

Diabetic Choroidopathy: Choroidal Vascular Density and Volume in Diabetic Retinopathy With Swept-Source Optical Coherence Tomography

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• **PURPOSE:** To compare choroidal vascular density (CVD) and volume (CVV) in diabetic eyes and controls, using en face swept-source optical coherence tomography (SS-OCT).

• **DESIGN:** Prospective cross-sectional study.

• **METHODS:** SETTING: Multicenter. PATIENT POPULATION: Total of 143 diabetic eyes—27 with no diabetic retinopathy (DR), 47 with nonproliferative DR (NPDR), 51 with NPDR and diabetic macular edema (DME), and 18 with proliferative DR (PDR)—and 64 age-matched nondiabetic control eyes. OBSERVATION PROCEDURES: Complete ophthalmologic examination and SS-OCT imaging. En face SS-OCT images of the choroidal vasculature were binarized. MAIN OUTCOME MEASURES: CVD, calculated as the percent area occupied by choroidal vessels in the central macular region (6-mm-diameter circle centered on the fovea), and throughout the posterior pole (12 × 9 mm). The central macular CVV was calculated by multiplying the average CVD by macular area and choroidal thickness (obtained with SS-OCT automated software). Multilevel mixed linear models were performed for analyses.

• **RESULTS:** Compared to controls (0.31 ± 0.07), central macular CVD was significantly decreased by 9% in eyes with NPDR + DME (0.28 ± 0.06 ; $\beta = -0.03$, $P = .02$) and by 15% in PDR (0.26 ± 0.05 ; $\beta = -0.04$, $P = .01$). The central macular CVV was significantly decreased by 19% in eyes with PDR ($0.020 \pm 0.005 \text{ mm}^3$, $\beta = -0.01$, $P = .01$) compared to controls ($0.025 \pm 0.01 \text{ mm}^3$).

• **CONCLUSIONS:** Choroidal vascular density and volume are significantly reduced in more advanced stages of diabetic retinopathy. New imaging modalities should allow further exploration of the contributions of choroidal vessel disease to diabetic eye disease pathogenesis, prognosis, and treatment response.

DIABETIC RETINOPATHY (DR) IS A MAJOR CAUSE OF visual loss worldwide and is estimated to affect up to 35% of patients with diabetes.¹ The pathogenesis of DR is primarily attributed to a dysregulation of the retinal vasculature, involving breakdown of the blood-retina barrier.² Recent evidence, however, also points to the presence of a diabetic choroidopathy.³⁻⁷ It has also been hypothesized that choroidal vessel abnormalities might be responsible for decreased vision in DR, even in the absence of foveal changes in the retina.⁴

The development of the enhanced depth imaging (EDI) protocol for spectral-domain optical coherence tomography (SD-OCT) has enabled detailed noninvasive imaging of the choroid. Several studies have been published to assess choroidal thickness (CT) in DR, with somewhat conflicting results, but mostly demonstrating a decrease in CT.⁸⁻¹⁸ Recently, our group described a decreased CT in eyes with proliferative DR, using swept-source OCT (SS-OCT).¹⁹ SS-OCT seems to have several advantages for the assessment of the choroid. It uses a different light source (wavelength-tunable laser) and detection method such that longer wavelengths can be used. This allows for deeper penetration and lower scattering from the retinal pigment epithelium.²⁰

In addition to CT, and to further assess the choroidal vasculature, the choroidal vascularity index (CVI) has recently been introduced and proposed as a marker for vascular health of this layer.²¹⁻²³ The CVI is obtained by binarizing EDI-OCT B-scan images, thus distinguishing lumens of choroidal vasculature from surrounding stroma. In healthy subjects, subfoveal CT was shown to be the

only significant association with CVI.²¹ In DR, a reduced CVI has been recently noted.²³

A similar index, the choroidal vascular density (CVD), can be obtained by binarizing high-resolution en face SS-OCT images. In control patients, this CVD has been shown to be significantly associated with advancing age and CT.^{24,25} CVD has been evaluated in other chorioretinal diseases, including age-related macular degeneration and central serous chorioretinopathy.^{26,27} Though there is evidence of localized choroidal microvascular changes in DR,²⁸ to our knowledge CVD has not been previously assessed in this disease.

