

# Macular Pigment Optical Density Measured by Dual-Wavelength Autofluorescence Imaging in Diabetic and Nondiabetic Patients: A Comparative Study

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**PURPOSE.** To compare macular pigment optical density (MPOD) in type 2 diabetic and nondiabetic patients by using dual-wavelength autofluorescence imaging and to investigate the correlation of MPOD with glycosylated hemoglobin (HbA1C) and serum lipid levels.

**METHODS.** Forty-three patients were divided into groups 1 (controls,  $n = 14$ ), 2 (diabetic without retinopathy,  $n = 17$ ), and 3 (diabetic with mild nonproliferative retinopathy,  $n = 12$ ). MPOD was measured with a modified confocal scanning laser ophthalmoscope and compared among groups (analysis of variance). The correlation of HbA1C and serum lipid (HDL, LDL, total cholesterol, and triglycerides) levels with MPOD was determined for each group (linear regression analysis).

**RESULTS.** Mean  $\pm$  SD ages in groups 1 ( $56.2 \pm 11.7$  years), 2 ( $58.6 \pm 11.5$  years), and 3 ( $62.8 \pm 9.8$  years) were similar ( $P = 0.324$ ). Mean MPOD averaged in a  $2^\circ$ -diameter circle around the fovea was significantly different between the three groups: 1, ( $0.29 \pm 0.07$  density units [DU]), 2 ( $0.22 \pm 0.09$  DU), and 3 ( $0.14 \pm 0.05$  DU) ( $P < 0.001$ ). There was a significant difference in mean MPOD levels at  $0.5^\circ$  between groups 1 ( $0.51 \pm 0.12$  DU) and 2 ( $0.24 \pm 0.11$  DU;  $P < 0.001$ ), but not between groups 2 and 3 ( $0.33 \pm 0.15$  DU;  $P > 0.05$ ). A significant inverse correlation was observed between HbA1C levels and mean MPOD, averaged at  $2^\circ$  around the fovea in all patients ( $r = -0.63$ ,  $P < 0.001$ ). No significant correlations were found between MPOD and serum lipid levels or age.

**CONCLUSIONS.** Type 2 diabetic patients, with or without retinopathy, had reduced MPOD when compared with that in nondiabetic patients. In addition, a significant inverse correlation between MPOD and HbA1C levels was observed. (*Invest Ophthalmol Vis Sci.* 2010;51:5840–5845) DOI:10.1167/iov.09-4695

Macular pigment (MP) consists of two dietary carotenoids (lutein and zeaxanthin) that act as an optical filter for wavelengths shorter than 550 nm and provide antioxidant protection to the human retina by inhibiting the peroxidation of long-chain polyunsaturated fatty acids.<sup>1–3</sup> The highest levels

of MP in the human body are measured in Henle's fibers at the fovea and in the inner nuclear layer in the perifoveal area.<sup>4–6</sup> As oxidative damage seems to be an important exacerbating factor in some retinal diseases,<sup>7–9</sup> the postulated protective role of MP in some disorders, such as age-related macular degeneration, has been investigated.<sup>3,10</sup>

The incidence of diabetes mellitus varies from 1.5% to 5.0% per year, with a variation in prevalence of 7.8% to 45% in different racial and ethnic groups.<sup>11–13</sup> In diabetics, an average prevalence of 52% for diabetic retinopathy has been reported.<sup>11</sup> Diabetic retinopathy is a major cause of blindness in the occidental world.<sup>11–13</sup> Hyperglycemia and oxidative stress are major factors involved in the pathophysiology of this threatening condition,<sup>14–17</sup> and an experimental study has demonstrated a reduction in retinal oxidative damage after carotenoid supplementation.<sup>18</sup> Finally, a negative relationship between dietary fat intake and MPOD<sup>19</sup> and between body fat content and MPOD within the retina has been demonstrated in other studies.<sup>20,21</sup>

Several objective techniques have been used to measure MPOD indirectly and noninvasively in vivo. They are divided into psychophysical (heterochromatic flicker photometry<sup>22</sup> and motion photometry<sup>23</sup>) or optical (autofluorescence spectrometry,<sup>24</sup> reflectometry,<sup>25</sup> imaging reflectometry,<sup>26</sup> and Raman spectrometry<sup>27</sup>) methods. They are well described in the literature, and each one has certain merits and limitations. Autofluorescence, one of the more recently introduced methods, allows objective and reliable measurements of MP distribution within the retina and is particularly easy to use in a clinical environment with patients.<sup>28,29</sup>

In this study, we used dual-wavelength autofluorescence imaging to investigate differences in MP optical density (MPOD) between diabetic and nondiabetic patients. In addition, we investigated whether those values would correlate with glycosylated hemoglobin (HbA1C) and serum lipid levels in both groups of patients.

