Multifocal Pupillography Identifies Changes in Visual Sensitivity According to Severity of Diabetic Retinopathy in Type 2 Diabetes

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Purpose. Retinal light sensitivity loss has been shown to occur prior to other signs of retinopathy and may predict the sight-threatening sequelae. A rapid, objective perimetric test could augment diabetes care. We investigated the clinical use of multifocal pupillographic objective perimetry (mfPOP) to identify patients with and without diabetic retinopathy.

Methods. Retinopathy severity was determined using the Early Treatment of Diabetic Retinopathy Study (ETDRS) standard for fundus photography. Pupillary responses were measured from both eyes of 25 adults with none to moderate diabetic retinopathy and 24 age-matched controls, using three mfPOP stimulus variants. Multifocal pupillographic objective perimetry stimulus variants tested 44 regions per eye arranged in a five-ring dartboard layout presented within either the central 30° or 60° of fixation. Receiver operator characteristic (ROC) curves were produced from contraction amplitudes and time to peak responses.

Results. Regression analysis revealed that mean amplitude deviations were larger with severity of early retinopathy. On average, the longest delays were measured in patients with no retinopathy. The brightest wide-field stimuli produced the highest area under the ROC curve for differentiating eyes with no retinopathy from nonproliferative diabetic retinopathy (NPDR) and from healthy eyes (100 ± 0.0%, mean ± SE). The asymmetry in local delay deviations between eyes tended to produce higher sensitivity and specificity than amplitude deviations.

Conclusions. Asymmetry in local response delays measured by mfPOP may provide useful information regarding the severity of diabetic retinopathy and may have clinical use as a rapid, noninvasive method for identifying functional loss even in the absence of NPDR.

Keywords: multifocal, objective perimetry, pupils, type 2 diabetes, diabetic retinopathy

Diabetes mellitus has been estimated to affect 346 million people globally[1] with approximately 30% having signs of diabetic retinopathy (DR).[2] Type 2 diabetes (T2D) is the leading cause of vision loss among working-age individuals in the developed world and its prevalence continues to rise.[3] Current treatments are mainly directed to advanced DR and may have unfavorable ocular[4] and vascular effects.[5] The advent of treatments for early-stage DR, such as angiotensin-converting enzyme (ACE) inhibitors and fenofibrate, and the consequent reversibility of early retinal lesions,[6] means that identifying prognostic functional markers, and monitoring progression from early to late stage retinopathy is increasingly relevant.[7,8]

Morphologic changes in the retinal nerve fiber[9,10] and ganglion cell layers[11,12] have been shown to occur before the onset of clinically evident DR such as reflected by microaneurysms and hemorrhages. These findings have been supported by perimetric[13–15] and electrophysiological[16,17] studies demonstrating neural retinal deficits preceding, and even predicting, the development of microvascular changes.[18] Clinician researchers are exploring additional structural measures of retinal vessel caliber as a means to predict vascular outcomes in people with T1D.[19–21]

Together these findings support the need for and use of functional visual testing, however, perimetric studies have been inconsistent in eyes with nonproliferative diabetic retinopathy (NPDR).[22,23] This disagreement may be due to the recognized variability inherent in subjective automated perimetry.[24–26]

Pupillography provides a noncontact method and studies have identified neurodegenerative changes in patients with diabetes that precede clinically visible changes,[27] however these methods are limited by the use of a single wide-field stimulus. A recent variant, multifocal pupillographic objective perimetry (mfPOP), permits noninvasive recording of responses from many locations of the visual field concurrently in both eyes. Damaged regions of the visual field produce both contraction amplitude and temporal response abnormalities. Several mfPOP studies from our laboratory have demonstrated visual-field impairment and high diagnostic accuracy in early-stage macular degeneration[28,29] and T2D.[30] Previous reports suggest that NPDR may be missed by simply imaging visible pathology within the seven standard field area.[31] Given the evidence that mfPOP may detect retinal dysfunction preceding microvascular changes, the present study compares the

Table 1. Stimulus Protocol Characteristics

Stimulus Protocol	Eccentricity, deg	Maximum Stimulus Luminance, cd/m²	Background, cd/m²	Mean Interval, s/region	Regions/Eye (Fig. 1)
Macula-bal	±15	288	10	3.98	44
Wide-bal	±30	288	10	3.98	44
Wide-old	±30	150	10	3.98	44

diagnostic accuracy of macular (central 15° diameter) and wide-field (central 30° diameter) stimulus presentations in patients with T2D with and without the presence of vasculopathy. We also compare an older mfPOP stimulus that had shown promise in T2D[30] with the two new variants employing a newer luminance balanced method.[29]

Methods

Subjects

Twenty-five T2D subjects (mean age ± SD, 54.9 ± 12.4 years, 12 females) were recruited from the Endocrinology Department at The Canberra Hospital (Canberra, Australia). Severity of DR was classified based on ETDRS scoring of fundus photographs and subjects were clustered in two groups as follows: no visible retinopathy (mean duration ± SD, 11.4 ± 4.5 years, $n = 15$) and mild to moderate NPDR ($n = 10$, 11.6 ± 4.4 years). The control group consisted of 48 eyes from 24 subjects (56.8 ± 7.2 years, 13 females). Control subjects were classified according to their fundus photography only and were excluded if they had a history of diabetes or evidence of retinopathy. Exclusion criteria for all subjects included: (1) best-corrected visual acuity (BCVA) worse than 6/9, (2) distance refraction greater than or equal to ± 5 diopters (D) and greater than or equal to ± 2 D cylinder, (3) IOP greater than or equal to 21 mm Hg, (4) evidence of ocular, neurological, or systemic disease that may affect retinal sensitivity, (5) medications that may affect pupillary responses, and (6) peripheral neuropathy with monofilament testing.

Total plasma cholesterol and HbA1C were measured at their clinical examination.

