foveola and drops to negligible levels in the parafoveal area. Lutein is more prevalent in the peripheral retina, and the ratio of lutein to zeaxanthin changes from approximately 1:2.4 in the central retina to 2:1 in the peripheral retina.[4] Lutein has strong antioxidant properties and also acts as a blue filter, protecting the retina from oxidative stress.[5] Macular pigment found in photoreceptor axons in the foveola, the internal and external plexiform layers in the macula, absorbs short-wavelength blue light harmful to the retina, protecting the macula against photo-oxidative stress at a prereceptive level.[6] Apart from filtering blue light and quenching free radicals, MP may also have another anti-inflammatory effect.[7] Because oxidative stress plays a major role in AMD pathogenesis, macular pigment optical density

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(MPOD) measurement in AMD patients has been researched in several studies.[3,8,9] Carotenoids are not synthesized *de novo* by animals and, therefore, MP is entirely of dietary origin.[10] Studies have shown that increased intake of the macular carotenoids lutein and zeaxanthin and food rich in these nutrients (e.g., spinach and collard greens) is associated with a decreased risk of neovascular AMD. It would seem, therefore, that any measures that might prevent, or even delay, AMD, such as dietary supplementation, should be thoroughly explored.[11,12] Some observational epidemiological and clinical studies have shown a reduced risk of AMD in subjects with higher intakes of lutein and zeaxanthin or with higher plasma concentrations of these compounds,[11–13] whereas other epidemiological studies have failed to show these protective effects.[14,15] There have been numerous studies investigating possible links between MPOD and AMD using a variety of measurement techniques. Some of these studies have supported an MPOD-AMD association,[3,9,16–19] and some have not.[8,20,21] Although low dietary and blood carotenoids were assumed to be modifiable risk factors for developing AMD, the protective effect of MPOD in the retina remains unclear.

The association of MP with AMD has been extensively studied in recent years, and interest in a rapid, objective, and accurate technique to measure MPOD has increased. Several techniques have been used to measure MPOD indirectly and noninvasively *in vivo*. These are categorized as either psychophysical or optical methods, and each one has merits and limitations. Psychophysical methods are the most common. Among them, heterochromatic flicker photometry is the most frequently used and is recognized for its accuracy and repeatability. It can measure visual sensitivity with a test wavelength that is maximally absorbed by MP and a reference wavelength that is not absorbed by MP.[22,23] Similarly, color perimetry (CP), one of the more recently introduced psychophysical methods, provides measurements of MP distribution and makes it possible to analyze MPOD across time. This is a simple and noninvasive method that does not require pupillary dilation. However, the repeatability of the technique with respect to measuring MPOD has not been investigated.

Since it has been asserted that MP is a protective factor against AMD, the question is whether there is any association between levels of MPOD in patients with AMD and normal healthy subjects. In the present observational study, we investigated the relationship between levels of MPOD and the presence of AMD in a case-control group of elderly subjects. We ruled out factors affecting the level of MPOD, such as ethnicity, color of the iris, and micronutrition supplementation, to analyze the relationship between AMD and MPOD in a homogeneous population. Our purpose was also to study the clinical applicability and repeatability of a commercially available technique. As far as we know, no other study has investigated the repeatability of CP.

MATERIALS AND METHODS

This cross-sectional comparative study included cases referred to Ankara University Department of Ophthalmology between October and March 2012 with wet AMD in one eye and dry AMD in the fellow eye or with bilateral dry AMD. Patients with medium drusen (≥63 but <125 μm), but without pigment abnormalities thought to be related to AMD, were considered to have early AMD. Patients with large drusen (≥125 μm) or with pigment abnormalities associated with at least medium drusen were considered to have intermediate AMD. Patients with lesions associated with neovascular AMD or geographic atrophy were considered to have late AMD.[24] All early and intermediate AMD cases were defined as dry AMD. Seventeen healthy subjects with no evidence of ocular disease were also enrolled in this study as a control group, and one eye from each was randomly assigned for analysis. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all patients before the clinical evaluation. The exclusion criteria included the intake of micronutrition supplementation containing retinal carotenoids; light-colored iris; patients who had exudative AMD or other retinal diseases; patients who had undergone cataract surgery, retinal surgery, or retinal photocoagulation; or patients who had any other disease that could have adversely affected visual acuity in the studied eye. All participants underwent complete ophthalmic examination at baseline, including the best-corrected visual acuity with a Snellen chart, biomicroscopic examination, fundoscopic examination, and intraocular pressure measurement. Smoking history and body mass index (BMI) were also noted for all participants. Spectral domain optical coherence tomography (Cirrus high-definition OCT; Carl Zeiss, Dublin, CA) examinations were performed at the same time as MP measurement. All exudative AMD patients had fundus fluorescein angiography (Heidelberg Retina Angiograph HRA2; Heidelberg Engineering, Germany) imaging. The diagnosis of AMD was confirmed by clinical examination, fluorescein angiography, and spectral domain optical coherence tomography.