Here, we use SS-OCT en face images to analyze CVD and introduce choroidal vascular volume (CVV) as a global index (combining CVD and CT) in eyes with diabetes as compared to eyes from healthy nondiabetic subjects.

METHODS

• **STUDY DESIGN:** This study was conducted as part of a cross-sectional, observational, multicenter study on retinal imaging performed at the Retina Service, Massachusetts Eye and Ear (MEE), Harvard Medical School, Boston, Massachusetts, USA; and the Ophthalmology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal, in collaboration with the Association for Innovation and Biomedical Research on Light and Image (AIBILI), Coimbra, Portugal. This research adhered to the tenets of the Declaration of Helsinki. The Institutional Review Boards of MEE and of AIBILI approved the study protocol. As required by law in Portugal, the Portuguese National Committee of Data Protection (CNPD) also approved this study. All included participants provided written informed consent.

• **STUDY SUBJECTS:** Consecutive diabetic patients were prospectively identified at CHUC/AIBILI and the MEE Retina Service, with the following exclusion criteria: refractive error greater than or equal to 6 diopters spherical equivalent; diagnosis of ocular hypertension or glaucoma with an optic nerve cup-to-disc ratio greater than 0.6; treatment in the 90 days prior to inclusion with laser capsulotomy, focal laser, panretinal photocoagulation, or intravitreal injections; any previous retinal surgery; diagnosis of other retinal or choroidal pathology, namely age-related macular degeneration, vitreomacular traction, epiretinal membrane, macular hole, uveitis; systemic diseases that might affect CT, such as uncontrolled hypertension, systemic lupus erythematosus, anemia, leukemia, and obstructive sleep apnea; and decreased media transparency that precluded appropriate OCT imaging.

Simultaneously, nondiabetic subjects without any diagnosed vitreoretinal disease were also included as a control group. The same exclusion criteria were applied.

• **CLINICAL STUDY PROTOCOL:** Our study protocol has been described in detail by Laíns and associates.¹⁹ Briefly, all participants underwent complete ophthalmologic examination and their medical charts were reviewed to collect clinical variables, such as age, sex, and comorbidities, among others.

Diabetic eyes were categorized according to the Early Treatment Diabetic Retinopathy Study (ETDRS),²⁹ as follows: no DR, no signs of DR; NPDR, nonproliferative DR without macular edema; NPDR + DME, NPDR with macular edema; and PDR, active proliferative DR or any previous treatment with panretinal photocoagulation (PRP).

• **SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY IMAGING:** At the same visit, all study subjects were imaged using the Topcon DRI OCT-1 Atlantis (Capelle aan den IJssel, The Netherlands).¹⁹ CT was obtained with the built-in software of the SS-OCT device (Topcon FastMap, version 9.12.003.04). User-independent thickness maps were created according to the conventional ETDRS grid.

For all subjects, an experienced, masked investigator (I.L., K.E.T., P.G., or J.G.) examined the position of the ETDRS grid, as well as the retinal and choroidal segmentations for all the obtained volume scans. Manual corrections were performed if the automated positioning or segmentation was deemed inaccurate.¹⁹

Finally, the obtained CT values in the 9 different ETDRS fields were registered. The average CT, defined as the arithmetic average of all sub-regions of the standard ETDRS grid, and the maximum CT, defined as the maximal choroidal thickness value of all sub-regions of the ETDRS grid, were recorded.

• **CHOROIDAL VASCULAR DENSITY AND VOLUME:** SS-OCT en face images of choroidal vasculature were obtained and flattened with the Bruch membrane (BM) as reference, using the en face tool included in the DRI OCT visualization software (Topcon, Tokyo, Japan). Images with substantial motion artifacts were excluded from analysis, as detailed in the Results section.

