

# Multidimensional Functional and Structural Evaluation Reveals Neuroretinal Impairment in Early Diabetic Retinopathy

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**PURPOSE.** To test whether quantitative functional tests and optical coherence tomography (OCT)-defined structure can serve as effective tools to diagnose and monitor early diabetic neuroretinal disease.

**METHODS.** Fifty-seven subjects with diabetes (23 without diabetic retinopathy [no DR], 19 with mild nonproliferative diabetic retinopathy [mild NPDR], 15 with moderate to severe [moderate NPDR]), and 18 controls underwent full ophthalmic examination, fundus photography, spectral-domain optical coherence tomography (SD-OCT), e-ETDRS (Early Treatment Diabetic Retinopathy Study) acuity, and the quick contrast sensitivity function (qCSF) method. Perimetry testing included short-wavelength automated perimetry (SWAP), standard automated perimetry (SAP), frequency doubling perimetry (FDP), and rarebit perimetry (RBP).

**RESULTS.** ETDRS acuity and RBP were not sensitive for functional differences among subjects with diabetes. AULCSE, a metric of qCSF, was reduced in diabetics with moderate compared to mild NPDR ( $P = 0.03$ ), and in subjects with no DR compared to controls ( $P = 0.04$ ). SWAP and SAP mean deviation (MD) and foveal threshold (FT) were reduced in moderate compared to mild NPDR (SWAP, MD  $P = 0.002$ , FT  $P = 0.0006$ ; SAP, MD  $P = 0.02$ , FT  $P = 0.007$ ). FDP 10-2 showed reduced MD in moderate compared to mild NPDR ( $P = 0.02$ ), and FDP 24-2 revealed reduced pattern standard deviation (PSD) in mild NPDR compared to no DR ( $P = 0.02$ ). Structural analysis revealed thinning of the ganglion cell layer and inner plexiform layer (GCL+IPL) of moderate NPDR subjects compared to controls. The thinner GCL+IPL correlated with impaired retinal function.

**CONCLUSIONS.** This multimodal testing analysis reveals insights into disruption of the neuroretina in diabetes and may accelerate the testing of novel therapies.

**Keywords:** diabetic retinopathy, retinal neurodegeneration, contrast sensitivity, visual fields, structure-function analysis, OCT

Diabetes mellitus (DM) is a growing global epidemic complicated by diabetic retinopathy (DR) and progressive vision loss. Current guidelines for diagnosis, staging, and management of DR are based on the identification of visible vascular changes such as hemorrhages, blood vessel leakage, and neovascularization.<sup>1,2</sup> Over the last two decades, overwhelming evidence has shown that DM affects the entire neurovascular unit of the retina, not merely the microvasculature.<sup>3-6</sup> In fact, recent findings suggest that dysfunction of the neuroretina may precede the characteristic vascular findings.<sup>7-10</sup>

The integrity of the neuroretina is not readily determined by clinical fundus exam in the absence of signs of vascular lesions or inflammation, but can be evaluated through both functional testing and imaging studies. Visual acuity is the standard test of visual function, but many studies have shown that psychophysical tests, including perimetry and contrast sensitivity, are more sensitive for retinal neuropathy. For example, white-on-

white standard automated perimetry (SAP) is a nonselective test of the visual pathways, while frequency doubling technology perimetry (FDP), short-wavelength automated perimetry (SWAP), and rarebit perimetry (RBP) are selective tests of inner retinal function, predominantly stimulating magnocellular, koniocellular, and parvocellular pathways.<sup>11-13</sup> Contrast sensitivity is a nonspecific test of the inner retina, but different spatial frequencies favor specific neural pathways.<sup>14,15</sup> Parravano et al.<sup>16,17</sup> showed that performance on multiple tests of inner retinal function, including FDP and SAP, is reduced in subjects with diabetes without evidence of DR. Additionally, Jackson et al.<sup>18</sup> demonstrated that reduced contrast sensitivity and decreased performance on FDP and SAP correlate with nonproliferative diabetic retinopathy (NPDR). Hellgren et al.<sup>19</sup> evaluated performance on SAP over 4 years and demonstrated progression of DR based on neuroretinal functioning and not microvascular-based grading. Bengtsson et al.<sup>20</sup> compared SAP



to SWAP and demonstrated that both tests are more sensitive to retinal impairment than Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity. Finally, several studies showed reduced performance on RBP testing in patients with diabetes, with and without retinopathy.<sup>21-23</sup>

Retinal neuropathy also presents with structural changes such as neuronal apoptosis and thinning of the inner retinal layers in diabetes.<sup>24-34</sup> Using spectral-domain optical coherence topography (SD-OCT) and automatic retinal layer segmentation, Vujosevic and Midena<sup>29</sup> showed evidence of early neurodegeneration by the thinning of the retinal nerve fiber layer (RNFL) in diabetic patients with no DR compared to healthy controls. Cabrera DeBuc and Somfai<sup>30</sup> also found thinning of the RNFL, as well as thinning of the ganglion cell layer and inner plexiform layer (GCL+IPL), in diabetic patients with mild NPDR compared to no DR. Subsequently, DeBuc et al.<sup>31</sup> demonstrated GCL+IPL and outer plexiform layer (OPL) thinning in mild NPDR compared to healthy controls, and additional thinning of the RNFL and outer retina in mild NPDR compared to no DR.

Several studies have shown that changes apparent on OCT imaging occur concomitantly with functional impairment in diabetic patients with early stages of DR. Verma et al.<sup>34</sup> studied diabetic patients without DR and demonstrated that reduced foveal and photoreceptor layer thickness on OCT was correlated with reduced sensitivity as measured by microperimetry. Montesano et al.<sup>35</sup> also found that reduced GCL+IPL thickness in diabetic patients without DR correlated with decreased sensitivity on microperimetry. Stem et al.<sup>36</sup> demonstrated that higher contrast sensitivity was associated with a more intact inner nuclear layer (INL) in patients both with and without DR. Additionally, Van Dijk et al.<sup>23</sup> showed that reduced performance on RBP in patients with diabetes correlated with reduced thickness of the ganglion cell layer. Thus, OCT-defined retinal structure together with quantitative assessment of visual function can reveal retinal neurodegeneration.

The contemporary understanding of the neurovascular unit and the natural history of retinal dysfunction mandates a revised clinical approach to DR. The imperative for reliable tests and endpoints for monitoring progression and treatment response in early DR was emphasized at the 2015 NEI/FDA Diabetic Retinopathy Clinical Trial Design and Endpoint Workshop.<sup>37</sup> Multiple functional and structural tests have demonstrated usefulness for detecting and monitoring retinal dysfunction, but their applicability to clinical practice has not yet been evaluated. Thus, the primary aim of the present work was to conduct a pilot study to compare SWAP, SAP, FDP, RBP, contrast sensitivity function, and OCT-defined retinal structure for their utility in detecting retinal impairment in early DR. Specifically, we were interested in determining which test(s) best detects subtle differences among diabetic patients with early evidence of neuroretinal degeneration.

## METHODS

This study was conducted at the University of Michigan W. K. Kellogg Eye Center. The protocol design and conduct were consistent with the tenets of the Declaration of Helsinki, approved by the Institutional Review Board of the University of Michigan Medical School (HUM 99155), and compliant with the Health Insurance Portability and Accountability Act. Patients were recruited from the University of Michigan clinics and University of Michigan Health Research Web site between March 2016 and January 2017. Written informed consent was obtained from all patients before participation in the study.

## Subject Enrollment and Evaluation

Subjects were enrolled into four groups based on clinical evaluation and fundus photography: nondiabetics (control group), diabetics without retinopathy (no-DR group), diabetics with mild nonproliferative retinopathy (mild NPDR group), and diabetics with moderate to very severe nonproliferative DR (moderate NPDR group). Inclusion criteria for the control group were (1) age  $\geq$  18 years, (2) no clinical diagnosis of diabetes, and (3) ETDRS DR severity level of 10, no detectable retinopathy. Inclusion criteria for the diabetic group were (1) age  $\geq$  18 years and (2) diabetes as defined by the American Diabetes Association criteria for diagnosis.<sup>38</sup> The mild NPDR group included patients with ETDRS DR grade 20 to 35. The moderate NPDR group included patients with ETDRS DR grade 43 to 53.

Exclusion criteria for all the groups were any neurologic or systemic disease (other than DM), any other ocular diseases (i.e., glaucoma and cataracts greater than 1+ nuclear sclerosis) that could impair vision, any drug intake that could impair vision, Snellen or equivalent best-corrected visual acuity (BCVA) worse than 20/40, spherical equivalent more than  $\pm$ 6.0 diopters (D), proliferative DR, clinically significant diabetic macular edema and cystic changes on OCT, pregnancy or nursing, and inability to give informed consent or to complete testing.