## METHODS

This prospective, comparative, and noninterventional protocol was conducted at the Department of Ophthalmology, The New York Eye and Ear Infirmary, after approval by the Infirmary's Institutional Review Board and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects before enrollment and examination.

## Patients

We enrolled consecutive eligible patients with or without type 2 diabetes. Patients evaluated in the comprehensive Ophthalmology Clinic who met our inclusion criteria were directed to the Retina Clinic, where a complete ophthalmic examination was performed. All

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diabetic patients were receiving regular medical (insulin or oral hypoglycemic) treatment. Exclusion criteria included spherical equivalent outside the range of  $\pm 4$  D, ocular diseases other than mild nonproliferative diabetic retinopathy, clinical evidence of diabetic macular edema, previous macular laser treatment, moderate or dense lens opacity, previous intraocular surgery or ocular trauma, and best corrected visual acuity (BCVA)  $\leq 20/40$ . If both eyes were eligible, one eye was randomly selected. Mild nonproliferative diabetic retinopathy was defined by the presence of microaneurysms only. After inclusion, the patients were divided into three groups. Group 1 comprised nondiabetic patients, group 2 diabetics without clinical evidence of retinopathy, and group 3 diabetics with mild nonproliferative diabetic retinopathy. Demographic data collected included age, sex, ethnicity, and systemic comorbidities, all self-reported by the patients.

## Procedures

The autofluorescence method for measuring MPOD has been extensively described elsewhere.<sup>24,28-31</sup> Briefly, it is based on the principle of the autofluorescence of lipofuscin, which is located in the RPE cells. This fluorescence is emitted in the 520- to 800-nm spectral range, and it can be excited in vivo by light between 400 and 570 nm.<sup>32</sup> In the fovea, since the excitation spectrum of foveal lipofuscin is strongly influenced by the absorption spectrum of MP, light absorbed by the carotenoids results in a central area of reduced lipofuscin fluorescence.<sup>28</sup> In measuring MPOD, the autofluorescence method compares results from the region of maximum MPOD overlying the fovea to an area with no optically appreciable MP several degrees eccentric to the fovea, using two excitation wavelengths that are differentially absorbed by the MP (488-nm, well-absorbed, and 512 nm, minimally absorbed). Quantitative imaging was performed with a modified scanning laser ophthalmoscope (SLO; Heidelberg Retinal Angiographer [HRA1], Heidelberg Engineering GmbH, Heidelberg, Germany).

All patients had their pupils dilated with tropicamide 1% and phenylephrine 2.5% drops before imaging, and each eye was tested three times. Each patient was positioned in front of the SLO camera and instructed to maintain steady fixation straight ahead. After the operator was comfortable with the patient's fixation and the focus of the SLO on the macular region, rapid sequences of 20° images were captured at 488 and 514 nm. MPOD maps were generated by digital subtraction of the log autofluorescence images, and the mean MPOD was calculated for 0.5°- and 2°-diameter circles centered on the fovea.

Also, the average MPOD in 1°, 2°, and 4°-diameter circles was measured. The reference area of no MPOD was set at 7.0° from the center of the fovea. For each eye tested, the average of two good-quality maps was used for the analysis. Criteria for good-quality images included sharp focus and accurate centration within the posterior pole.

Blood samples were obtained from all patients for laboratory analysis of HbA1C and serum lipid (HDL, LDL, total cholesterol, and triglycerides) levels on the same day as the MPOD measurement. Patients were instructed to fast for 12 hours overnight, and blood collection was performed early in the morning on arrival at the Infirmary.

## Statistical Analysis

The  $\chi^2$  test was used to compare categorical data of the three groups. The D'Agostino-Pearson test for normal distribution was performed. Parametric variables were compared between groups by analysis of variance (post hoc analysis was performed with Student-Newman-Keuls test). Nonparametric data were compared by using the Kruskal-Wallis and Mann-Whitney tests whenever appropriate. Pearson's and Spearman's rank correlation tests were performed to correlate the patient's baseline characteristics (age, duration of diabetes, HbA1C and serum lipid levels) with MPOD whenever appropriate. We used the mean MPOD from only one location (2°-diameter circle) to avoid multiple analyses of correlation. Statistical significance was set at  $P < 0.05$  (MedCalc software; MedCalc, Inc., Mariakerke, Belgium).