All subjects were given a thorough eye examination involving detailed history, frequency-doubling technology (FDT) perimetry C-20 threshold field tests, slit-lamp examination of the anterior segment, fundus photography (CR-2 Retinal Camera; Canon, Inc., Tokyo, Japan), and applanation tonometry. All subjects had their pupils dilated following mfPOP testing using 0.4% oxybuprocaine and 1.0% tropicamide. Five 45° fundus images were acquired equivalent to the seven 30° photos of the ETDRS protocol.[32] Grading of retinopathy was completed by the Retinal Vascular Imaging Centre (Centre for Eye Research Australia, Melbourne, Australia) who were masked to the subjects' identity and medical status. The potential risks of participation were advised to all subjects before testing and written informed consent was obtained. This research was approved by the ANU Human Research Ethics Committee (ANU 2010/194) and adhered to the tenets of the Declaration of Helsinki.

Stimuli and Data Acquisition

All subjects were tested in one visit with three randomized mfPOP stimulus, with stimulus methods (protocols) presented on a prototype of the Food and Drug Administration (FDA)–cleared nuCoria Field Analyzer (nCFA; nuCoria Pty Ltd., Acton, ACT, Australia). Subjects were instructed to fixate on a small central red cross. A dim starburst radial grating with a long, thin vertical white line bisecting the fixation point was presented in the background to help maintain fusion. Before testing adjustments were made to ocular vergence to align the image with their distance phoria.

Yellow multifocal stimuli were presented dichoptically on two liquid crystal displays (LCD) operating at 60 frames/s. The resolution of LCD screens was 1024 × 768, and pixel size equated to 0.0797° of visual angle. The International Commission on Illumination (CIE) x, y coordinates for yellow protocol test regions were (0.377, 0.464) and the background (0.408, 0.515). The multifocal method presented independent stimuli to each eye and allowed for both eyes to be tested concurrently producing direct and consensual responses concurrently from each visual field region. Details of the method are given elsewhere.[33] The protocols were chosen because earlier studies had demonstrated high signal to noise ratios (SNR) in the central visual field for both normal[34] and T2D subjects.[30] Protocol parameters were identical except for stimulus luminance and eccentricity (Table 1; Fig. 1). The rings of stimuli are not cortically scaled; they are scaled to give approximately equal pupillary responses. This information has been given in detail in previous papers.[34,35]

All protocols used a 44-region array arranged in a dartboard layout, each stimulus having blurred margins to minimize the effects of ametropia on responses.[36] The accommodative pupil response was overcome by maintaining the contrast of the stimulus elements to within 1.5 D (spherical equivalent) of uncorrected distance refractive error. Stimuli were presented at optical infinity and trial lenses were fitted to correct the subject's distance prescription. The pseudorandomly-cued stimuli were each presented for 33 ms with a mean interval of 3.98 seconds between presentations. No adjacent or overlapping regions between eyes were activated simultaneously. Test duration for each protocol was 6 minutes, consisting of nine 40-second recording blocks with short rest breaks between blocks to allow blinking. Therefore, each region received a total of 90 stimulus presentations.

A luminance balancing technique, described elsewhere,[37] was employed to produce similar pupillary response amplitudes and SNRs across the visual field. This was achieved by deliberately reducing the luminance presented to highly responsive regions (Fig. 1). In brief, protocol Macula-bal and Wide-bal had peak luminances in the least responsive regions of 288 and 150 cd/m², and a mean luminance across regions of 250 cd/m² and 120 cd/m², respectively. Wide-old presented all activated elements at a peak uniform luminance of 288 cd/m², matching our previous study.[30] Separate video cameras for each eye recorded pupil responses at 30 frames/s under infrared illumination. Pupil diameter was measured by fitting a circle to the lower three-fourths of the pupil allowing some tolerance to ptosis. Fixation was monitored online and data recorded during blinks and fixation losses during a segment were deleted. If the data loss exceeded 15% the segment was repeated.

Analyses were performed in MATLAB (2012b; Mathworks, Inc., Natick, MA, USA). The mean pupil response for each region was obtained from raw pupillary response waveforms by a multiple regression analysis.[38] Signal to noise ratios were determined for