All subjects underwent a comprehensive ophthalmologic examination that included a slit-lamp examination, applanation tonometry, and measurement of BCVA with Snellen and electronic visual acuity (EVA) testing using the e-ETDRS protocol, color fundus photography, spectral-domain optical coherence tomography (SD-OCT), and contrast sensitivity using the quick contrast sensitivity function (qCSF) method.<sup>39</sup> Six visual field methods were tested; Swedish interactive threshold algorithm (SITA) blue-on-yellow SWAP and white-on-white SAP, frequency doubling perimetry (FDP) using the 24-2 and the 10-2 strategies, and RBP using the fovea and central (inner and outer) field testing strategies.

One eye of each subject was selected; if both eyes met eligibility criteria, the eye with more severe retinopathy was chosen. If both eyes were eligible for the same retinopathy group, then the eye with the better visual acuity was selected.

## Fundus Photography and SD-OCT

Color fundus photographs were taken using nonsimultaneous stereoscopic, on-axis, nonsteered, 200° ultrawide field (UWF) imaging (Optos 200TX; Optos plc, Dunfermlie, UK), and the images were magnified to the equivalent field dimensions of seven standard fields of the ETDRS scale. Spectral-domain optical coherence tomography (Spectralis HRA+OCT; Heidelberg Engineering, Inc., Heidelberg, Germany) was performed using the following scan acquisition parameters: macular scan volume, 37 B-scans, each spaced 120  $\mu$ m, 15°  $\times$  15°, automatic real-time (ART) mean of 12 in high-resolution (HR) mode. For quality control, all OCT scans were performed by VMC and KAJ, with the same technique and parameters used for both cases and controls. The retinal layers on each SD-OCT scan were segmented semiautomatically using the built-in software of the Heidelberg Spectralis. The boundaries of all segmented layers were carefully reviewed by two reviewers (VMC and KAJ) and adjusted when necessary. Retinal thickness was analyzed using the ETDRS grid, which included the 1-mm central fovea, 3-mm inner ring, and 6-mm outer ring (parafovea). The inner and outer rings were sectioned into superior, inferior, temporal, and nasal quadrants. The retinal thickness was recorded for the total retina, RNFL, GCL+IPL, inner nuclear layer together with outer plexiform layer

(INL+OPL), outer nuclear layer (ONL), and between the external limiting membrane (ELM) and RPE.

### Contrast Sensitivity

Contrast sensitivity was evaluated using the qCSF method on the AST Platform (Adaptive Sensory Technology, San Diego, CA, USA), a new computerized method for evaluating the contrast thresholds over a wide range of contrast (0.002%–100%) and spatial frequency (approximately 1–27 cyc/deg).<sup>39,40</sup> The test consisted of 25 trials, with a Bayesian adaptive algorithm selecting the frequency–contrast combinations of each trial sampling various grating frequencies with a high test–retest reliability greater than 92.4%.<sup>41</sup> The duration for each test was approximately 2 minutes. The test stimuli are spatially filtered optotypes that modulate both frequency and contrast, unlike the traditional Pelli-Robson chart, which does not test more than one spatial frequency.<sup>39,42,43</sup> All participants were tested monocularly following measurement of BCVA while the untested eye was covered with a patch. The sensitivity at various spatial frequencies (1.5, 3, 6, 12, and 18 cyc/deg) and the area under the log CSF (AULCSF)—integrated from 1.5 to 18 cyc/deg—served as metrics of contrast sensitivity function and were used for statistical analysis.

### Visual Fields

Subjects performed the following visual field tests; SWAP and SAP (Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA, USA), FDP (Humphrey Matrix 715 Visual Field Analyzer; Carl Zeiss Meditec, Inc.), and RBP (Lars Frisen, Goteborg, Sweden). The visual field tests were performed in the same order with a 5- to 10-minute break between tests. All visual fields were tested on two separate visits within 3 weeks. SWAP, SAP, RBP, and FDP were considered reliable when fixation losses, false-negative errors, and false-positive errors were less than 33%. Only subjects with two reliable tests for each strategy were included. The second of these two reliable tests was used for statistical analysis.

**Short-Wavelength and Standard Automated Perimetry.** SWAP was performed using the 24-2 SITA-SWAP strategy (version 4.1) on the Humphrey Field Analyzer II. Each narrow-band blue (440-nm wavelength) Goldmann size V target was presented for 200 ms on a 100 cd/m<sup>2</sup> yellow background. The average testing time was approximately 3.8 minutes per eye. SAP was performed using 24-2 SITA-standard strategy (version 4.1) on the Humphrey Field Analyzer II. Each white light stimulus was a Goldmann size III target, which was presented for 200 ms on a white background illuminated to 10 cd/m<sup>2</sup>. The average testing time was approximately 5 minutes per eye. Fifty-two test locations (54 minus the 2 locations at the blind spot) were evaluated for both SWAP and SAP. Lens correction was automatically calculated by built-in technology of the Humphrey Field Analyzer II. Foveal threshold (FT), mean deviation (MD), and pattern standard deviation (PSD) were recorded and used for statistical analysis. FT is the increment threshold sensitivity at the fovea, MD is a global index of the age-adjusted average deviation from the mean across all test locations, and PSD is a global index of the uniformity of the deviation compared to age-matched controls. Subjects with reduced retinal function have more depressed MD values (negative) and higher PSD values (positive).

**Frequency Doubling Perimetry.** The FDP 24-2 strategy was performed on the Humphrey Matrix 715 Visual Field Analyzer. The stimulus was a 0.25 cyc/deg monochrome sinusoidal grating of vertical gray stripes that was phase

reversed at 18 Hz. The minimum contrast threshold of the 5° diameter stimulus was measured at each of 55 test locations. The testing time was approximately 5.2 minutes per eye. The 10-2 strategy was also performed with a stimulus of 0.25 cyc/deg monochrome sinusoidal grating of vertical gray stripes that was phase reversed at 12 Hz, testing flicker sensitivity. The minimum contrast threshold of the 2° diameter stimulus was measured at each of 44 test locations. The testing time was approximately 4.3 minutes per eye, and the subjects wore their own prescription glasses. FT, MD, and PSD were recorded. Additionally, the parafoveal threshold was calculated for FDP 10-2 as the average of the increment threshold sensitivities in the 10° around the fovea.

**Rarebit Perimetry.** Rarebit perimetry was designed to detect subtle visual field defects that may be missed with other strategies that have large stimuli testing overlapping receptive fields.<sup>13</sup> The RBP (version 4) visual field testing was performed on a standard computer with a 15-inch liquid crystal display screen using a wireless single mouse and keyboard. The software was downloaded in Windows (Microsoft, Redmond, WA, USA) format from the author (lars.frisen@neuro.gu.se). The test was set up in a dark room, and the screen background and target luminance were set at 1 and 150 cd/m<sup>2</sup>, respectively. The fovea testing strategy was performed at 2.0 m, with a corresponding addition of +0.5 D correction using trial lenses. The targets (one or two high-contrast microdots separated by 1°) were presented at random positions on a testing matrix consisting of 10 square test areas, each 1.5° by 1.5°, for 200 ms. Fifteen repeat runs were performed, and the total testing time was approximately 4 minutes. The inner central field test was performed at 0.5 m with a +2.0 D correction, and the outer central field test was performed at 1 m with a +1.0 D correction. The inner test covered four inner test areas while the outer test covered 20 outer test areas (6° × 8° wide in the center and 6° × 14° wide in periphery), extending 30° horizontally and 20° vertically from the fixation point. The target for these tests was two microdots separated by 4° presented at random positions for 200 ms. Fifteen repeat runs were performed for each test, and the total testing time was approximately 13 minutes. The mean hit rate (MHR), which represents the average number of targets detected out of all the targets presented across the field map, was used for statistical analysis.

### Statistical Analysis

Demographic data were summarized as means ± standard deviations for continuous variables, and frequencies for categorical variables. Data distribution was assessed graphically and with the Shapiro-Wilk test for normality. Analysis of variance (ANOVA) was used to compare continuous parametric variables, and the Kruskal-Wallis test was used to compare continuous nonparametric variables. The Tukey-Kramer HSD test (parametric) and Wilcoxon rank sum test (nonparametric) were used for post hoc analysis. Test sensitivity was calculated as the percent of subjects falling below the 10th percentile of the control group. Note that the 90th percentile was used for PSD values, as higher PSD values correspond to greater retinal dysfunction. McNemar's test was used to evaluate redundancy between tests. The Bonferroni correction for multiple comparisons was applied to functional analyses and spatial contrast sensitivity data such that the statistical significance would occur at  $P \leq 0.0031$  and  $P \leq 0.01$ , respectively. For other tests,  $P < 0.05$  was considered statistically significant. Statistical analysis was performed with JMP, Version Pro 12, 1989-2007 (SAS Institute, Inc., Cary, NC, USA).