The image analysis procedure described herein is depicted in the [Figure](#). The en face images were exported every 2.6 μm from BM to the choroidal-scleral interface (CSI) and subsequently imported to ImageJ (National Institutes of Health, Bethesda, Maryland, USA) as an image stack. The image stack was converted to binary images using the auto-thresholding function of the software (ImageJ command “Image > Adjust > Threshold > Apply”) so as to distinguish the choroidal vasculature from choroidal stroma. The binarization of the en face image was done using the Otsu method, which is an automatic threshold selecting algorithm using gray-level histograms.³⁰ Noise in the resulting binarized images was then removed (ImageJ command “Process > Noise > Remove Outliers”). The signal from the optic disc, retinal blood vessels, and any minor artifact was accounted for by creating a mask from the

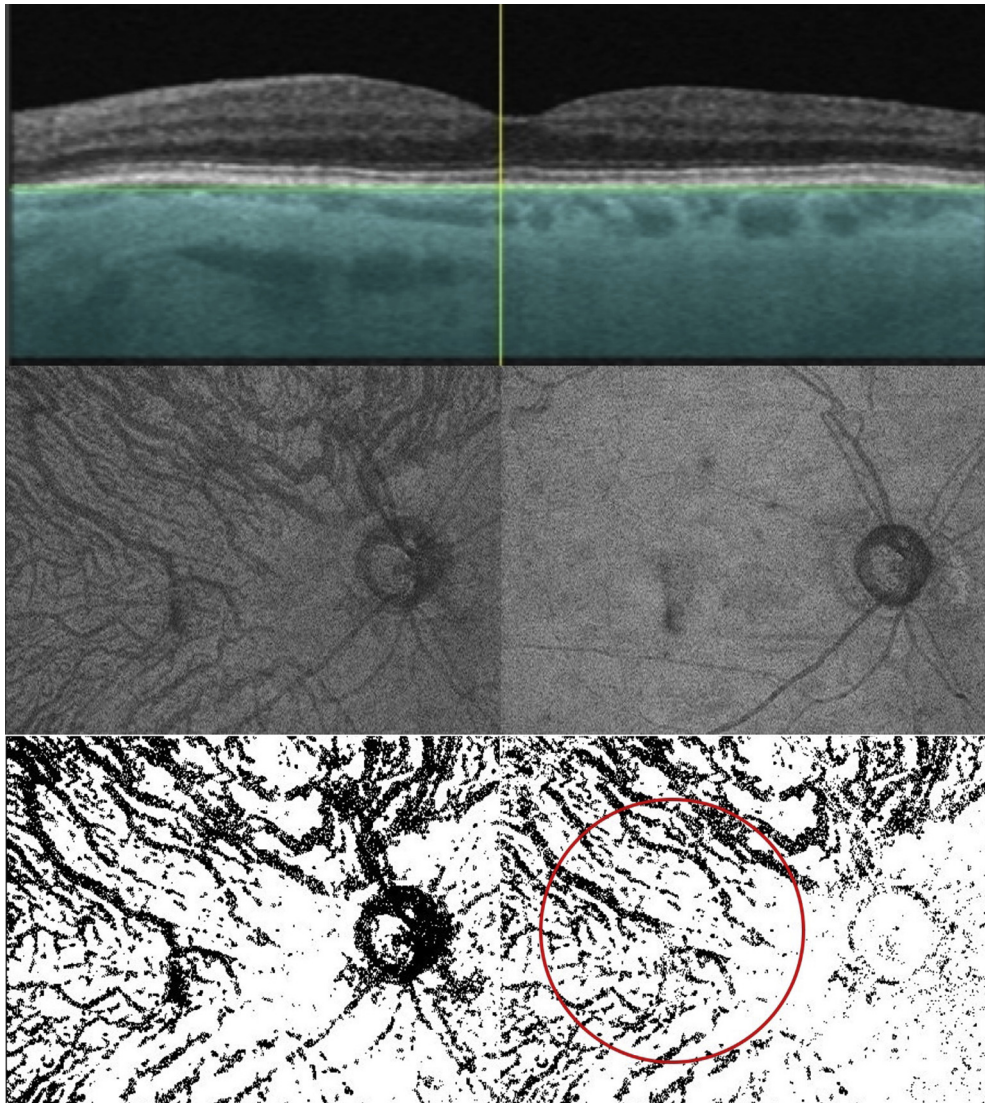


FIGURE. Representative example of swept source optical coherence tomography (SS-OCT) en face images and image processing. (Top) SS-OCT B-scan after flattening of the Bruch membrane and extraction of en face images from the Bruch membrane to the choroidal-scleral interface. (Middle left) Representative en face SS-OCT image 70 μm below Bruch membrane; the optic disc and retinal vessels are visible in addition to the choroidal vasculature. (Bottom left) The same image after binarization. (Middle right) En face SS-OCT image at the level of the Bruch membrane showing optic nerve and retinal blood vessels that is used as a mask and applied to all subsequent images. (Bottom right) The same representative image after masking. The optic nerve and blood vessels are no longer visible after masking. The red circle is 6 mm in diameter centered on the fovea; this region is used for macular subanalysis.