The dry AMD group consisted of 52 eyes and was divided into two groups based on the status of the fellow eyes. The control group consisted of 17 eyes of 17 healthy individuals. The MPOD in all participants was measured with CP (Metrovision, France), and results were compared between the eyes with dry AMD and the healthy controls as well as within the dry AMD group according to the status of the fellow eyes.

Short-Term Repeatability of the Test

To test the short-term repeatability of the technique, a total of 20 eyes of 20 young healthy subjects aged between 27 and 42 years (mean, 33.1 ± 3.6 years) were measured three times on three consecutive days. Testing was scheduled between 3:30 and 5:00 p.m. Intraobserver repeatability for clinical measurements was assessed using the intraclass correlation coefficient (ICC) and the Bland-Altman plot. The ICC is an analysis-of-variance (ANOVA)–type correlation that measures the relative homogeneity within groups (between the repeated measurements) as a ratio to the total variation.[25] The ICC will approach 1.0 when there is no variance within repeated measurements, indicating that the total variation in measurements is caused solely by variability in the parameter being measured. The analysis of intersession repeatability is a calculation of the difference in MPOD obtained for each subject in two test sessions conducted by the same observer. The degree of intersession repeatability is the range over which 95% of the differences—the 95% limits of repeatability—are equal to the mean difference ±1.96 × standard deviation (SD) of the differences. The limits of agreement were

TABLE 1.
Demographic characteristics of the different groups

Groups		Both eyes with dry-type group	Wet AMD in one eye–dry AMD in the other eye group	Control group	p
Mean age, yr		69	70	69	0.57
Sex, %	Male	48	65	41.2	0.32
	Female	52	35	58.8	
Smoking, %		0.12	0.14	0.11	0.56
BMI		27.8 ± 2.2	28.71 ± 2.56	26.85 ± 2.08	0.40

*p < 0.05 statistically significant.
AMD, age-related macular degeneration; BMI, body mass ındex.

calculated as the mean difference in the measurements obtained by each observation ±1.96 × SD of the differences. The limits of repeatability are shown and were also plotted as the difference versus the mean of the MPOD in the two test sessions. This method uses graphing to assess whether there is agreement between the measurements.[26] A Bland-Altman plot was performed using MedCalc demo version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

Macular Pigment Optical Density Measurements

This program evaluates the density of the macular pigment by comparing the thresholds of perception of blue light and red light using a staircase technique similar to the technique used in automated perimetry. Luminance differential thresholds were measured for two stimuli: a blue stimulus (450 to 480 nm), which is absorbed by the MP, and a red one (620 nm), which is not absorbed. The stimuli were presented at the fovea and at six peripheral locations, with an eccentricity of 3 to 10 deg. The optical density of the MP was estimated as the difference between the thresholds of blue and red stimuli at the fovea and perifovea areas. In normal subjects, blue thresholds present a relative attenuation of about 0.6 log units at the fovea, indicating the presence of MP, which absorbs blue light. A correction for blue absorption by the lens was made based on the difference between the thresholds of blue and red stimuli at 10 deg eccentricity.