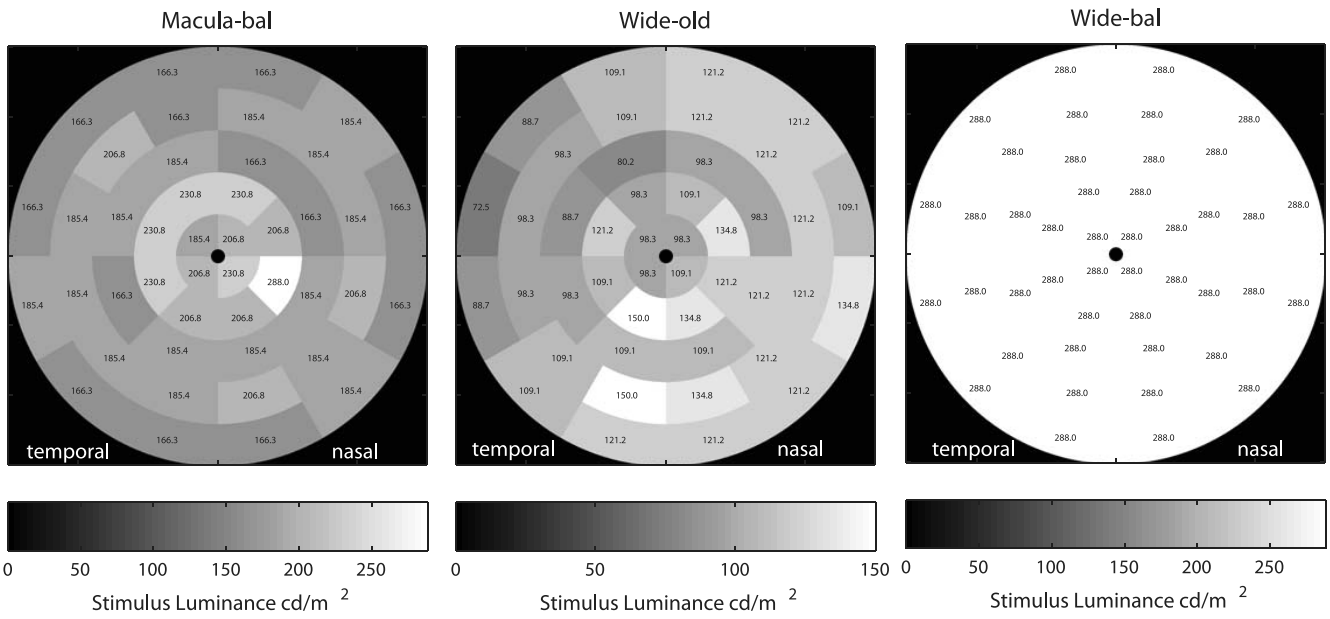

Figure 1. Multifocal stimulus patterns and luminance characteristics arranged in a dartboard layout as presented to left eyes; right eye luminances were mirror-symmetric. All protocols consisted of 44 visual field test regions per eye. Stimulus protocol Wide-old displayed all test regions at 288 cd/m^2. Stimuli Macula-bal and Wide-bal varied the luminance presented at each region depending on the location in the visual field in an attempt to make responses more even in amplitude (so-called luminance balancing).

both the direct and consensual responses for each stimulus field and the pupil response that produced the largest SNR was used in the analysis. Responses to the stimuli were reduced to the peak contraction amplitude given in decibels and the time (delay) to the peak contraction; details are given elsewhere.[30,39]

Differences between controls and diabetic groups were assessed using multivariate linear mixed-models coded in MATLAB (Table 2). We used liner mixed-effects models to examine the subject-wise effects of stimulus protocol and disease severity on pupillary contraction amplitudes and time to peak. To further overcome the effects that multiple measurements have on responses that are correlated between eyes, inputs to the mixed model were the mean responses of all regions in each ring producing five data points per subject and eye, allowing effects of visual field eccentricity to be quantified. The effects were fitted as contrasts to the reference value (mean direct response of a healthy male subject) for each response parameter. The independent effects of potential confounders (such as sex and consensual response) upon contraction amplitudes were found to be multiplicative, thus the transformation to decibels was appropriate and also stabilized the variance. The diagnostic power of the stimulus protocols were assessed by ROC analysis with bootstrapping (n resamples = 100) and presented as the area under the curve (AUC). The ROC analysis used per region deviations from the normative data.[30,39] The normative data was computed as the median result at each

Table 2. Clinical and Demographic Information (Mean ± SD) of Control Subjects and Patients with Diabetes

	Control	Type 2 Diabetes No NPDR	Type 2 Diabetes Mild/Mod NPDR	Type 2 Diabetes Differences *P* Value
N	24	15	10	
Age, y	56.8 ± 7.2	58.1 ± 9.8	52.2 ± 13.2	0.437
Sex, female/male	13/11	9/6	3/7	0.438
Duration of diabetes	-	11.4 ± 4.5	11.1 ± 4.4	0.871
HbA_{1C}, %	-	7.3 ± 0.7	8.5 ± 1.8	0.129
HbA_{1C}, mM/mol	-	56.5 ± 8.0	68.9 ± 20.0	0.135
Total cholesterol, mM	-	4.9 ± 1.7	4.7 ± 0.7	0.608
Triglycerides, mM	-	2.2 ± 2.8	4.1 ± 1.6	0.475
HDL-cholesterol, mM	-	1.4 ± 0.3	1.2 ± 0.4	0.503
LDL-cholesterol, mM	-	2.2 ± 0.6	2.9 ± 1.6	0.417
Body mass index, kg/m^2	-	32.7 ± 9.9	30.6 ± 7.4	0.688
Systolic BP, mm Hg		126.2 ± 17.3	132.4 ± 19.6	0.573
Diastolic BP, mm Hg		76.8 ± 7.2	84.0 ± 5.8	0.056
Current smoker (%)	-	0 (0)	3 (30)	0.01
Ocular measures				
ETDRS score	-	Level 10	Level 20–43	
BCVA†	1.0	0.8 ± 0.08	0.9 ± 0.1	0.01
FDT mean deviation (dB)	2.2 ± 1.7	1.4 ± 1.6	0.03 ± 3.2	0.416

* Best-corrected decimal visual acuity.

visual field location of the control eyes, flipping right eye data to achieve a single normative template. Deviations from the normative data were then calculated for every field of every subject. This process was redone for the asymmetry between the anatomically conjugate left and right eye visual field locations compared with normal between-eye asymmetry, providing a disease measure based upon variability across the field compared with healthy controls. For both types of data, ROC plots were then redone for the worst point in each field, the mean of the two worst points in each field, and so on.

Results

Summary demographic and patient characteristics for both groups are presented in Table 2. There was no significant difference between the patient groups across all components except for BCVA and current smokers.