TABLE 1. Demographics of the Cohort

	Control, <i>n</i> = 18	No DR, <i>n</i> = 23	Mild NPDR, <i>n</i> = 19	Moderate NPDR, <i>n</i> = 15	<i>P</i> Value
Age, y (SD)	51.7 (14.3)	53.7 (12.2)	57.4 (12.7)	59.9 (11.6)	0.2457
Sex					
Male, <i>n</i> (%)	5 (27.8)	13 (56.5)	14 (73.7)	12 (80.0)	0.007
Female, <i>n</i> (%)	13 (72.2)	10 (43.5)	5 (26.3)	3 (20.0)	
Diabetes type					
Type 1, <i>n</i> (%)	NA	5 (21.7)	6 (31.6)	1 (6.67)	0.170
Type 2, <i>n</i> (%)		18 (78.3)	13 (68.4)	14 (93.3)	
Duration of diabetes, y (SD)	NA	9.35 (8.67)	20.8 (12.5)	17.5 (11.4)	0.003
BMI (SD)	26.2 (4.44)	32.9 (7.93)	30.4 (5.14)	35.7 (6.29)	0.001
HbA <sub>1c</sub> % (SD)	5.45 (0.26)	7.75 (1.99)	8.05 (1.03)	7.87 (1.54)	0.0005
Cholesterol (SD)	195.3 (44.3)	161.6 (34.7)	160.3 (28.4)	173.5 (43.3)	0.065
Triglycerides (SD)	86.3 (23.8)	118.7 (18.4)	124.5 (21.3)	200.2 (22.9)	0.018

SD, standard deviation.

## RESULTS

### Demographics

Seventy-five participants were enrolled and included in the analysis: 23 subjects with diabetes and no DR, 19 with mild NPDR, 15 with moderate NPDR, and 18 healthy controls. Subject demographics are listed in Table 1. The cohort consisted of 68.4% males and 78.9% subjects with type 2 diabetes. Subjects with mild and moderate NPDR had a longer duration of diabetes than subjects with no DR ( $P = 0.002$  and  $P = 0.016$ , respectively). Diabetic subjects had higher body mass index (BMI) and HbA<sub>1c</sub> than control subjects ( $P = 0.002$  for both). Subjects with moderate NPDR also had higher

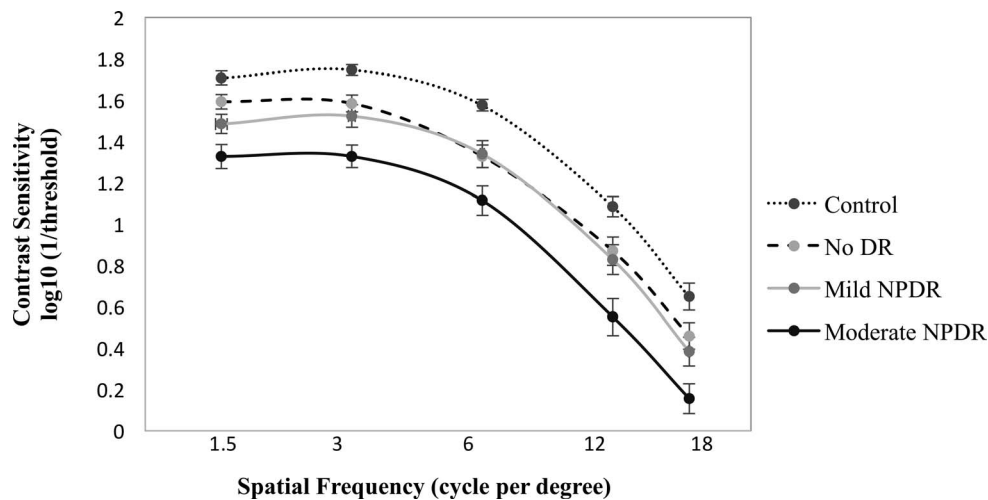
triglyceride levels than controls ( $P = 0.001$ ). There was no difference in age, type of diabetes, or cholesterol levels among the groups.

### Function Analysis

Eight quantitative tests of visual function were employed to evaluate controls and subjects with diabetes, with and without retinopathy. Results for each test are presented in Table 2. ANOVA and Kruskal-Wallis tests were used to determine if there were differences among groups. Bonferroni correction for multiple comparisons was applied such that the statistical significance would occur at  $P \leq 0.0031$ . AULCSF (a measure of

TABLE 2. Comparison of Visual Function Outcomes

	Control, <i>n</i> = 18	No DR, <i>n</i> = 23	Mild NPDR, <i>n</i> = 19	Moderate NPDR, <i>n</i> = 15	<i>P</i> Value All Groups, Corrected	<i>P</i> Value Control, No DR	<i>P</i> Value No DR, Mild NPDR	<i>P</i> Value Mild, Moderate
ETDRS logMAR (Snellen equivalent)	-0.11 (20/16)	-0.03 (20/20)	0.02 (20/20)	0.04 (20/20)	0.008	0.317	0.595	0.949
AULCSF (SD)	1.60 (0.12)	1.40 (0.25)	1.34 (0.27)	1.11 (0.26)	<0.001	0.044	0.847	0.027
SWAP 24-2								
MD (SD)	-2.14 (1.91)	-3.47 (4.26)	-4.21 (4.46)	-9.39 (3.97)	<0.0001	0.979	0.448	0.002
PSD (SD)	2.40 (0.48)	2.71 (1.32)	2.80 (0.74)	4.04 (1.98)	0.001	0.454	0.383	0.013
FT (SD)	24.2 (3.65)	23.4 (4.92)	22.4(4.73)	16.1(4.23)	<0.0001	0.946	0.881	0.0006
SAP 24-2								
MD (SD)	-0.56 (0.72)	-1.27 (2.47)	-1.81 (2.43)	-4.95 (3.76)	<0.0001	0.808	0.838	0.015
PSD (SD)	1.38 (0.24)	1.95 (1.93)	2.72 (2.34)	3.42 (2.24)	<0.0001	0.199	0.046	0.401
FT (SD)	34.7 (0.45)	34.4 (1.85)	34.3 (0.44)	32.3 (0.49)	0.001	0.556	0.749	0.007
FDP 24-2								
MD (SD)	0.605 (1.95)	0.016 (1.99)	-0.74 (2.97)	-3.61 (5.54)	0.002	0.938	0.874	0.057
PSD (SD)	2.53 (0.36)	2.74 (0.95)	3.37 (0.79)	3.88 (1.17)	<0.0001	0.977	0.019	0.584
FT (SD)	31.7 (2.35)	30.1 (4.07)	28.7 (4.30)	25.5 (5.51)	0.0009	0.464	0.748	0.262
FDP 10-2								
MD (SD)	0.12 (2.28)	0.11 (2.29)	-1.26 (2.87)	-4.40 (4.63)	<0.0001	0.994	0.505	0.016
PSD (SD)	2.43 (0.28)	2.86 (0.67)	0.66 (0.69)	3.83 (1.08)	0.0001	0.101	0.8	0.161
10° FT (SD)	32.1 (2.13)	31.2 (3.36)	30.1 (3.02)	26.3 (5.74)	0.0004	0.588	0.666	0.109
Rarebit fovea	<i>n</i> = 17	<i>n</i> = 18	<i>n</i> = 14	<i>n</i> = 7				
MHR (SD)	94.6 (4.70)	96.4 (4.70)	94.4 (4.27)	83.9 (10.45)	0.005	0.521	0.309	0.0936
Rarebit central	<i>n</i> = 17	<i>n</i> = 18	<i>n</i> = 13	<i>n</i> = 6				
MHR (SD)	93.7 4.83	93.1 5.24	93.2 4.92	90.0 4.77	0.338	0.989	0.999	0.411



**FIGURE 1.** Contrast sensitivity was measured over a range of spatial frequencies (1.5, 3, 6, 12, and 18 cyc/deg) using the qCSF method. Error bars represent the standard error of the mean. Contrast sensitivity appears to be impaired in subjects with diabetes, with greatest impairment found in subjects with moderate NPDR. Diabetic subjects without signs of retinopathy showed significant impairment at the low and intermediate spatial frequencies (1.5, 3, 6, 12 cyc/deg). Differences were detected among all groups at 1.5 cyc/deg.

contrast sensitivity), SWAP, SAP, FDP 24-2, and FDP 10-2 showed statistically significant differences among groups. A subanalysis was performed for the subjects with type 2 diabetes, which showed similar differences among groups in AULCSF ( $P < 0.0001$ ), SWAP (MD  $P = 0.0001$ , PSD  $P = 0.0001$ , FT  $P = 0.001$ ), SAP (MD  $P = 0.0002$ , PSD  $P = 0.0001$ , FT  $P = 0.001$ ), FDP 24-2 (MD  $P = 0.02$ , PSD  $P = 0.0002$ , FT  $P = 0.001$ ), and FDP 10-2 (MD  $P = 0.0002$ , PSD  $P = 0.0003$ , FT  $P = 0.0005$ ). Post hoc analysis on all subjects was performed to compare each group to one another (see below). Multiple aspects of visual function appear to be affected with increasing severity of DR.