Before starting the procedure, the untested eye was occluded with a patch and optical correction for near vision was placed in front of the tested eye. The heads of all patients were positioned properly and comfortably. The position of the examined eye was adjusted to the level of the eye marks. The examiner could see whether the eye being tested was within the rectangular control area by means of a camera in the monitor. The visual stimulator displayed a circle, in the middle of which blue and red stimuli were presented. The physician asked the patient to press a button every time a stimulus was presented. In the second step of the measurement process, the visual stimulator displayed a central fixation dot. The patient was asked to fixate on the central dot and press the button every time a point appeared on the periphery. All measurements were performed in collaboration with the patients under the direction of the attending physician. First measurements were not recorded to allow participants to become familiar with the device and adapt to the procedure. The procedure was repeated in cases experiencing problems with cooperation during the measurements. Every individual was able to comprehend the tasks and follow the instructions. All MPOD measurements of the eyes were performed by an experienced ophthalmologist.

All analyses were conducted with the SPSS 15.0 software package (SPSS Inc., Chicago, IL). Data were expressed as numbers (percentage) for categorical variables and as mean ± SD for continuous variables. A value of $p < 0.05$ was considered to be statistically significant. The ANOVA comparison test was used to compare the mean values of more than two different groups, and an independent samples t-test was used to compare the mean values of two different groups in terms of MPOD and the demographic characteristics of patients.

RESULTS

The mean age was 70.4 ± 6.7 years in patients with dry AMD and 69.1 ± 7.8 years in the healthy control group. The 52 eyes of the 52 patients with dry AMD were divided into two groups according to the status of their fellow eyes. Of these 52 eyes, 31 had dry AMD and 21 had wet AMD in their fellow eyes. No significant difference in age ($p = 0.56$), sex ($p = 0.3$), smoking status ($p = 0.564$), and BMI ($p = 0.398$) was found between the groups (Table 1).

The mean values for MPOD were 5.59 ± 2.06 dB in cases in which both eyes had dry AMD, 5.25 ± 2.72 dB in cases in which one eye had wet AMD and the studied eye had dry AMD, and 5.97 ± 2.14 dB in the eyes of the healthy control group. The mean value was slightly lower in cases in which the fellow eye had wet AMD; however, no significant difference in MPOD was found between the three groups ($p = 0.659$, ANOVA) or between the group with dry AMD in both eyes and the healthy control group ($p = 0.977$, t-test). Also, no statistically significant difference was found between males and females in terms of MPOD ($p = 0.411$) (Table 2).

TABLE 2.
The mean MPOD values of the groups

Groups	Mean MPOD value, dB	p*
Both eyes with dry-type group	5.59 ± 2.06	0.66
Wet AMD in one eye–dry AMD in the other eye group	5.25 ± 2.72	
Control group	5.97 ± 2.14	

*p < 0.05 statistically significant.
AMD, age-related macular degeneration; MPOD, macular pigment optical density.

TABLE 3.
ICC of the measurements

Measurement	ICC	95% CI	p
First–Second	0.696	0.321–0.873	<0.001
First–Third	0.485	0.092–0.754	0.008
Second–Third	0.822	0.603–0.926	<0.001
First–Second–Third	0.669	0.437–0.839	<0.001

The intraobserver repeatability of the MPOD measurements of the healthy young subjects on three consecutive days was evaluated with ICC. The overall evaluation of the results showed that, clinically, each of the three measurement results had a low agreement (ICC, 0.669). The consistency between the second and last measurements was the highest (ICC, 0.822) (Table 3). The differences depicted in the Bland-Altman plots of days 1 to 2 (Fig. 1), days 1 to 3 (Fig. 2), and days 2 to 3 (Fig. 3) did not show a systematic distribution around the zero point, and no clear relationship was evident between the averages and differences.

The graph of the measurement results from the Bland-Altman plots of the first and second measurements (Fig. 1) and the first and third measurements indicates that the mean difference was 0.5 dB. Furthermore, the width of the limits of agreement was 3.3 dB for first to second, 4.7 dB for first to last, and 3.1 dB for second to last measurement, so the best agreement was between the last two. This was an indication of a learning period of the test.