## RESULTS

A total of 43 patients (43 eyes) were included. There were 14 nondiabetic patients (group 1), 17 diabetics without retinopathy (group 2), and 12 diabetics with mild nonproliferative retinopathy (group 3). A comparison of the baseline characteristics of each group is shown in Table 1.

Mean  $\pm$  SD age in each group was  $56.2 \pm 11.7$ ,  $58.6 \pm 11.5$ , and  $62.8 \pm 9.8$  years, respectively ( $P = 0.324$ ). The patients with type 2 diabetes had a higher prevalence of systemic hypertension than did the nondiabetic patients. HbA1C and HDL levels differed significantly between groups ( $P \leq 0.01$ ). The mean MPOD in a 2°-diameter circle around the fovea was significantly different between the three groups: 1 ( $0.29 \pm 0.07$  density units [DU]), 2 ( $0.22 \pm 0.09$  DU), and 3 ( $0.14 \pm$

TABLE 1. Baseline Characteristics of Study Patients\*

	Group 1 (controls) <i>n</i> = 14	Group 2 (type 2 diabetic without DR) <i>n</i> = 17	Group 3 (type 2 diabetic with DR) <i>n</i> = 12	<i>P</i>
Variables				
Age, y	56.2 $\pm$ 11.7	58.6 $\pm$ 11.5	62.8 $\pm$ 9.8	0.32†
Sex: M/F, <i>n</i>	3/11	9/8	5/7	0.24‡
Race: Caucasian, Hispanic, African- American, <i>n</i>	11/2/1	3/9/5	4/6/2	0.08‡
DM duration, y	N/A	6.1 $\pm$ 4.7	19.3 $\pm$ 16.2	<0.01§
Systemic comorbidities				
Hypertension, %	3 (21.4)	12 (70.6)	9 (75)	0.01‡
Dyslipidemia, %	6 (42.8)	8 (47.1)	7 (58.3)	0.63‡
HbA1C, %	6.2 $\pm$ 0.5	7.6 $\pm$ 1.1	8.9 $\pm$ 1.2	<0.01†
Triglycerides, mg/dL	113.4 $\pm$ 106.9	116.3 $\pm$ 52.0	145.7 $\pm$ 84.8	0.54§
HDL cholesterol, mg/dL	57.4 $\pm$ 11.8	43.8 $\pm$ 10.2	47.4 $\pm$ 13.2	0.01†
LDL cholesterol, mg/dL	129.4 $\pm$ 24.6	109.9 $\pm$ 30.0	100.6 $\pm$ 29.1	0.07†
Total cholesterol, mg/dL	209.1 $\pm$ 25.5	176.6 $\pm$ 37.3	176.7 $\pm$ 40.2	0.03†

DR, diabetic retinopathy; C, Caucasian; H, Hispanic; AA, African-American; DM, diabetes mellitus.

\* Data are expressed as the mean  $\pm$  SD, unless otherwise indicated.

† Analysis of variance.

‡  $\chi^2$  test.

§ Kruskal-Wallis test.

0.05 DU); ( $P < 0.001$ ). There was a significant difference in the mean MPOD at  $0.5^\circ$  from the center of the fovea between groups 1 ( $0.51 \pm 0.12$  DU) and 2 ( $0.24 \pm 0.11$  DU;  $P < 0.001$ ), but not between groups 2 and 3 ( $0.33 \pm 0.15$  DU;  $P > 0.05$ ). Examples of MPOD maps from each group are given in Figure 1.

A significant inverse correlation was found between HbA1C levels and mean MPOD at  $2^\circ$  ( $r = -0.63$ ,  $P < 0.001$ ) around the fovea in the entire study population. When only diabetic patients were analyzed, this correlation remained significant ( $r = -0.53$ ,  $P = 0.003$ ). There was a trend toward a statistically significant association when these analyses were performed for each diabetic group separately (group 2,  $r = -0.45$ ,  $P = 0.07$ ; group 3,  $r = -0.56$ ,  $P = 0.06$ ). There was no significant correlation for the control group ( $P = 0.9$ , Fig. 2). No significant correlation was found between mean MPOD and age, duration of diabetes, or serum lipid levels ( $P \geq 0.08$ ; Table 2).