image slice at the level of BM, and then applying that mask to all subsequent images, thereby eliminating any signal from those locations throughout the entire image stack (ImageJ command “Process > Image Calculator > Multiply”). A 6-mm-diameter circular macular region centered on the fovea was cropped from the wider posterior pole image. Finally, the area occupied by choroidal vessels was analyzed (ImageJ command “Analyze > Measure”), and the choroidal vascular density was calculated as a percent area occupied by the choroidal vessels for each slice.

The average of the choroidal vascular densities of all image slices between the Bruch membrane and corresponding to

the maximal CT was recorded as the average CVD. The overall average CVD was calculated from the whole 12×9 -mm wider posterior pole image, whereas the average macular CVD was calculated from the 6-mm-diameter circular macular region centered on the fovea. The macular CVV volume was calculated by multiplying the average CVD by the macular area and maximal CT.

- **STATISTICAL ANALYSIS:** The study population demographic, clinical, and structural characteristics were summarized with traditional descriptive methods. Considering the inclusion of both eyes of the same patients,

TABLE 1. Demographic and Clinical Characteristics Relevant to Diabetes of Study Subjects Including Controls and Patients With Different Stages of Diabetic Retinopathy

	Controls (N = 64)	No DR (N = 27)	NPDR (N = 47)	NPDR + DME (N = 51)	PDR (N = 18)
Age, years (mean ± SD)	63.9 ± 13	68.1 ± 9.9	65 ± 9.8	64.9 ± 6.2	67.2 ± 10.1
Female sex, n (%)	43 (67%)	5 (19%)	13 (28%)	19 (37%)	7 (39%)
Left eye, n (%)	34 (53%)	13 (48%)	20 (43%)	25 (49%)	11 (61%)
Spherical equivalent (mean ± SD)	-0.43 ± 1.3	0.66 ± 1.2	0.3 ± 1.2	-0.1 ± 1.8	+0.13 ± 1.3
Intraocular pressure (mean ± SD)	16.8 ± 1.6	17.3 ± 3.5	16.7 ± 2.5	17.1 ± 2.9	16.2 ± 3.5
LogMAR BCVA (mean ± SD)	0.09 ± 0.18	0.04 ± 0.15	0.09 ± 0.2	0.3 ± 0.27	0.33 ± 0.23
Type of diabetes, n (%)					
Type 1	-	2 (7%)	4 (9%)	0 (0%)	0 (0%)
Type 2	-	25 (93%)	43 (91%)	51 (100%)	18 (100%)
Type of diabetes medication, n (%)					
Oral	-	24 (89%)	27 (57%)	14 (27%)	5 (28%)
Insulin	-	3 (11%)	4 (9%)	2 (4%)	4 (22%)
Both	-	0 (0%)	16 (34%)	35 (69%)	9 (50%)
Years with DM (mean ± SD)	-	14.8 ± 9.9	20.2 ± 7.2	17 ± 8.7	23.3 ± 13.6
Hemoglobin A1C (mean ± SD)	-	6.4 ± 0.9	8.0 ± 1.7	8.1 ± 2.1	8.1 ± 1.2
DME at time of imaging, n (%)	-	-	0 (0%)	51 (100%)	9 (50%)
Eyes that received anti-VEGF injections, n (%)	-	-	2 (4%)	9 (18%)	7 (39%)
Total number injections (mean ± SD)	-	-	5 ± 2.8	6.7 ± 7.1	2.6 ± 1.6
Months since last injection (mean ± SD)	-	-	12 ± 8.5	9.3 ± 3.7	23 ± 20
Focal laser, n (%)	-	-	3 (6%)	20 (39%)	8 (44%)
Number of sessions (mean ± SD)	-	-	1.3 ± 0.6	2.1 ± 2.0	2.3 ± 0.5
Total spots (mean ± SD)	-	-	118 ± 6.4	130 ± 110	134 ± 62
Months since last (mean ± SD)	-	-	6 ± 5	35 ± 32	23 ± 19
PRP, n (%)	-	-	-	-	13 (72%)
Number of sessions (mean ± SD)	-	-	-	-	4.9 ± 3.9
Total spots (mean ± SD)	-	-	-	-	5300 ± 4000
Months since last (mean ± SD)	-	-	-	-	24 ± 25