DISCUSSION

The macular pigment is thought to play a protective role in AMD by reducing oxidative stress and the deleterious effects of short-wavelength light on the retina.[3–5] Hammond et al.[12] observed an increase in MP density within 4 weeks of dietary modifications consisting of increased intake of natural sources of lutein and zeaxanthin for most, but not all, subjects studied, a result indicating that the response to dietary carotenoids varies among individuals. The Carotenoids in Age-Related Eye Disease Study,[27] the Blue Mountain Eye study,[13] the Age-Related Eye Disease Study (AREDS),[28] and the Rotterdam Study[29] demonstrated that diets rich in lutein or zeaxanthin decrease the risk of AMD. Furthermore, various studies have shown that MPOD values are lower in subjects with AMD than in healthy individuals.[3,9,17,19] Obana et al.[30] determined that macular carotenoid levels are lower in patients with AMD than in healthy individuals; they also demonstrated that MPOD levels are much lower in advanced-stage AMD than in earlier stages of the disease. The authors emphasized the rapid progression of AMD in patients with low MPOD values. With this information, MP is thought to play a protective role against the development and progression of AMD. However, in the current study, the MPOD values in all groups did not show a significant difference, and the important role of MPOD in AMD was not demonstrated.

Growing evidence suggests a relationship between levels of MP and risk of age-related eye diseases, although a direct link has yet to be established. In addition, the known risk factors for AMD, such as cigarette smoking, light-colored irises, age, obesity, and female sex, have been associated with low levels of MPOD.[5] In our cross-sectional study, when the MPOD values of individuals with similar characteristics for risk factors, such as smoking, cataract surgery, BMI, color of the iris, and micronutrition supplementation, were investigated, no statistically significant difference was found between the three groups, although the mean MPOD value

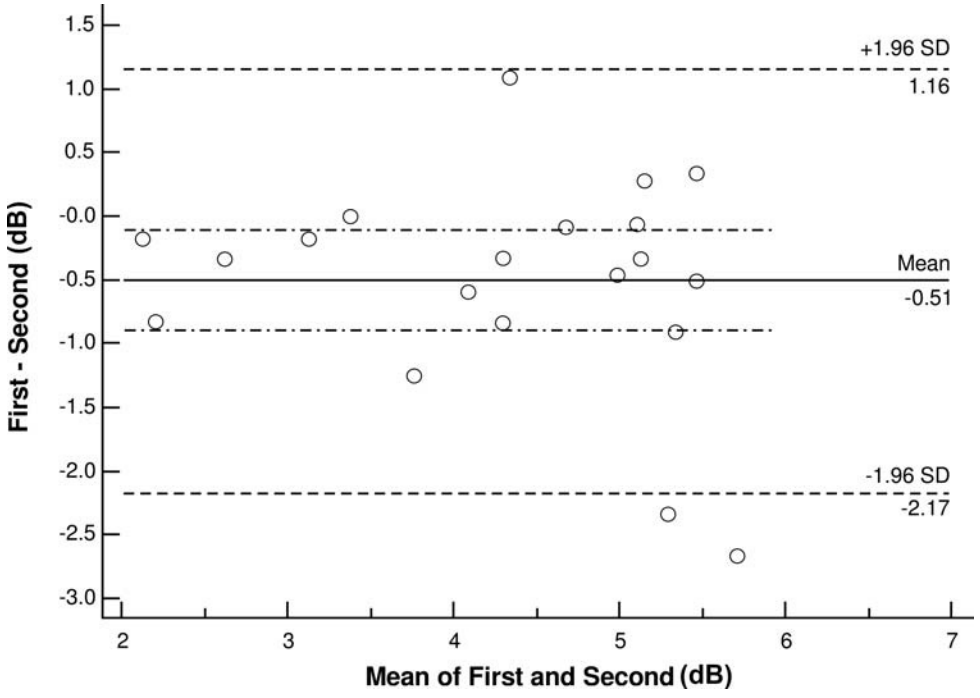


FIGURE 1.
The differences did not show a systematic distribution around zero point, and it is seen that there was no clear relationship between the averages and differences. The average difference graph of the measurement results indicates that one person was close to the limits ±1.96 SD and two people were outside the limits ±1.96 SD, which constitute 15% of the entire group. According to these findings, there is low consistency in the results between the first and second measurements.