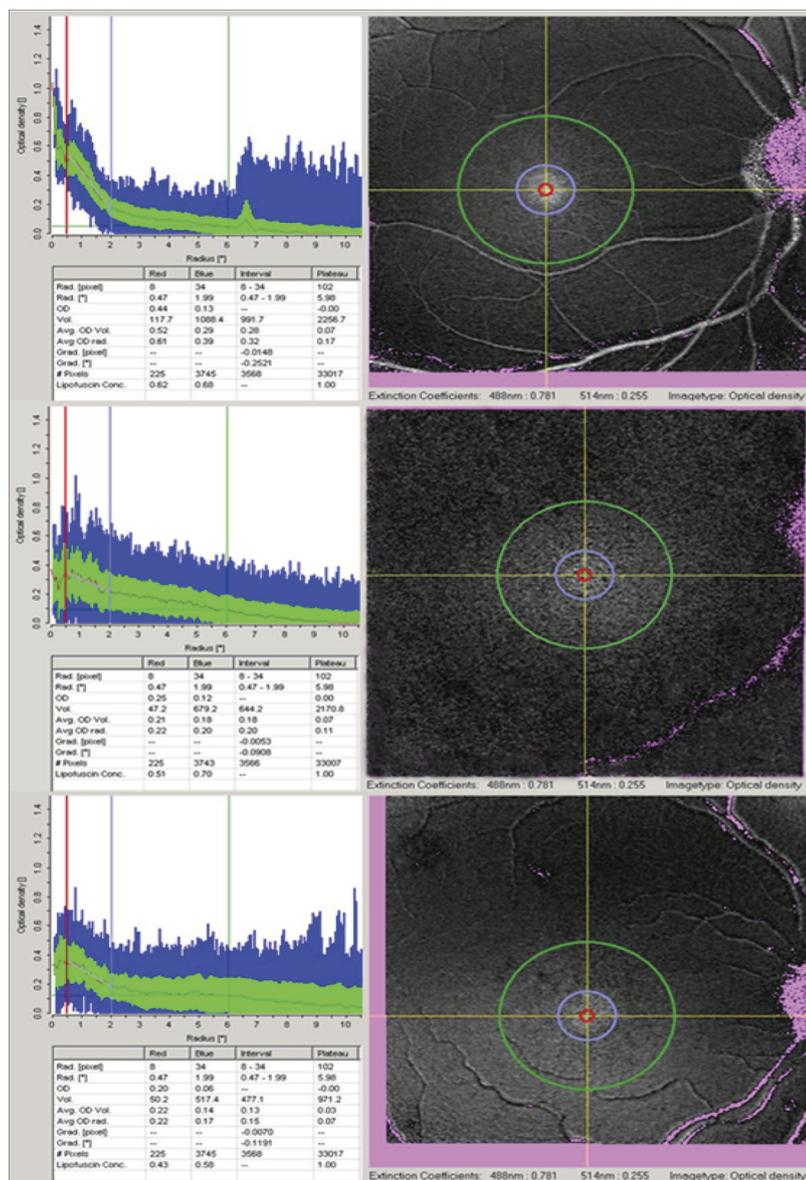
A subanalysis was performed to evaluate the ratio of MPOD averaged in  $1^\circ$  and  $4^\circ$  circles around the fovea. There was a large variation between subjects within each group based on 95% CI, mainly in the group comprising diabetic patients with mild retinopathy (Fig. 3). In addition, there were no significant

differences in the mean ratios between groups: group 1, 3.66 (95% CI, 3.28–4.03); group 2, 3.27 (95% CI, 2.8–3.73); group 3, 3.6 (95% CI, 1.89–5.3);  $P = 0.755$ ). Finally, no significant correlations were found between these ratios and HbA1C levels in each group ( $P > 0.05$ ).