**Early Treatment Diabetic Retinopathy Study Visual Acuity.** Visual acuity as measured by e-ETDRS is the standard functional outcome measure in many DR research studies and clinical trials, but in this study it did not detect a difference among groups following Bonferroni correction (statistical significance occurring at  $P \leq 0.0031$ ). In our subanalysis of subjects with type 2 diabetes, ETDRS acuity also did not detect a significant difference following Bonferroni correction ( $P = 0.004$ ). The relatively young ages of the subjects and visual acuities better than 20/40 reduced the likelihood that differences in early cataract development would confound the results.

**Contrast Sensitivity.** Contrast sensitivity function as measured with the Pelli-Robson chart is decreased in diabetic subjects with retinopathy.<sup>18</sup> This study employed the automated qCSF method on the AST Platform, which allows for measurement of contrast sensitivity function across a range of spatial frequencies.<sup>40</sup> The AULCSF was decreased in diabetic subjects with and without retinopathy compared to controls. Lesmes et al.<sup>39</sup> demonstrated that with 25 trials, AULCSF has a mean bias  $< 5\%$  with a coefficient of variation  $< 15\%$ . Subjects with moderate NPDR showed reduced AULCSF compared to subjects mild NPDR ( $P = 0.027$ ), and diabetic subjects with no DR had significantly reduced AULCSF compared to controls ( $P = 0.044$ ).

Contrast sensitivity at different spatial frequencies was evaluated separately to determine which spatial frequencies are differentially affected by diabetes. Figure 1 depicts the contrast sensitivity curves over 1.5, 3, 6, 12, and 18 cyc/deg. The Bonferroni correction for multiple comparisons was applied such that the statistical significance would occur at  $P$

$\leq 0.01$ . There were differences among the four groups at each spatial frequency (1.5 cyc/deg,  $P < 0.0001$ ; 3 cyc/deg,  $P < 0.0001$ ; 6 cyc/deg,  $P < 0.0001$ ; 12 cyc/deg,  $P = 0.0004$ ; 18 cyc/deg,  $P = 0.0002$ ). Diabetic subjects without evidence of DR showed statistically significant reductions in contrast sensitivity compared to controls at 1.5, 3, 6, and 12 cyc/deg (1.5 cyc/deg,  $P = 0.006$ ; 3 cyc/deg,  $P = 0.0009$ ; 6 cyc/deg,  $P = 0.001$ ; 12 cyc/deg,  $P = 0.038$ ). Reduced contrast sensitivity in the moderate NPDR group compared to the mild NPDR group was apparent across all spatial frequencies (1.5 cyc/deg,  $P = 0.039$ ; 3 cyc/deg,  $P = 0.021$ ; 6 cyc/deg,  $P = 0.028$ ; 12 cyc/deg,  $P = 0.035$ ; 18 cyc/deg,  $P = 0.021$ ). Diabetic subjects had decreased contrast sensitivity across all spatial frequencies, but only the contrast sensitivity at the lowest spatial frequency (1.5 cyc/deg) was markedly different among all groups. Thus, diabetes affects contrast sensitivity early in the course of diabetic retinal sensory neuropathy.

**Short-Wavelength and Standard Automated Perimetry.** SWAP and SAP revealed neuroretinal impairment in subjects with clinical evidence of retinopathy. Both tests found a statistically significant decrease in MD and FT values in subjects with moderate compared to mild NPDR (SWAP, MD  $P = 0.002$ ; SWAP, fovea  $P = 0.0006$ ; SAP, MD  $P = 0.015$ ; SAP, fovea  $P = 0.007$ ). SWAP PSD values that reflect variability across the field also differentiated among subjects with moderate and mild NPDR ( $P = 0.013$ ). With SAP, PSD values differentiated between diabetic subjects with no DR and with mild NPDR ( $P = 0.046$ ). SAP appears to have advantages over SWAP in detecting retinal dysfunction early in NPDR, but SWAP was able to detect differences in later-stage NPDR with a slightly reduced testing time.

**Frequency Doubling Perimetry.** FDP testing revealed defects of inner retinal processing and is highly sensitive to early-stage DR.<sup>17,18</sup> To gain further insight into the utility of FDP, the 24-2 and 10-2 field programs were compared. FDP 10-2 testing showed that MD values were significantly reduced in subjects with moderate NPDR compared to mild NPDR ( $P = 0.016$ ). However, the variance in FDT 10-2 MD values of the moderate NPDR group were greater compared to both SAP and SWAP. Additionally, FDP 24-2 testing revealed that PSD values were reduced in subjects with mild NPDR compared to no DR ( $P = 0.019$ ), and the variance of the diabetic patients testing with FDT 24-2 was less than with SAP. The two FDP strategies

**TABLE 3.** Percentage of Subjects Outside of the Normal Reference Range (At or Below the 10th Percentile of the Control Group)

	No DR	Mild NPDR	Moderate NPDR
ETDRS logMAR	21.7	36.8	40
AULCSF	47.8	47.4	85.7
SWAP 24-2			
MD	26.1	42.1	86.7
PSD	17.4	31.6	66.7
FT	17.4	26.3	73.3
SAP 24-2			
MD	30.4	42.1	86.7
PSD	21.7	63.2	93.3
FT	13	15.8	40
FDP 24-2			
MD	8.7	21.1	53.3
PSD	21.7	57.9	73.3
FT	39.1	52.6	80
FDP 10-2			
MD	8.7	31.6	73.3
PSD	47.8	57.9	78.6
10° FT	26.1	42.1	73.3
Rarebit fovea	<i>n</i> = 18	<i>n</i> = 14	<i>n</i> = 7
MHR	5.56	14.29	42.86
Rarebit central	<i>n</i> = 18	<i>n</i> = 13	<i>n</i> = 6
MHR	11.1	7.69	33.3

were not equivalent in distinguishing between diabetic subjects in this cohort. SWAP and FDT 24-2 used together appear to detect the subtle differences (with least variance) among diabetic subjects with a cumulative testing time of less than 10 minutes.

**Rarebit Perimetry.** Only 74.7% of subjects were able to successfully complete the foveal and central testing strategies on RBP due to the long testing protocol (approximately 18 minutes, in contrast to the other tests, which were much shorter). Ages were similar among groups ( $P = 0.189$ ). There was no significant difference among the groups using either the foveal or central rarebit algorithms (inner and outer together). Subanalysis of just subjects with type 2 diabetes also did not detect any differences among groups. Thus, contrast sensitivity, SAP, SWAP, and FDP appear to be particularly useful for detecting functional differences among diabetic subjects with early stages of DR.

**Test Sensitivity.** The sensitivity of each functional test was calculated as a clinical estimate of effect size. Table 3 shows the test sensitivities as the percentage of subjects whose test performance was outside of the normal reference range (below

the 10th percentile of the control group). As expected, subjects with moderate NPDR had the greatest impairment across all groups on all tests of visual function. The sensitivities suggest that impairment was greatest for subjects with moderate NPDR using contrast sensitivity, SWAP, and SAP. These were all approximately 2-fold more sensitive for moderate NPDR than for mild NPDR. The impairment of the mild NPDR group compared to the no-DR group was most evident using FDP and SAP. Additionally, FDP, SAP, and AULCSF detected the greatest impairment in diabetic subjects without evidence of retinopathy. Taken together, AULCSF and SAP detected large impairments in subjects with no DR, and in moderate NPDR compared to mild NPDR. SWAP was sensitive to moderate NPDR, while FDP detected the greatest impairment among subjects with no DR.