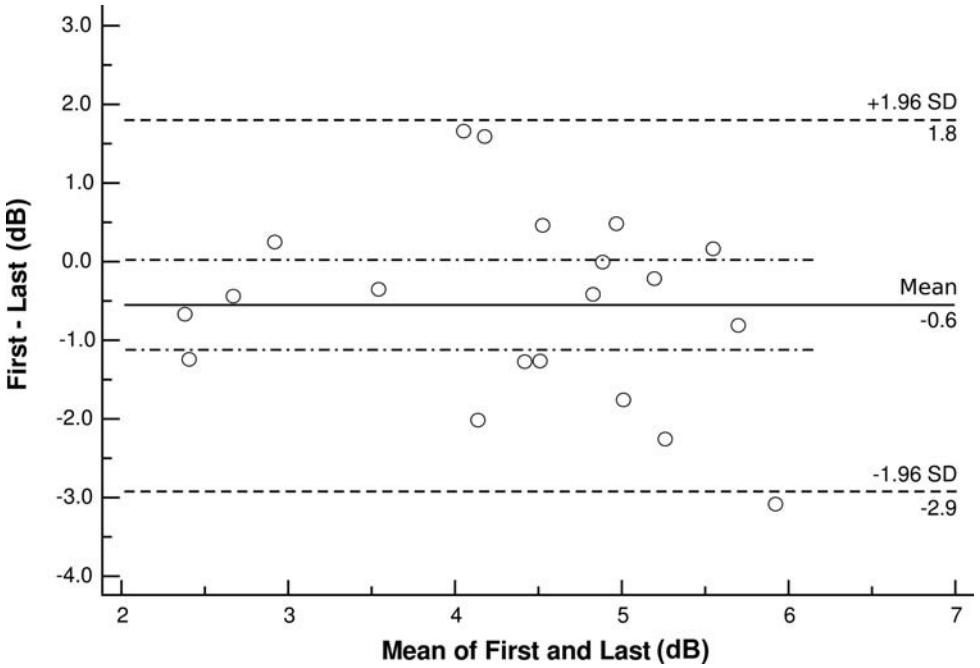
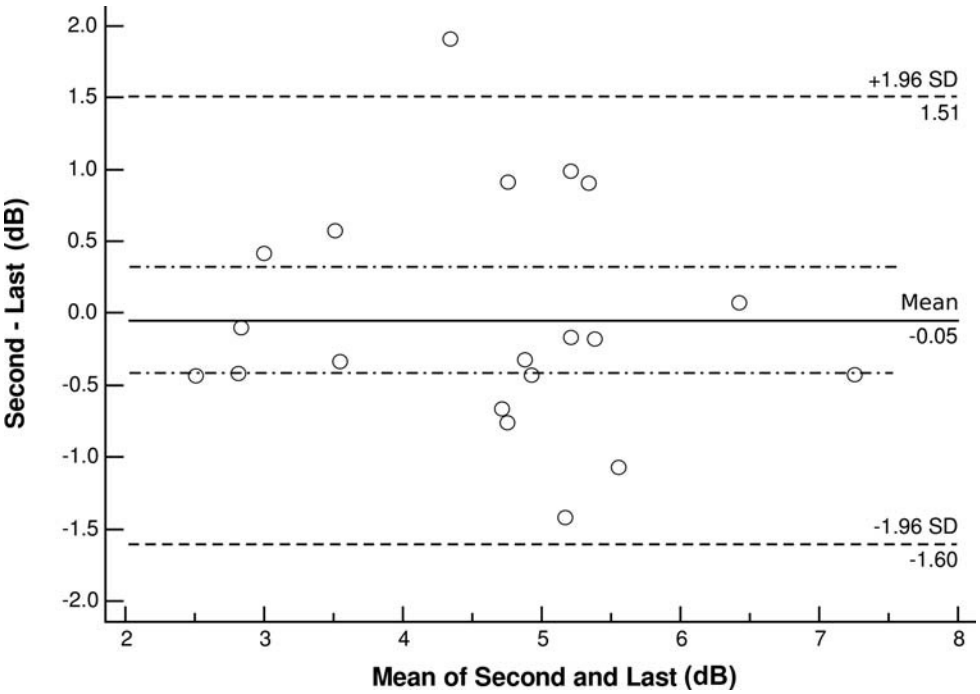


FIGURE 2.