In general, mean response delays between controls and diabetic patients were similar (444 ± 0.015 to 445 ± 0.015 ms, respectively), suggesting that there were no response delays due to neuropathy of the irises. Figure 2 shows an example of mean pupillary contraction waveforms for a control (1) and a subject with bilateral no NPDR (2) and mild/moderate NPDR (3) to a ± 30° stimulus protocol. Direct and consensual responses were produced from regional amplitudes corresponding to the location of the visual field where stimuli were presented to each eye. In agreement with the mean ring effects according to disease severity (Fig. 3), response amplitudes tended to increase in eyes with no NPDR and then reduce with increasing severity to NPDR. Figure 3 illustrates the mean sensitivities and delays according to eccentricity, in other words, the mean responses for each of the five rings of stimuli. Such a ring analysis is common in multifocal studies and was suggested by our previous results from retinal diseases.[29,30,37] The ring averages were computed for controls and for diabetic eyes with and without retinopathy and entered into a linear mixed-effects model. The linear mixed-model was used to compare pupillary responses between the different groups, where the eye would be a random effect, accounting for the correlation between eyes and pupils. Figure 3 demonstrates that the pattern of response changes differs between the two DR groups. Recall from the Methods that the coefficient for each ring quantifies the difference from the reference condition (mean response of ring 1 for male control subjects). Mean contraction amplitudes of both groups are significantly larger than control eyes across the central and peripheral visual field locations, and the largest effects were produced by the no NPDR group. Stimulus Macula-bal produced the largest response sensitivity deviations in the field ranging from 0.96 ± 0.39 dB (t-stat = 2.5) for no NPDR at 6° eccentricity to 0.68 ± 0.33 dB (t-stat = 1.2) for mild/moderate NPDR at 8° eccentricity, respectively (Figs. 3B, 3C). Thus, mean response size tended to first rise, then fall with increasing disease severity. Deviations in mean time to peak response delay showed an initial increase with the trend that the longest delays were measured in the no-NPDR patient eyes ranging from 1.7 ± 7.5 ms (t-stat = 0.23) to 10.7 ± 8.0 ms (t-stat = 1.3) for stimuli Wide-bal at 12° eccentricity and Wide-old at 8° eccentricity, respectively. The independent effect of sex on subjects' responses added a small offset to the normative data of females of 2.17 ± 1.11 ms. Mean consensual responses were smaller than direct responses but did not reach significance for Macula-bal (–0.16 ± 0.09 dB, P = 0.91). However, both Wide-old and *Wide-Bal* stimulus protocols reached significance for mean consensual responses (–0.30 ± 0.09 dB, $P < 0.05$ and –0.28 ± 0.07 dB, $P <$ 0.05, respectively). The SNR for controls and patients, expressed as t-statistics, were 2.81 ± 0.38 and 2.70 ± 0.39 (median ± SE). Compared with controls, the average effect of T2D on FDT mean deviation did not reach significance in eyes with no NPDR and approached significance in mild/moderate NPDR (t-stat = 1.88, t-stat = 2.07, respectively).

We next examined the diagnostic accuracy of each protocol in terms of the AUC for ROC quantifying the difference between each of the two severities of patient eyes and healthy control eyes. In general, the mean of the one or two most deviating locations in each field produced the highest AUC values, and examples of each are presented in Table 3. Given the results in Figure 3, we examined both negative deviations from normal (suppressed and delayed responses) and positive deviations from normal (enhanced and more rapid responses). Table 3 gives the results for response amplitude deviations and shows that on balance enhanced responses are more diagnostic than suppressed, as would be expected from Figure 3; however, asymmetry outperforms either. In the case of delay deviations (Table 3) asymmetries were also best and gave consistently high AUC values across the two patient groups. Interestingly, between the no and mild/moderate NPDR groups the most diagnostic delays flipped from the few most delayed regions, to the few quickest regions.

Figure 4 gives examples of individual ROC plots for time to peak and amplitude for between-eye asymmetry.[30] Figures 4A and 4B are for diabetic patients with no retinopathy, Figures 4C and D are for mild to moderate DR. As expected, higher diagnostic accuracy was seen with more severe disease (Figs. 4C, D). When delay deviations were considered, stimulus Wide-old achieved the highest AUC values for eyes with 100% ± 0.0% SE across all patient groups. The Wide-old stimulus also achieved the highest diagnostic accuracy when amplitude deviations for eyes with no retinopathy were considered (88.2% ± 6.5%).

We next explored %AUC as a function of three different measures of disease severity (risk factors): current percent HbA1c level, duration of diabetes, and FDT perimeter C20 mean deviation. We developed severity ratings for each measure by dividing the patients into three groups for each measure, where the cut-off points (Table 4, left column) were selected to give a near-equal numbers of patients for each measure. These comparisons are useful because we have no gold standard by which we can judge the true severity of neuropathy of these retinas, so we have used reasonable proxies for diabetic damage, and examined if the mfPOP results track these severity markers.

Table 4 shows the AUCs for discriminating patients in each disease severity group from healthy subjects for between-eye asymmetry in the time to peak deviations. They are reported as %AUC for readability. Stimulus Wide-old produced the highest AUC values, which increased consistently with disease severity for all three measures. In patients with the lowest categories of HbA1c, disease duration and FDT mean deviation severity, classifying them into groups based on levels HbA1c produce the best diagnostic discrimination from control subjects, producing AUCs ranging between 56.5% ± 8.3% SE for the Wide-bal protocol and 92.9% ± 4.8% SE for the Wide-old protocol. The cutoff HbA1c levels are given in Table 4. Interestingly, FDT mean defects were almost as good as HbA1c, and it was the eyes with more positive mean deviation values (>2 dB) that produced the highest %AUC values (83.8% ± 8.7% SE), in line with the results for mfPOP (Fig. 3; Table 3). The %AUCs for contraction amplitudes were not tabulated here, as they were generally lower than time to peak deviations. These results were consistent with the effect seen in Figure 3 (i.e., response delays were more informative than amplitudes in differentiating eyes with no retinopathy from healthy eyes). In general, the dimmest stimulus (Wide-bal) produced the lowest diagnostic accuracy across protocols for the lowest categories of severity.