Redundancy among tests was evaluated to determine whether the tests that showed high sensitivity and statistically significant differences among groups were identifying the same subjects. McNemar's test was used to compare subjects who fell below the 10th percentile based on AULCSF and the MD index of SWAP, SAP, and FDP. Table 4 shows that there was no significant difference in diabetic subjects detected by SWAP, SAP, and AULCSF. AULCSF detected 9 of 23 subjects with no DR missed by both FDP 24-2 and 10-2 ( $P = 0.027$  and  $P = 0.003$ , respectively). Both SAP and SWAP detected 4 of 19 subjects with mild NPDR ( $P = 0.046$  for both) and 5 of 15 subjects with moderate NPDR ( $P = 0.025$  for both) missed by FDP 24-2. The subjects detected by central and foveal RBP were also compared with the subjects detected by FDP 24-2 and 10-2, SAP, and SWAP. We found no difference in detection of moderate NPDR subjects, but SAP and SWAP detected subjects with mild NPDR who were missed by the central RBP. In general, SAP is a nonspecific test of retinal function, whereas both FDP and SWAP were developed to stimulate different populations of ganglion cells within the inner retina. Our results suggest more overlap between SAP and SWAP than with FDP. AULCSF, also a nonspecific test of retinal function, appears to also have more overlap with SAP and SWAP than FDP in detecting neuroretinal impairment.

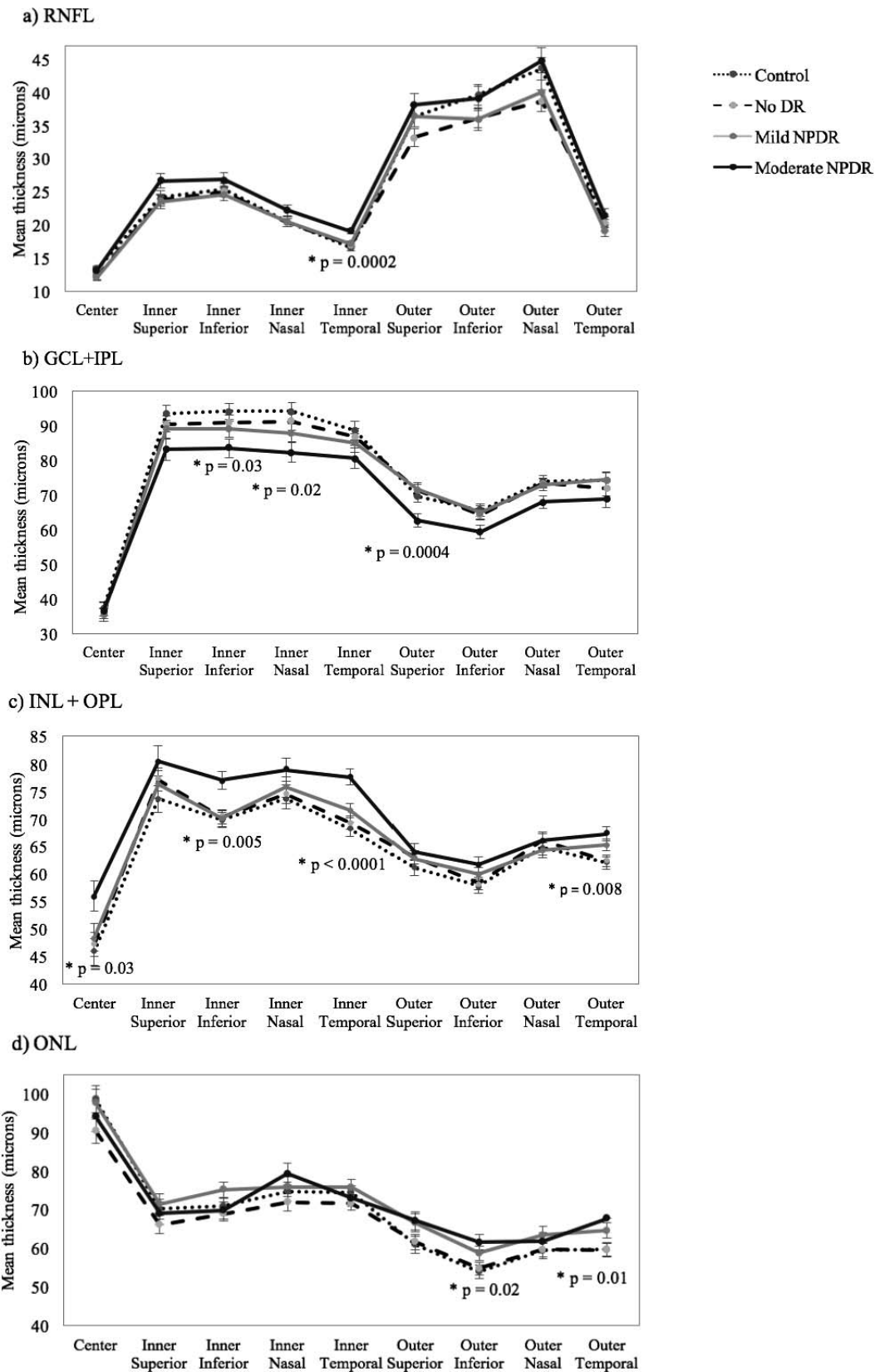
### Structural Analysis

Retinal thickness was measured with SD-OCT to compare structural disruptions between subjects with diabetes and healthy controls. Macular OCT scans were obtained and segmented semiautomatically to determine thickness measurements for the RNFL, GCL+IPL, INL+OPL, ONL, outer retina, and total retina in the 1-mm central fovea, 3-mm inner ring, and 6-mm outer ring of the ETDRS grid. Figure 2 shows the results

**TABLE 4.** Comparison of Subjects Detected by AULCSF, SWAP, SAP, and FDP Using McNemar's Test

	SWAP, MD	SAP, MD	FDP 24-2, MD	FDP 10-2, MD
AULCSF	No DR, $P = 0.132$ Mild, $P = 0.705$ Moderate, $P = 1.000$	No DR, $P = 0.206$ Mild, $P = 0.705$ Moderate, $P = 1.000$	No DR, $P = 0.027^*$ Mild, $P = 0.025^*$ Moderate, $P = 0.059$	No DR, $P = 0.003^*$ Mild, $P = 0.180$ Moderate, $P = 0.414$
SWAP, MD		No DR, $P = 0.654$ Mild, $P = 1.000$ Moderate, $P = 1.000$	No DR, $P = 0.046^*$ Mild, $P = 0.046^*$ Moderate, $P = 0.025^*$	No DR, $P = 0.157$ Mild, $P = 0.157$ Moderate, $P = 0.157$
SAP, MD			No DR, $P = 0.025^*$ Mild, $P = 0.046^*$ Moderate, $P = 0.025^*$	No DR, $P = 0.059$ Mild, $P = 0.157$ Moderate, $P = 0.157$
FDP 24-2, MD				No DR, $P = 1.000$ Mild, $P = 0.157$ Moderate, $P = 0.083$

\* Mean deviation (MD) values were used for statistical analysis.



**FIGURE 2.** OCT scans were acquired and retinal layer thicknesses were measured in the central macula, inner ring, and outer ring of the ETDRS grid. Graphs are shown for the retinal layer thickness in controls and diabetic subjects with no DR, mild NPDR, and moderate NPDR. (a) Nerve fiber layer (RNFL); (b) ganglion cell layer and inner plexiform layer (GCL+IPL); (c) inner nuclear layer and outer plexiform layer (INL+OPL); (d) outer nuclear layer (ONL); (e) external limiting membrane to the retinal pigment epithelium (defined as outer retina); (f) total retina. Error bars represent the standard error of the mean.