The differences did not show a systematic distribution around zero point, and it is seen that there was no clear relationship between the averages and differences. The average difference graph of the measurement results indicates that one person was outside the limits ± 1.96 SD, which constitutes 5% of the entire group. So, it is considered that there was moderate consistency between the first and last measurements.

of the group of patients with wet AMD was slightly lower. The association between the risk factors for AMD and a relative lack of MP has prompted us to think that low MPOD values in AMD patients might be attributable to the risk factors for low MPOD levels present in AMD patients. In AMD patients who have no other risk factors, their MPOD levels might remain the same throughout their lifetime because of autoregulatory mechanisms. Our results showed that MPOD and AMD have no direct relation. These findings are similar to those in other studies.[8,20,21] Supplemental lutein has been shown to augment MP in all AMD and normal subjects. Also, it has been indicated that dry AMD retinas can accumulate xanthophyll to the same extent



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as eyes with no retinal pathology.[11] Bone and Landrum[31] et al. conducted a study to determine the effect of different doses of lutein supplements on serum lutein concentration and MPOD. They found that age does not significantly influence serum lutein uptake or MPOD response. Further longitudinal studies are required to compare the incidence of AMD in eyes with high and low MPOD to provide definitive evidence on the influence of MPOD on AMD progression.

Age and advanced disease in the fellow eye are the two most important risk factors for AMD. The reduced choroidal circulation seen in AMD can impair the delivery of important nutrients to the macula.[32] Current evidence, as well as the research the authors have cited, indicates that a systematic reduction in choroidal perfusion with an increase in the severity of AMD features is associated with the risk for the development of choroidal neovascularization.[33] Impaired choroidal perfusion will adversely affect the metabolic transport of nutrients across the retinal pigment epithelium to the retina.[32,33] Hence, altered circulation in eyes with exudative AMD should result in less transport of lutein and zeaxanthin across the retinal pigment epithelium and low MPOD in both eyes. Previously, an AREDS-1 study found that the risk of developing advanced AMD in the fellow eye of patients with advanced AMD in one eye was 43% across a span of 5 years.[34] Therefore, these dry-type eyes with advanced AMD in their fellow eyes might have low MPOD. Recently, Tsika et al.[8] investigated this hypothesis and showed some interesting results. They found that the MPOD of the fellow eye of patients with unilateral wet AMD was higher than that of patients with bilateral dry AMD, and women had higher MPOD than men. The authors did not find the lowest MPOD in the fellow eye of patients with unilateral wet AMD. The current study also compared the MPOD in dry AMD patients whose fellow eyes have either wet or dry AMD. We found that those patients with unilateral wet AMD have slightly lower levels of MPOD in their fellow eye compared with patients with bilateral dry AMD or with healthy subjects. However, the differences between the groups were not statistically significant. Similar to Tsika et al.,[8] we were not able to show that patients with wet AMD in one eye have low levels of MPOD in their fellow eye.

Two general approaches are used to measure lutein and zeaxanthin in the retina, namely, direct analytical measurements on autopsied eyes and indirect MPOD measurements using, for example, heterochromatic flicker photometry.[22,23] Bone et al.[17] showed significant differences in the MPOD values between AMD and non-AMD control groups by using high-performance liquid chromatography. However, details about the stage of AMD in the studied eyes were not mentioned. We believe that some advanced AMD patients might have scar tissue over the layers of the photoreceptors, so their MPOD values would have been much lower than those for the control group. An accurate assessment of the amount of MP, expressed as MPOD, is necessary to investigate the role of carotenoids and their assumed protective functions. Several psychophysical techniques have been proposed to estimate the spatial distribution of MP density. These techniques follow a common basic principle, which is to compare the patient's sensitivity to blue light with his/her sensitivity to another wavelength, such as red light, which is not absorbed by the pigment. One technique to measure this sensitivity is the heterochromatic flicker photometry technique, which has been frequently used in recent studies. Another technique is the CP technique, which is similar to the classic visual field test, except for the use of chromatic stimuli. High repeatability and reliability are especially important to monitor patients in studies that investigate the effect of disease processes or diet and nutritional lutein and zeaxanthin supplements on MPOD. *Reliability* is defined as the repeatability of measurements or the consistency of repeated measurements. The most common type of reliability in medical research is intrarater/interrater reliability. When the measurements are continuous, ICC is used to evaluate intrarater/interrater reliability. The current study is the first to independently evaluate an alternative CP technique for MPOD measurement, which is commercially available as a part of the Metrovision test suite (Metrovision, France).