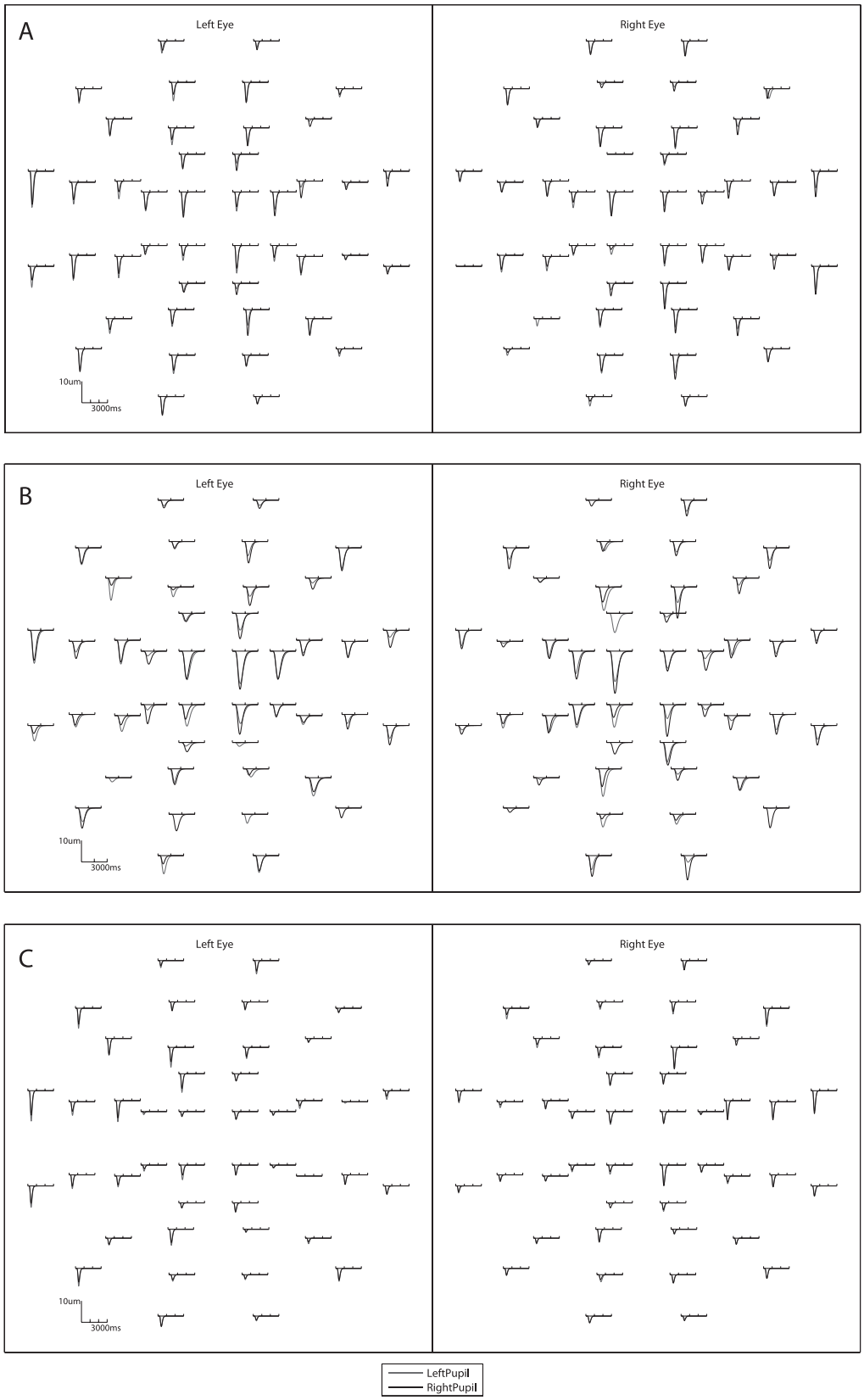

Figure 2. Example of mean direct and consensual regional response recordings for a control subject (**A**) and a patient with bilateral no NPDR (**B**) and NPDR (**C**). An initial increase can be observed in response amplitudes in eyes with no NPDR and then a reduction with increasing severity.

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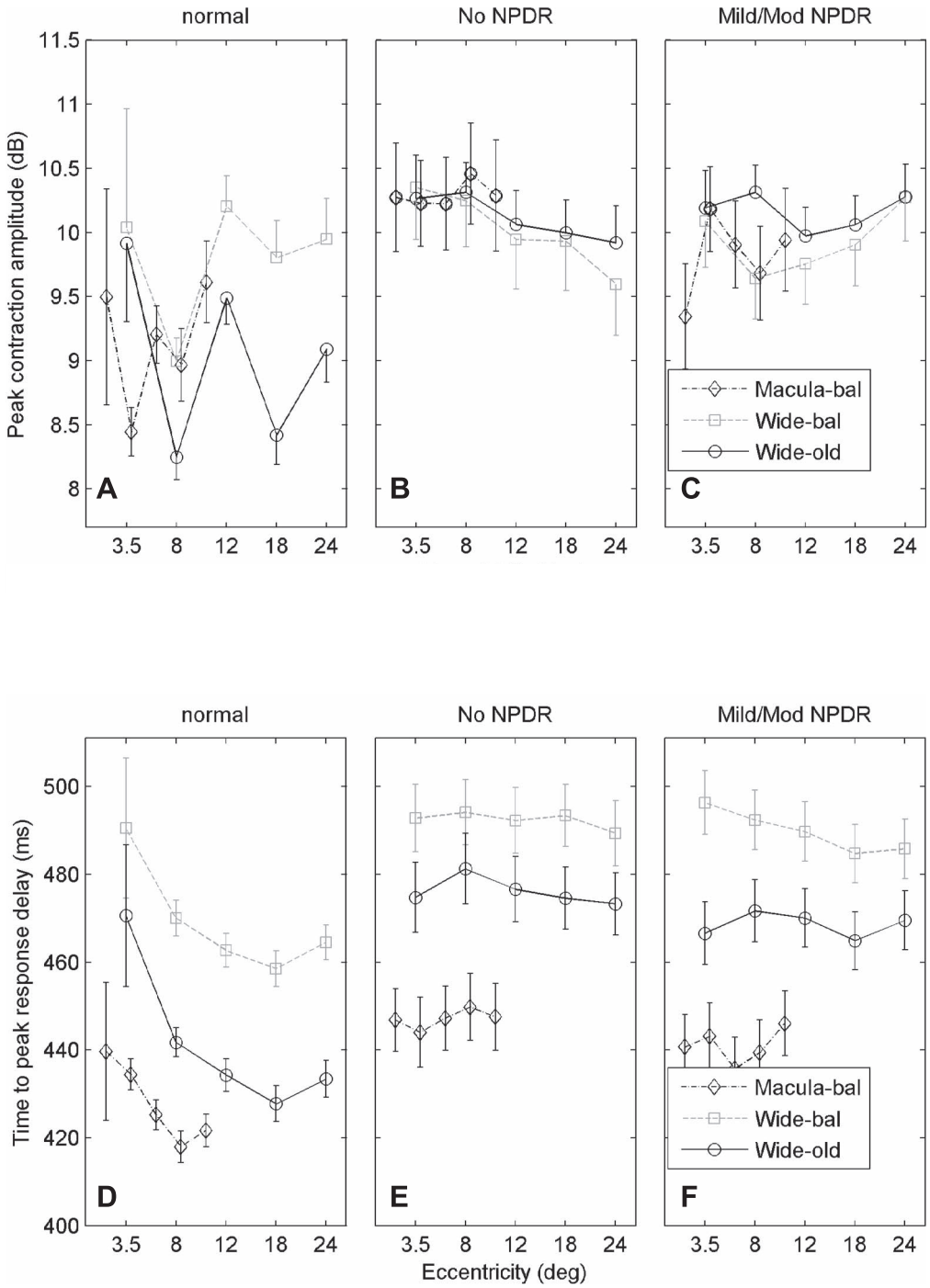