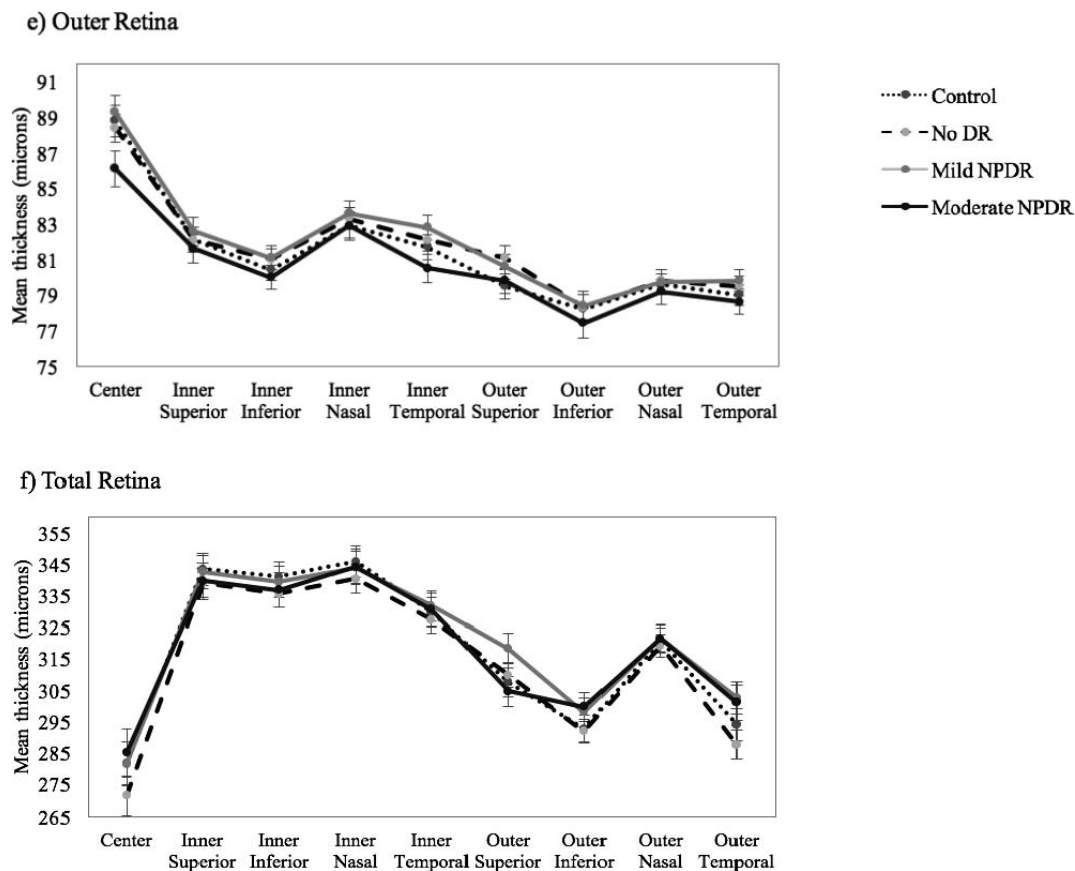


FIGURE 2. Continued.

of the OCT analysis. The RNFL thickness in the temporal zone of the inner ring was increased in diabetic subjects with moderate NPDR compared to healthy controls, diabetics with no DR, and diabetics with mild NPDR by 13.2%, 11.1%, and 9.9%, respectively ( $P = 0.0002$ ). The GCL+IPL was thinner in diabetic subjects with moderate NPDR compared to controls by 12.8% and 14.5% in the inferior and nasal sections of the inner ring, respectively ( $P = 0.03$  and  $P = 0.02$ , respectively). The GCL+IPL was also thinner in the superior section of the outer ring in moderate NPDR compared to controls, no DR, and mild NPDR by 11.2%, 14.0%, and 14.4% ( $P = 0.0004$ ). By contrast, the INL+OPL thickness was increased in the moderate NPDR group compared to controls by 18.3%, 9.3%, 12.3%, and 7.8% in the central, inner inferior, inner temporal, and outer temporal zones, respectively ( $P = 0.03$ ,  $P = 0.005$ ,  $P < 0.0001$ ,  $P = 0.008$ ). The ONL thickness was also increased in the moderate NPDR group compared to controls by 12.4% and 11.8% in the outer inferior and outer temporal areas of the ETDRS grid, respectively ( $P = 0.002$  and  $P = 0.01$ , respectively). There were no differences in the outer retinal or total retinal thicknesses between controls and diabetics.

A subanalysis was performed on subjects with type 2 diabetes with similar results. There was an increase in the inner temporal RNFL ( $P = 0.0006$ ) and a decrease in the GCL+IPL thickness in the inner inferior, inner nasal, and outer superior sections ( $P = 0.05$ ,  $P = 0.03$ , and  $P = 0.02$ , respectively). The INL+OPL was thicker in the central ( $P = 0.04$ ), inner inferior ( $P = 0.003$ ), inner temporal ( $P < 0.0001$ ), outer inferior ( $P = 0.05$ ), and outer temporal sections ( $P = 0.0002$ ). The ONL was also thicker in the outer inferior and outer temporal ( $P = 0.006$  for both). There were no differences among groups for the outer retinal thickness and total retinal thickness. These OCT

data reveal that diabetes exerts variable effects on the macular layer thickness rather than a uniform pattern as might be expected.

### Structure–Function Analysis

Structurally, there were several discernible differences between controls and diabetic subjects with varying degrees of early neuroretinal impairment. The GCL+IPL and INL+OPL showed the most areas of thickness differences as well as the greatest relative differences. Figures 3 and 4 show the correlations of retinal function with GCL+IPL and INL+OPL, respectively. AULCSF and SAP MD values were used as markers of retinal function as they showed the greatest impairment in diabetic patients with and without retinopathy. Thinner areas of GCL+IPL correlated with decreased contrast sensitivity and performance on SAP, suggesting that a thinner, less intact retina is associated with greater functional impairment. Interestingly, there were no significant correlations between the thicker INL+OPL and retinal function as assessed by these tests.

The functional performance of subjects with the greatest GCL+IPL thinning was further evaluated to better comprehend the correlation between retinal structure and function. Eight subjects of the cohort had GCL+IPL thickness in the lower 10th percentile in at least two of the three areas found to be significantly different among groups (inner inferior, inner nasal, and outer superior sections of the ETDRS grid). There was no difference in age ( $P = 0.21$ ), sex ( $P = 0.32$ ), type of diabetes ( $P = 0.09$ ), duration of diabetes ( $P = 0.62$ ), or HbA<sub>1c</sub> % ( $P = 0.35$ ) when compared to the rest of the cohort. The eight subjects had diabetes with varying clinical evidence of retinopathy; one had no DR; three had mild NPDR, and four had moderate



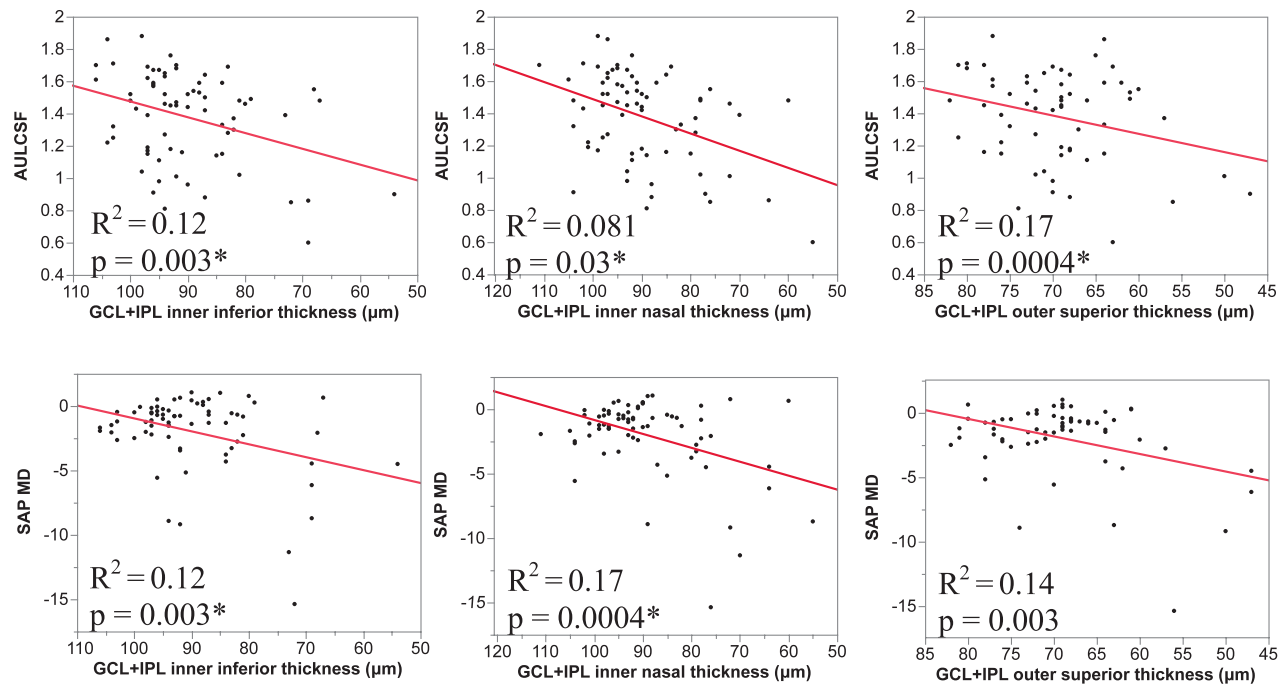


FIGURE 3. Correlations of OCT-derived GCL+IPL thickness with retinal function assessed by AULCSF (contrast sensitivity) and SAP mean deviation. Functional performance appears to be correlated with GCL and IPL thickness.