We used ICC to test repeatability. In the literature, the ICC values for the studies that used the HPF technique were reported with a range in values from 0.06 to 0.58.[35] The ICC in this study, which used CP, was found to be 0.669, which indicates low agreement between the results.[36] In addition, the Bland-Altman plots of data for days 1 to 2 showed low agreement between the measurements.[25,26] However, the best agreement was between the last two measurements. We think that this moderate agreement might be related to the learning effect of the test. The test-retest variability with CP might be variable in AMD patients until they become familiar with how to use it. It could be used to measure MPOD longitudinally in subjects with AMD rather than for one or two measurements for an observational clinical study.

Some studies have investigated new areas in which CP can be applied such as for early detection of glaucoma.[37] The literature shows that CP can also be used to determine visual adaptation and retinal eccentricity profiles, especially in patients with retinal diseases, such as age-related maculopathy or diabetes.[38] However, the current study investigated MPOD value detection with CP as well as its repeatability. Although no calculated value for MPOD with CP has been reported in the literature, values for MPOD in another study were reported as 0.44 log units with heterochromatic flicker photometry,[18] 0.41 log units with fundus autofluorescence,[18] and 0.50 log units with fundus reflectometry.[22] We found that the values with CP were within the range of values (5.44 ± 2.14) previously reported with other techniques, a result that agrees with those of previous research. Our findings on SD were similar to those of other studies as well. The standard deviation was 0.20 to 0.30 log units with CP. It was 0.26 log units for psychophysics in a study reported by Delori et al.[22] and 0.21 log units for heterochromatic flicker photometry in the study of Hammond et al.[18] The CP technique is similar to other techniques with regard to how it estimates MPOD. Although the patients with dry AMD did not show any differences in MPOD based on the status of the fellow eye, the mean MPOD value is slightly lower in the healthy age-matched control group than in the AMD group in this study. This does not definitively mean that there is no relationship between the development of AMD and MPOD. The study lacks the statistical power to adequately answer this question and the new test may not be sensitive enough to differentiate existing variations in MPOD between groups. We were not able to show the statistical significance between the groups with a difference of 0.07 log units. However, one study reported statistically significant results between the groups with a 0.04 density unit difference in a larger study sample.[9] Another

study found a difference of 0.16 log units, which was a large enough effect size to reach statistical significance with fewer subjects than in our study and a similar SD.[3] We calculated the *post hoc* power and effect size of the current study. Based on a 0.19 effect size, 0.05 error probability, 69 total sample size, and three different groups, the power of the current study was detected as 0.26. To fix the study power at 0.80, the effect size needed for that power was found to be 0.38. We believe that sample size is the limitation of the study, and further research with a larger sample size is necessary to reach a definitive conclusion on the relationship between MPOD and AMD.

Similar to other techniques, the CP technique provides an estimation of MPOD. The absence of pupil dilatation and the control of fixation with a camera are advantages of the CP technique, which can be used with standard visual perimetry equipment with natural pupils. Other advantages of the CP test are the ability to test several test locations in the periphery and the follow-up program. This test has been produced as an alternative to the heterochromatic flicker photometry method because of its rapid applicability; the staircase thresholding technique is much easier for patients than the heterochromatic flicker technique. However, the fact that the test-retest variability was high in the young healthy control subjects indicates that CP might be more suitable after a learning period. Learning effects may contribute to measured test-retest variability, and CP would be suitable for repeated measurements during long-term follow-up with the same patient. To the best of our knowledge, this study is the first to test the repeatability of the CP technique. Further studies are needed to establish the clinical applicability of this technique.

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