Figure 3. The ring-wise mean response data for each stimulus protocol calculated for contraction amplitudes (**A–C**) and time to peak response (**D–F**). Mean responses were calculated for each ring of the stimulus array using a mixed-effects model, which also included small independent effects for sex and consensual response. *Error bars* are 95% confidence limits. (**A–C**) Mean contraction amplitudes of both groups show statistically significant hypersensitivity across the central and peripheral visual field locations. Diabetic eyes with mild to moderate retinopathy show less deviation from control eyes. (**D–F**) Mean effects on time to peak responses show greater delays in patients with no NPDR.

Comparing Patient Groups

We next investigated the ability of the various stimuli to distinguish between diabetic patients with and without NPDR. For all three stimuli between eye asymmetry in response amplitudes were 100 ± 0.0% for the three worst locations in the field, worst meaning the most deviating locations from no-NPDR fields. The mean performance across the stimuli fell to 86.4 ± 8.9% based upon the mean of the worst five locations in the fields. For asymmetry in response delay, Wide-old achieved the highest AUC at 100 ± 0.0% for any of the means of the single worst to the four worst locations in the field. In terms of a per-eye diagnosis, the best measure was the reduction in delay compared with no-NPDR eyes. Across the three stimuli, the mean AUC was 85.6 ± 7.5% for the single most deviating location, with the macular stimulus performing best at 88.1 ± 7.8%.

Table 3. Percentage AUC ± SE for Each mfPOP Protocol

	Macula-bal			Wide-bal			Wide-old		
	Within-eye			Within-eye			Within-eye		
Amplitude deviations									
Severity	NegDev	PosDev	Asym	NegDev	PosDev	Asym	NegDev	PosDev	Asym
No retinopathy	47.5 ± 7.9	70.6 ± 7.1	80.2 ± 7.3	49.8 ± 8.5	72.2 ± 7.4	81.6 ± 6.8	58.1 ± 8.2	72.6 ± 7.1	88.2 ± 6.5
Mild/mod retinopathy	47.2 ± 12.1	77.8 ± 13.7	100 ± 0.0	36.5 ± 13.4	73.4 ± 11.1	100 ± 0.0	33.3 ± 13.5	81.7 ± 9.7	61.1 ± 17.3
Delay deviations									
Severity	NegDev	PosDev	Asym	NegDev	PosDev	Asym	NegDev	PosDev	Asym
No retinopathy	66.3 ± 8.1	48.5 ± 8.6	69.6 ± 8.0	74.4 ± 7.4	48.5 ± 8.4	84.1 ± 6.1	78.7 ± 7.4	45.4 ± 8.6	100 ± 0.0
Mild/mod retinopathy	50.8 ± 13.8	65.9 ± 13.4	60.3 ± 14.1	41.3 ± 13.4	74.2 ± 8.9	65.1 ±12.6	49.2 ± 14.6	69.8 ± 11.6	100 ± 0.0

Receiver operator characteristic estimates were obtained from response amplitudes (sensitivities in dB) and delays (ms) for both the negative (NegDev) and positive (PosDev) deviations from normal. Response deviations were measured for each eye (Within-eye). Alternatively, we used the difference in response (amplitude or delay) at the anatomically similar region in the fellow eye (i.e., between-eye asymmetries in the fields [Asym]). Both within-eye and between eye measures were compared with healthy control eyes. Estimates are the mean %AUC of the two-worst deviations from any position in the visual field.

Discussion

This study is the first to investigate the diagnostic accuracy of mfPOP for comparing nondiabetic controls and patients with T2D with or without mild/moderate DR. We showed that pupillary responses became significantly faster and smaller as early-stage retinopathy increased. Response delays became shorter in the mild/moderate NPDR group compared with no-NPDR group but more delayed than normal. Evidence of significant delays in mfPOP time to peak responses compared