NPDR. Table 5 shows the functional analysis of these eight subjects compared to the rest of the cohort. Contrast sensitivity and performance on SWAP, SAP, and FDP were all markedly decreased in the subjects with the thinnest GCL+IPL. These results support the conclusion that subjects with thinning of the inner retina have a greater corresponding functional impairment.

## DISCUSSION

Extensive evidence now shows that retinal neurodegeneration is an early event in the evolution of DR.<sup>10</sup> The recent emphasis to develop early interventions to prevent vision loss from diabetes includes the need for reliable endpoints associated with the early-stage disease.<sup>37</sup> Therefore, this pilot study evaluated multiple quantitative functional tests and OCT imaging for effectiveness in detecting neuroretinal impairment in early DR, prior to onset of diabetic macular edema or neovascularization. Notably, this study was the first multimodal evaluation of early-stage DR comparing OCT-defined retinal structure and functional performance using ETDRS visual acuity, contrast sensitivity using an automated qCSF method, and six visual field tests.

Each functional test revealed evidence of neurosensory retinal dysfunction in patients with diabetes. ETDRS visual acuity was not as sensitive for detecting subtle functional differences among patients with diabetes. Contrast sensitivity, as assessed by AULCSF and contrast sensitivity measurements at various spatial frequencies on the AST Platform, was the most sensitive test for detection of subtle functional differences in diabetic patients both with and without retinopathy. Inner retinal function, as assessed by SWAP, SAP, and FDP, was impaired among diabetic patients without evidence of retinopathy, with mild NPDR, and with moderate-to-severe NPDR. Between the different visual field testing strategies, SAP detected the greatest impairment among diabetic patients without retinopathy and with moderate-to-severe NPDR. SWAP

was also sensitive for moderate-to-severe NPDR while FDP was more sensitive to patients without evidence of retinopathy. Interestingly, SWAP and SAP detected a different subpopulation of NPDR patients than FDP, which could reflect dysfunction of different neuronal pathways as cellular changes progress in diabetes. Based on our findings and with further input from future longitudinal studies, functional tests may serve as a more practical tool than electroretinography for identifying differences in neuroretinal dysfunction among patients with diabetes.

Retinal thinning has been suggested to correlate with retinal dysfunction in diabetic patients.<sup>23,35,36,44</sup> Similar to previous studies, we found structural evidence of retinal neurodegeneration by the thinner GCL+IPL in diabetic patients.<sup>10,25,30,31,35</sup> We also found that diabetes differentially affects other layers of the retina. Like Vujosevic and Midena,<sup>29</sup> we detected a significantly increased thickness of the INL+OPL in patients with NPDR compared to healthy controls. This could be explained by the hypertrophy of Müller cells located in the INL, in early stages of DR,<sup>8,9,45</sup> but histologic confirmation of OCT measurements is needed. The majority of changes we detected were in the fovea, but the changes in the temporal INL+OPL extended to the parafovea. Unlike what was seen in other studies,<sup>10,29,32,33</sup> we detected an increased thickness of the inner temporal quadrant of the RNFL, and outer inferior and temporal quadrants of the ONL, in diabetic patients with moderate-to-severe NPDR. These findings were exclusive to one or two quadrants of the ETDRS grid and were proportionally smaller than the findings for the GCL+IPL and INL+OPL. As the processes of the Müller cells extend to the base of the RNFL and the ONL, we hypothesize that these changes may also be due to gliosis.<sup>8,9,45</sup> In consideration of the study's limitations, it is important to note that the Heidelberg SD-OCT built-in algorithm used to obtain the retinal thicknesses has variability and bias with off-axis acquisition in detecting subtle changes in retinal thickness.<sup>46-48</sup> Future studies should consider a higher-density volume scan and fractal-based analysis of retinal thickness, which may be able to provide

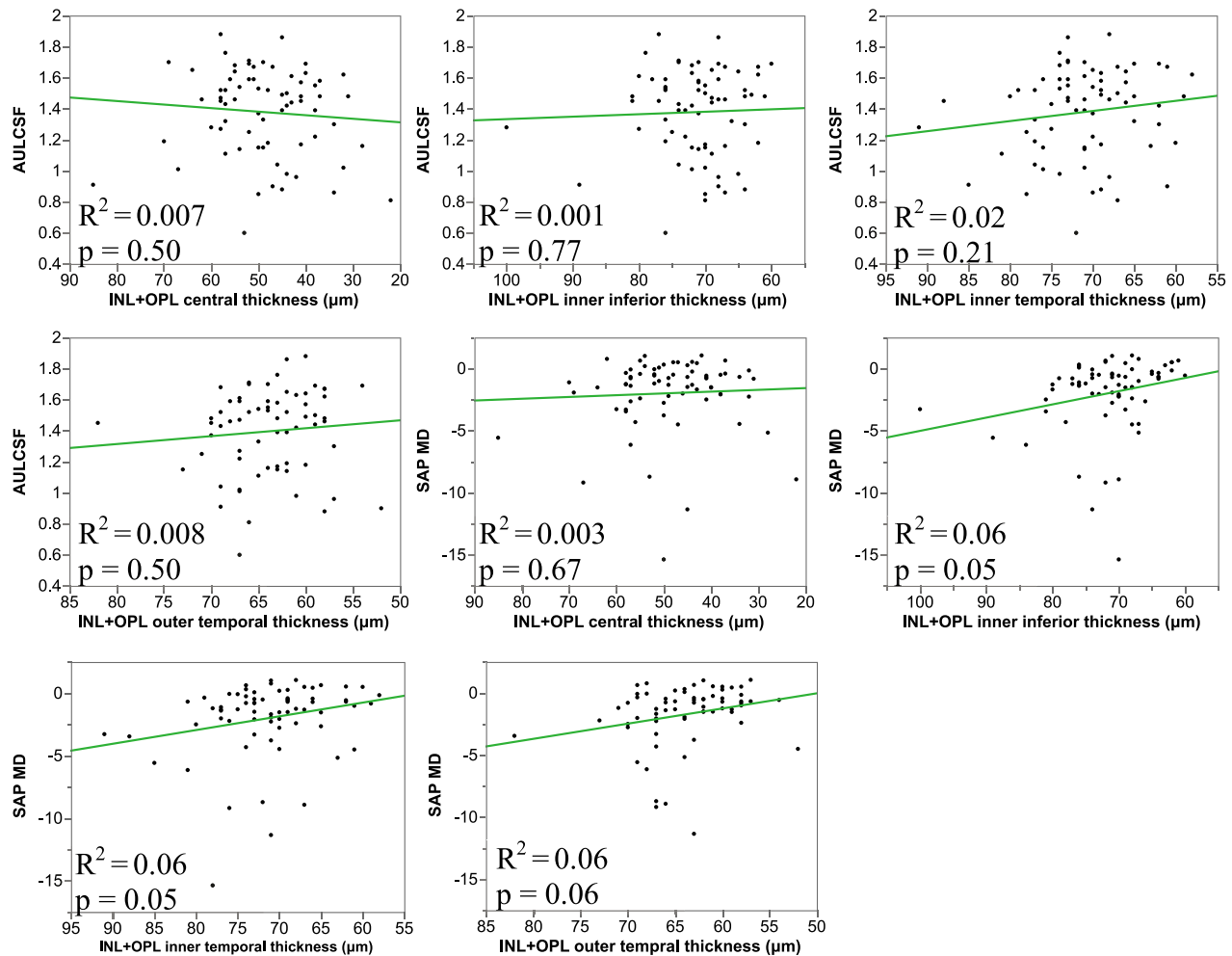


FIGURE 4. Correlations of OCT-derived INL+OPL thickness with retinal function assessed by AULCSF (contrast sensitivity) and SAP mean deviation. There were no significant correlations between increased INL+OPL thickness and functional performance.

better sensitivity and reproducibility for OCT-defined structural changes in early DR.<sup>49</sup>

In diabetes, just as in glaucoma, the correlation between function and structure may be limited,<sup>50-52</sup> as we found markedly more pronounced functional differences than structural differences among groups. It is possible that retinal function was affected before significant structural changes related to neurodegeneration occurred. Nonetheless, the importance of structural integrity is supported by the

correlations of the GCL+IPL thickness and contrast sensitivity and SAP performance. In accordance with previous studies,<sup>10,23,35,36</sup> our results support the conclusion that diabetes disrupts the inner retina, which is associated with functional evidence of neuroretinal impairment. When we evaluated patients with the most extensive GCL+IPL thinning (lower 10th percentile), we found pronounced functional impairment. These patients had MD values ranging from  $-5$  to  $-9$  dB on various visual field tests, and by glaucoma standards these

TABLE 5. Functional Analysis of Subjects With GCL+IPL Thickness Below the 10th Percentile Compared to Subjects Above the 10th Percentile

	Subjects With GCL+IPL Thickness Above 10th Percentile, $n = 67$	Subjects With GCL+IPL Thickness Below 10th Percentile, $n = 8$	<i>P</i> Value
AULCSF	1.40 (0.25)	1.10 (0.36)	0.006
SWAP 24-2			
MD (SD)	$-3.99$ (4.19)	$-8.91$ (5.21)	0.003
SAP 24-2			
MD (SD)	$-1.49$ (2.15)	$-6.01$ (5.03)	<0.0001
FDP 24-2			
MD (SD)	$-0.22$ (2.51)	$-5.25$ (6.71)	<0.0001
FDP 10-2			
MD (SD)	$-0.65$ (2.75)	$-5.16$ (5.35)	0.0002

results would be considered moderate disease and treated accordingly. Thus, there is dire need to establish similar endpoints for diabetes to test novel neuroprotective treatments.