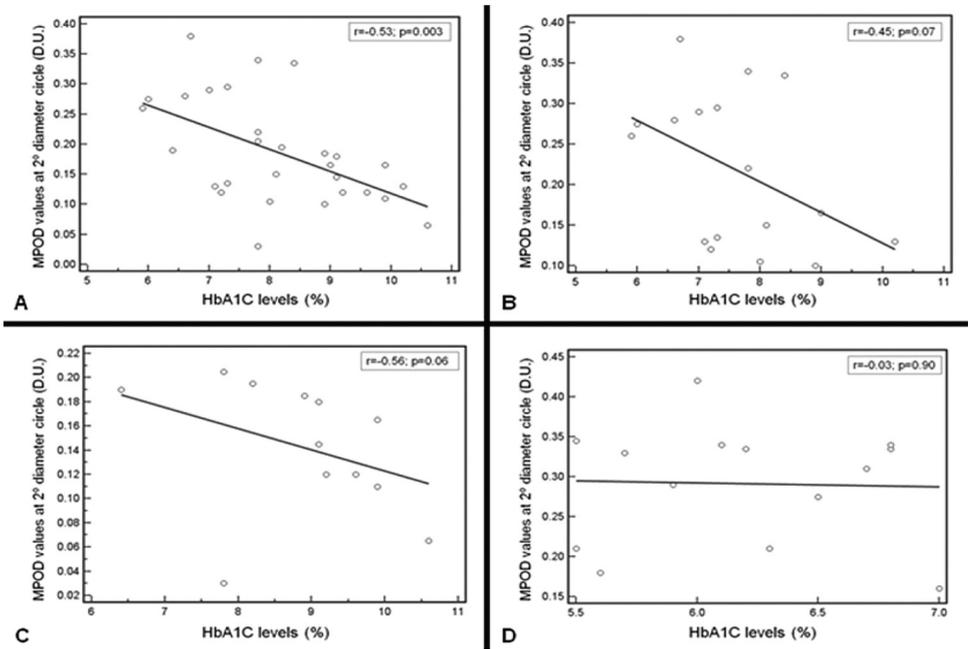
## DISCUSSION

Type 2 diabetic patients with or without retinopathy were found to have significantly reduced MPOD when compared with nondiabetic patients. In addition, MPOD correlated significantly with HbA1C levels in all patients. To our knowledge, we are the first to use dual-wavelength autofluorescence imaging to demonstrate a significant difference in MPOD between nondiabetic and diabetic patients.

In a few studies, the relationship between diabetes mellitus and MPOD has been evaluated. Using a different instrument (foveal reflection analyzer) and the spectral and directional reflectance techniques, Zagers et al.<sup>33</sup> showed that, although patients with type 1 or type 2 diabetes had a strong reduction in the amplitude of the directional reflection (indicating



**FIGURE 1.** MPOD maps of three eyes included in the study (superior, center and inferior). The superior image is from a nondiabetic patient. The image analyzed is demonstrated on the right side of the dialog. Yellow lines: the point used for the radial analysis. Red and blue circles: areas of  $0.5^\circ$  and  $2.0^\circ$  from the center of the fovea, respectively. On the top left of the dialog is a diagram with a normal radial distribution of MPOD around the center of the fovea. Bottom left: the box shows numerical values for MPOD in the specific locations analyzed. Middle image: the results for a diabetic patient without diabetic retinopathy. On the top left of the dialog, the diagram shows a reduced radial distribution of MPOD around the center of the fovea. Bottom image: derived from a diabetic patient with diabetic retinopathy. On the top left side of the dialog there is a diagram evidencing lower radial distribution of MPOD around the center of the fovea than in the other examples.



**FIGURE 2.** Scatterplots of correlation between MPOD averaged at a 2°-diameter circle around the fovea and HbA1C levels for diabetic patients (A) and for each group separately: (B) group 2; (C) group 3; (D) group 1.

changes in the integrity of the foveal cone photoreceptors), their MPOD was not significantly lower. Also, they demonstrated that the lens density increased at a substantially higher rate in diabetics when compared with the normal age-related increase in the control subjects. In contrast with our population, most diabetic patients in Zagers et al. presented with abnormalities in the foveal region, such as edema and ischemia that can in part explain the differences in the results. Another study, in which the optical density of the lens and MP were measured in diabetic patients by color matching, showed that the ocular media of diabetics are abnormal, with significantly increased lens and reduced MP optical density.<sup>34</sup> Similarly, that study showed an inverse relationship between grade of maculopathy and MPOD in those patients and suggested a possible role of oxidative stress in the development of diabetic maculopathy.

According to previous reports, different mechanisms could lead to reduced MPOD in diabetic patients, including a genetic influence,<sup>35</sup> a deficiency of lutein and zeaxanthin in the diet or a reduced absorption from the gut,<sup>36,37</sup> and finally a reduced rate of incorporation into the retinal tissue or an increased rate of removal from the retina. Specific alterations in diabetes mellitus, such as thickening of basement membranes of the

retinal capillaries,<sup>38-41</sup> increased affinity of oxygen for glycosylated hemoglobin,<sup>42</sup> a redox shift due to the effects of hyperglycemia on glycolysis and sorbitol metabolism,<sup>43</sup> and abnormal vasculature in the parafoveal capillaries<sup>44</sup> imply that diabetic retinas are under continuous oxidative stress. Analysis of retinal tissues from primate and human eyes for oxidation products of lutein and zeaxanthin showed that these pigments play a role as antioxidants protecting the macula against photo-oxidative stress.<sup>45</sup> In the present study we found that diabetic patients with or without retinopathy had significantly lower mean MPOD values than did the control subjects. Diabetic patients with mild retinopathy had even lower levels than did those without retinopathy. In addition, higher HbA1C levels correlated significantly with reduced MPOD. These findings suggest that the presence of retinopathy and poor glycemic control may contribute to the reduced MPOD observed in diabetic patients.