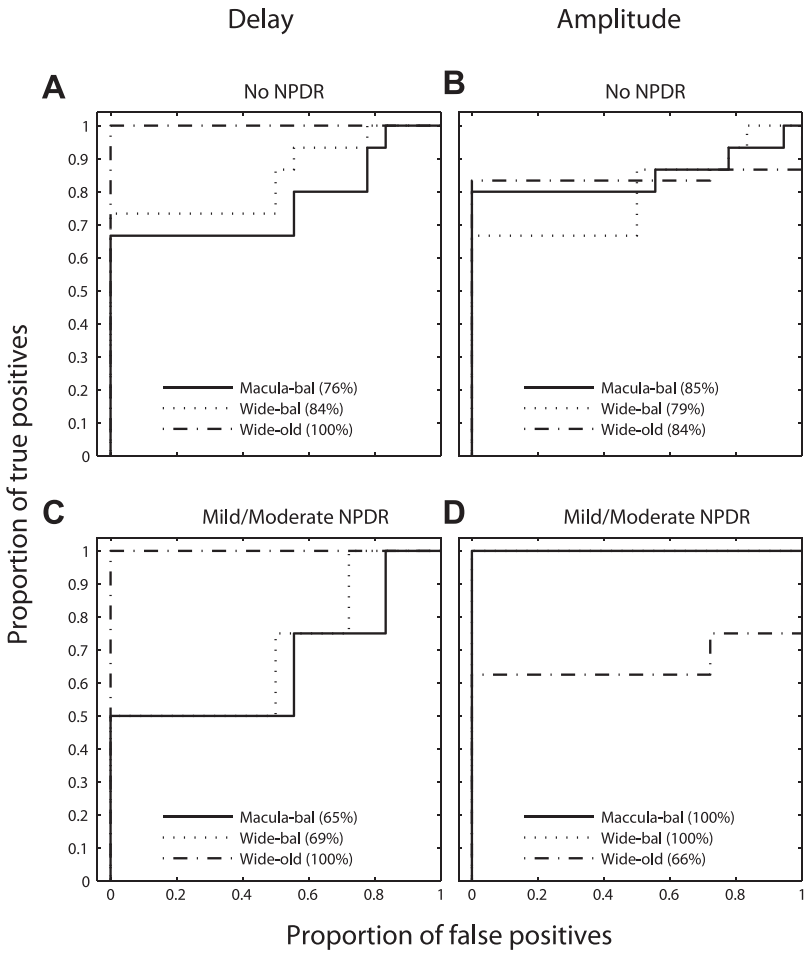

Figure 4. Receiver operator characteristic plots for the single worst deviation from the normative data according to stimulus protocol and retinopathy status for (**A**, **C**) response delays and (**B**, **D**) amplitudes. Receiver operator characteristic analysis used asymmetric deviations by computing the difference between deviations from controls obtained from left and right visual field regions. Receiver operator characteristic estimates for each curve are reported in each legend as percent AUC. An AUC of 50% represents random classification and an AUC of 100% represents the correct diagnosis of all diabetic and healthy subjects (100% sensitivity and specificity).

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Table 4. Diagnostic Accuracy of mfPOP Stimulus Protocols for Each Diabetes Severity Rating

	Macula-bal			Wide-bal			Wide-old		
Severity Rating	**%AUC**	**SE**	***P***	**%AUC**	**SE**	***P***	**%AUC**	**SE**	***P***
HbA1c									
5.6% to 7.3% ($n = 8$)	74.4	8.9	-	56.5	8.3	-	92.9	4.8	-
7.3% to 7.8% ($n = 7$)	62.3	9.8	0.039	65.2	9.2	0.074	100	0	0.007
7% to 12.9% ($n = 4$)	88.6	7.3	0.015	89.1	7.0	<0.01	100	0	0.007
Duration									
5 to 7.8 y ($n = 6$)	72.7	9.0	-	44.9	9.4	-	90.6	6.2	-
7.8 to 12 y ($n = 8$)	81.8	8.1	0.05	82.1	6.5	<0.01	100	0	0.005
12 to 23 y ($n = 5$)	59.1	11.9	0.04	67.8	9.5	0.07	100	0	0.02
FDT mean deviation									
−4.8 to 0.06 dB ($n = 6$)	65.3	10.6	-	69.2	9.7	-	89.7	6.7	-
0.06 to 1.97 dB ($n = 6$)	73.1	10.0	0.12	74.6	9.3	0.19	100	0	0.005
1.97 to 4.82 dB ($n = 6$)	83.8	8.7	0.01	61.2	10.3	0.11	100	0	0.01

Diagnostic performance was assessed using time to peak deviations. Receiver operator characteristic estimates are the %AUC values obtained from the mean of the two regions showing the most asymmetry between eyes compared with healthy visual fields. Thus, *n* refers to the number of OS/OD pairs of eyes for which the per region asymmetry was calculated. The diagnostic power of stimulus Wide-old was greater across all parameters and severity classifications. Receiver operator characteristic values calculated from time to peak responses performed better than other parameters and so have only been included here. Values reported for *P* are the comparison between the first column of each category and each diabetes severity level rather than the difference from the control group. There were some missing values for some of the measures.

with control subjects confirms our earlier finding for diabetic subjects without any detectable sign of retinal vasculopathy.[30] Here mfPOP tests that extended to peripheral regions of the retina appeared to be a more sensitive measure of retinal function than those restricted to the macula, achieving an AUC of 100% when response delays were considered. Eyes with greater time to peak deviations also had higher HbA1c levels and longer duration of diabetes (Table 4). Neuropathy of the pupils would create an overall loss of sensitivity and could not generate localized visual field changes as reported here and in our previous study.[30] In addition, we excluded subjects with evidence of peripheral diabetic neuropathy.

The between patient comparisons were between relatively few eyes. All the ROC analyses benefited from a leave one out (LOO) cross-validation method, by which the fields of control group eyes were only compared with a normative data set that did not include their own eye.[33] This relatively conservative method tended to reduce the AUC values by a few percent. For the no-NPDR versus NPDR comparisons, the asymmetry in response amplitude identified the severity of the patients' disease well, while shorter delays in individual eyes appeared to indicate more severe disease. Taken together, these factors appear to provide good ability to rate disease severity.