The AST Platform qCSF method, which uses 25 forced-choice questions with three different contrast letters along its own illumination, is a new tool for rapid evaluation of contrast sensitivity in function of spatial resolution.<sup>40,41</sup> Unlike the Pelli-Robson chart, it identifies frequency-specific contrast sensitivity deficits.<sup>39,42,43</sup> Multiple studies have demonstrated that spatial contrast sensitivity is reduced in diabetic patients with and without DR.<sup>53-55</sup> The present study found that contrast sensitivity was decreased in 48% of patients with no clinical evidence of retinopathy, and was correlated with severity of retinopathy. Furthermore, we found that contrast sensitivity at the low spatial frequency of 1.5 cyc/deg was markedly different among all study groups, making it the most sensitive test for early-stage DR. Its ability to detect subtle differences among all groups could prove to be useful for screening and monitoring progression of neuroretinal impairment. Several other studies have also found that the low spatial frequency is particularly affected by diabetes.<sup>53-55</sup> Additionally, Katz et al.<sup>56</sup> found that low spatial frequencies were particularly affected, but under mesopic conditions. In general, spatial contrast sensitivity function is mediated by the magnocellular pathway at low and intermediate spatial frequencies, and the parvocellular pathway at high spatial frequencies.<sup>14,15,57,58</sup> However, with grating stimuli and varying duration of letter presentation there is a processing overlap between the two pathways.<sup>15</sup> In diabetes, where there is a generalized retinal dysfunction of the inner retina, further characterization of the multiple pathways involved in contrast processing must be evaluated to better understand the changes that occur in DR. Additionally, it is important to note that the contrast sensitivity and spatial resolution measured by qCSF involve only the foveal area, where spatial resolution is highest due to anatomic characteristics.<sup>59</sup> Nonetheless, the qCSF measurements provided the most sensitive approach to detect foveal impairment in diabetic patients in our study.

Various visual field strategies have been used to evaluate the neuroretina in patients with diabetes. Neuroretinal dysfunction has been reported by several studies: Hellgren et al.<sup>19,60</sup> using SAP; Parravano et al.,<sup>17</sup> Jackson et al.,<sup>18</sup> and Pinnila et al.<sup>61</sup> using FDP; Afrashi et al.<sup>62</sup> using SWAP; and Nilsson et al.<sup>21</sup> using RBP. In the present study we did not find a difference among diabetic patients using RBP. The high-contrast microdot stimuli are meant to stimulate no more than one receptive field,<sup>13</sup> but did not detect neuroretinal impairment differently than FDP, SWAP, or SAP. With the long test duration (approximately 18 minutes), many patients were unable to maintain focus for the entire length of the exam. SWAP, SAP, and FDP were all considerably shorter in testing duration and have built-in gaze tracking to maintain fixation. For SWAP, unlike other studies that reported on its usefulness in patients without retinopathy,<sup>61,62</sup> we found that it may be more sensitive for subtle impairment differences among patients with NPDR. In our study, the findings for SAP and FDP parallel the results of previous publications. Similar to Hellgren et al.<sup>19</sup> and Pinnila et al.,<sup>61</sup> we found that SAP and FDP were sensitive for functional impairment in diabetic patients prior to clinical evidence of retinopathy. Like Pinnila et al.,<sup>61</sup> we found that PSD, rather than MD values, were different among groups measured by FDP 24-2. The criteria for visual field changes in diabetic patients were based on the glaucoma classification,<sup>63-65</sup> so it is important to recognize how the neuroretinal pathophysiology differs between the two diseases; in glaucoma, ganglion cell death begins near the optic disc and functionally affects large areas of the retina,<sup>66</sup> but in diabetes

there may be small foci of dysfunction in the inner retina responsible for low sensitivity and the high PSD on FDP. To further delineate the retinal dysfunction in diabetes, a frequency-of-seeing curve (FOS) is needed for patients with and without DR.<sup>67,68</sup>

Only a few studies have compared two or more visual field testing strategies in patients with diabetes. Bengtsson et al.<sup>20</sup> demonstrated that SAP was superior to SWAP in detecting defects among diabetic patients with retinopathy, but patients with mild cataracts were included in the study, which may have particularly affected SWAP results.<sup>69</sup> Similarly to our study, Bengtsson et al.<sup>20</sup> concluded that ETDRS visual acuity was not as useful as perimetry testing for detecting subtle difference in patients with diabetes. In addition, Jackson et al.<sup>18</sup> found that FDP was more sensitive than SAP for detecting retinopathy and evaluating mild NPDR. We found that FDP and SAP had similar sensitivities for detecting impairment in diabetic subjects without retinopathy, but SAP was more sensitive for patients with moderate NPDR. Compared to the study by Jackson et al., we evaluated older patients who mostly had type 2 diabetes. Overall, SAP appears to detect subtle differences better than SWAP and FDP, but SWAP and FDP may be more specific for late and early stages of neuroretinal impairment, respectively. These findings suggest that different tests may be able to detect different subsets of patients, just as in glaucoma.<sup>70-72</sup>

The major advantage of the present study is that we compared six visual field testing strategies in the same cohort of diabetic patients with varying severity of retinopathy. Although the scales vary between different visual fields, our study allowed for broad comparison of clinical sensitivity among the tests. The results of this study warrant confirmation by longitudinal studies comparing AULCSF, SAP, SWAP, and FDP for progression in diabetes. We hypothesize that contrast sensitivity testing and SAP may be good potential endpoints for neuroretinal impairment, but considering that different tests may be more useful for different stages of disease, longitudinal studies would be important to determine how to best proceed with patient follow-up. For future studies, it would also be interesting to compare SAP, SWAP, and FDP by comparable units, similar to the approach by Sun et al.<sup>73</sup> for glaucoma.

One limitation of this pilot study was its relatively small sample size and cross-sectional nature. Future studies will increase sample size and balance the sex of subjects with longitudinal follow-up. Additionally, with a larger sample of patients, more sophisticated statistical techniques such as a principal components and latent class analysis may be applied to determine which visual field test is most important for clinically separating and classifying neuroretinal dysfunction in diabetic patients with various stages of retinopathy. Finally, future studies of contrast sensitivity in diabetic patients might also include color contrast sensitivity and measurements of contrast sensitivity in mesopic conditions.<sup>56,58,74-76</sup>

Late-stage DR is a debilitating disease, similar to glaucoma. Progressive visual field loss is a well-studied consequence of glaucoma and is used to define disease severity, follow progression, and adjust therapy. As contrast sensitivity and visual field loss are becoming better understood in the pathophysiology and progression of DR, qCSF and perimetry testing could serve as reliable, affordable, and accessible screening tools for the identification and treatment of diabetic patients. Just as in glaucoma, these tools could potentially be used to identify patients likely to progress and prevent the need for aggressive treatments, associated costs, and complications. However, an important question remains to be answered: What change in visual field or contrast sensitivity is clinically significant for patients with diabetes? In glaucoma,

where visual field progression has been studied for over 20 years, there is still no standard definition of visual field progression; change in MD values on SAP ranging from  $-3$  to  $-6$  dB have been used to characterize disease progression.<sup>77,78</sup> Most of the moderate NPDR patients in the present study had MD depression greater than 3 dB, and yet there is little consensus on what that means. Prospective longitudinal studies are needed to determine the clinical impact of these functional tests, and it is imperative to better understand the cellular basis of impaired retinal function in diabetes so tests can be designed to assess the pathophysiology of retinal neurovascular unit disintegration.<sup>79</sup>

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