The implications of hyperglycemia and oxidative stress in the development and progression of diabetic retinopathy has been investigated. Previous studies have documented that sustained hyperglycemia is the instigating factor in the disruption

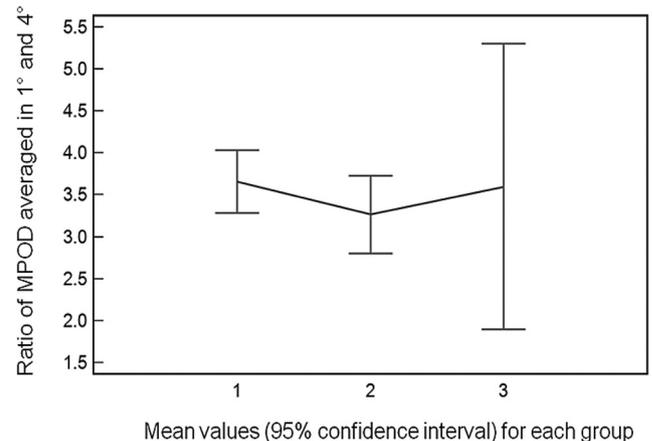
**TABLE 2.** Baseline Characteristics Associated with Mean MPOD at a 2°-Diameter Circle around the Fovea

Variables	Average OD Volume 2°	
	r*	P
Age	0.05	0.73
HbA1C, %	-0.63	<0.001
DM duration, y	-0.07†	0.71
Triglycerides, mg/dL	-0.28†	0.08
HDL cholesterol, mg/dL	0.29	0.08
LDL cholesterol, mg/dL	0.26	0.10
Total cholesterol, mg/dL	0.20	0.19

OD, optical density; HbA1C, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

\* Pearson's correlation coefficient.

† Spearman's rank correlation-coefficient.



**FIGURE 3.** Ratios of MPODs averaged in 1° and 4° circles around the fovea in the control subjects (1), diabetic patients without retinopathy (2), and diabetic patients with mild retinopathy (3).

of normal cellular metabolism that leads to the development of retinopathy.<sup>46</sup> Diabetes increases oxidative stress, which appears to be one of the key regulators in the development of diabetic complications.<sup>47-49</sup> Results of studies have shown that administration of antioxidants prevents diabetes-induced oxidative and nitrate stress and the development of retinopathy in diabetic rats.<sup>48,50</sup> Kowluru et al.<sup>18</sup> demonstrated that zeaxanthin supplementation in diabetic rats significantly inhibited diabetes-induced retinal oxidative damage and elevation in vascular endothelial growth factor and adhesion molecules, which play important roles in the pathogenesis of diabetic retinopathy.

It is important to discuss certain aspects of our study. First, although we found significant results, we have a relatively small sample size. Second, ocular comorbidities could have influenced our findings. Therefore, it is important to emphasize that all patients were carefully examined to exclude any factor that could alter the quality of the images and data analysis, such as macular edema. Finally it is known that age-related and possibly diabetes-related lens density changes (accumulation of advanced glycosylated end products, AGEs) can influence MPOD analysis by autofluorescence.<sup>51,52</sup> Although we excluded patients with poor BCVA ( $\leq 20/40$ ) and with moderate or dense cataracts, the influence of these factors should be considered when interpreting our results.

In conclusion, patients with type 2 diabetes, with or without retinopathy, were found to have significantly reduced MPOD when compared with nondiabetic patients. In addition, higher HbA1C levels correlated significantly with reduced MPOD, suggesting that the poor glycemic control may play a role in MP absorption and incorporation in the retinal tissue. Further studies are warranted to clarify the relationship between MPOD and the development of diabetic retinopathy. A controlled, prospective trial with dietary modifications, serial measurements of serum lutein and zeaxanthin, and correlation with MPOD<sup>53</sup> in diabetic patients is currently in the planning stages.

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