Recently, melanopsin retinal ganglion cells (mRGC) have been identified in primate retina and are proposed to mediate most of the slow pupillary response to luminance.[40] In addition to receiving input from cones (yellow-ON/blue-OFF), they respond intrinsically to melanopsin with a peak sensitivity at 482 nm. This combination of pigments results in a fast, transient, cone-driven response and a sluggish melanopsin–mediated response. Abnormalities of the intrinsically photosensitive retinal ganglion cells were first reported in diabetic patients by Feigle et al.[27] Using a single 7.15°-wide stimulus, they observed significant reductions in both the mRGC and cone-driven pupil response in diabetic eyes with no retinal vasculopathy. They also reported increasing mRGC impairment with increasing duration of disease; however, the study contained only seven patients. The use of short wavelength light may be inadvisable due to the reduced blue sensitivity from accelerated lens brunescence in diabetic eyes. Activated microglia has also been identified in DR before the appearance of vasculopathy,[41,42] and this process may contribute to the neural cell damage[43,44] identified by changes in the temporal component of mfPOP responses.

We have previously shown, in a series of patients with T2D, evidence of local retinal dysfunction in the absence of retinal vasculopathy.[30] In that study, yellow transient stimuli were used to minimize the influence of intrinsically photosensitive mRGC on responses and reduce the effect of brunescence. On average, response amplitude deviations were located predominately beyond the borders of macula and displayed hypersensitivity relative to controls as reported here. We now extend those results by investigating the effect of microvascular changes on regional pupillary response parameters. Confirming the results of Bell et al.,[30] we found larger than normal responses (Table 3) in diabetic eyes with no retinopathy; however, amplitudes became progressively reduced as retinopathy appeared and we observed a bias toward peak hypersensitivity in the macular area. In agreement with recent mfERG studies,[45] increases in response delay appear to be relatively more informative than amplitude changes in diabetic eyes with no retinopathy. Response delays appeared to occur uniformly across the field (Figs. 3D–3F), with responses becoming quicker with progression to retinopathy, yet still significantly delayed compared with controls. A recent study in our lab has found that patients with T1D presenting with proliferative retinopathy had significantly faster responses than controls across nearly one-half the visual field regions tested (19/44 regions) with one-third of the regions corresponding to regions with amplitude loss; however, the average effect across the visual field was not significantly quicker than controls (1.2 ± 2.3 ms, $P = 0.06$). These findings are illustrated in Supplementary Figure S1.

Those findings agree with our previous mfPOP studies identifying a changing dominance between delay and amplitude with progression of macular degeneration.[29,46,47] Such results have been verified in patients measured on the same day using mfPOP[48] and multifocal visual evoked potentials (mfVEPs).[37] The hypersensitivity may be more common under photopic than scotopic conditions where the light signal is more limited.[47] Overall, several studies now confirm that hypersensitivity in the peripheral retina may be a potential mechanism for compensating loss of retinal sensitivity more centrally in patients with AMD.[37,47,48] In the case of early DR, hypersensitivity peaks centrally before the appearance of

vasculopathy. In agreement with a previous study in patients with exudative AMD who were tested with both mfPOP and mfVEPs on the same day, we found shorter time to peak contractions in regions corresponding with amplitude loss in the central visual field.[37,48] Although we cannot confirm that faster and smaller responses are predictive of retinopathic progression, it seems reasonable that ramping up responses from healthy regions of the retina could be associated with neural retinal precursors to retinal vasculopathy. Whether these functional retinal changes are predictive of a higher risk of DR warrants further investigation.

The limitations to our study included the small number of diabetic subjects with more severe retinopathy. Although we excluded subjects with peripheral neuropathy, reports of retinal sensitivity loss predominantly affecting the peripheral retina have been documented.[49] An unpublished mfPOP study from our lab on patients with T1D showed that only quite severe peripheral neuropathy affected mfPOP results.[50] Also, note that pupil neuropathy cannot produce focal changes in sensitivity in the visual fields. The source of pupillary response changes is unknown, but early-stage experimental diabetes produces significant thinning of both inner and outer retinal nuclear layers and up to 25% loss of retinal ganglion cells.[51] Furthermore, structural and functional alterations of Müller cells have been documented and are believed to contribute to the development and progression of diabetic eye disease.[8,11]

There have been contrasting reports of visual field loss measured by standard automated perimetry (SAP) in diabetic patients with and without retinopathy. Previous reports using white-on-white SAP found that subjects with minimal DR had no significant visual field defects.[22,52] By contrast, Bengtsson et al.[23] reported that up to 24% of diabetic subjects with mild retinopathy had significantly depressed fields. In patients with no retinopathy, blue-on-yellow SAP has been reported to yield reduced mean deviations in patients with T1D[53,54] but no significant changes in T2D.[13,55] Furthermore, compared with white-on-white perimetry, flicker[15] and frequency doubling[56] perimetry have been found to be more sensitive to visual field loss in patients with type 1 and 2 diabetes but no retinopathy. Some of the discrepancy among reports may be due to the inherent variability in SAP results.[24,25] One trend that can be identified from the literature is evidence of greater sensitivity loss in the peripheral (beyond $\pm 10^{\circ}$ eccentricity) visual field[15,34,54] in diabetic eyes with no retinopathy. Our data showed that visual field tests that extended to peripheral regions of the retina (Fig. 4) were diagnostic in such eyes.

In summary, this study demonstrates an improvement in diagnostic accuracy for peripheral versus central mfPOP visual field tests with and without retinopathy. These functional test results suggest that response delays are a more sensitive measure of retinal dysfunction in early diabetic retinal disease than amplitude deviations. We are currently conducting a longitudinal study in a similar diabetic patient group to determine prognostic biomarkers to identify eyes at risk of retinopathy progression. Further improvements to the mfPOP methods have also made the effects more robust. In addition, examining spatial associations between microvascular changes and retinal dysfunction measured by mfPOP may further clarify the structure–function relationship in diabetic retinopathy. In the future, such retinal function testing may be a clinically useful adjunct for the diagnosis and monitoring, including of therapeutic responses in people with diabetes.

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