BCVA = best-corrected visual acuity; DM = diabetes mellitus; DME = diabetic macular edema; DR = diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; PRP = panretinal photocoagulation; VEGF = vascular endothelial growth factor.

for all analyses we used multilevel mixed-effect linear models. By definition, these models are appropriate for research designs where data for participants is nested.³¹ In this study, the units of analysis were considered to be the eyes (at a lower level), which are nested within patients—contextual/aggregate units (at a higher level).

Using these models, our statistical approach was centered on overall CVD (ie, the entire wider posterior pole image), macular CVD (ie, only the 6-mm-diameter circle centered on the fovea), and macular CVV (ie, only the 6-mm-diameter circle centered on the fovea). We started with univariate analyses for all potential covariates (Table 1) and all the parameters with a *P* value ≤ .250 were included in the initial multivariate model. Other parameters that did not meet this statistical criterion but with known clinical relevance for choroidal vasculature (such as spherical equivalent) were also considered.^{32–35} A backward (step-down) elimination procedure was then used to achieve the multivariate models presented.

All statistics were performed using Stata version 12.1 (StataCorp LP, College Station, Texas, USA), and *P* values < .05 were considered statistically significant.

RESULTS

• **DEMOGRAPHICS AND CLINICAL CHARACTERISTICS:** A total of 233 eyes of 128 subjects were included in the study (157 eyes of 90 diabetic subjects and 76 eyes of 38 control subjects). Thirty percent of the eyes were included at MEE (*n* = 71 eyes) and the remaining at CHUC/AIBILI (*n* = 162 eyes). Owing to significant motion artifacts that precluded appropriate image analysis, we then excluded 12 control eyes and 14 diabetic eyes. The overall clinical and demographic characteristics for both groups are summarized in Table 1.

TABLE 2. Summary of Choroidal Vascular Indices and Choroidal Thickness Values in Control Eyes and in Eyes With Different Stages of Diabetic Retinopathy

	Controls (N = 64)	No DR (N = 27)	NPDR (N = 47)	NPDR + DME (N = 51)	PDR (N = 18)
Average CVD (mean ± SD)	0.23 ± 0.03	0.24 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.20 ± 0.02
Average macular CVD (mean ± SD)	0.31 ± 0.07	0.34 ± 0.05	0.28 ± 0.06	0.28 ± 0.06	0.26 ± 0.05
Macular CVV (mean ± SD, mm ³)	0.025 ± 0.01	0.028 ± 0.01	0.021 ± 0.01	0.023 ± 0.01	0.020 ± 0.01
Average macular CT (mean ± SD, mm)	201 ± 82	219 ± 49	181 ± 75	189 ± 63	153 ± 45

CT = choroidal thickness; CVD = choroidal vascular density; CVV = choroidal vascular volume; DME = diabetic macular edema; DR = diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

• **CHOROIDAL VASCULAR INDICES AND THICKNESS:** Choroidal vascular indices—overall average CVD, average macular CVD, macular CVV, and choroidal thickness stratified by stage of DR—are displayed in Table 2. Compared to control eyes, the overall average CVD was significantly reduced by 6% in eyes with NPDR, by 7% in eyes with NPDR + DME, and by 12% in eyes with PDR. Similarly, the average macular CVD was significantly reduced by 9% in eyes with NPDR + DME and by 15% in eyes with PDR compared to control eyes. Finally, the macular CVV was significantly reduced by 19% in eyes with PDR compared to control eyes.

• **UNIVARIATE ANALYSES:** Univariate analysis revealed that increasing severity of diabetic retinopathy was significantly associated with decreased choroidal vascular indices. Specifically, when compared to the control group, eyes with NPDR presented decreased overall average CVD ($\beta = -0.01$, $P = .034$); eyes with NPDR + DME also showed decreased overall average CVD ($\beta = -0.02$, $P = .007$) and average macular CVD ($\beta = -0.02$, $P = .033$); and eyes with PDR presented a decreased overall average CVD ($\beta = -0.03$, $P = .005$), average macular CVD ($\beta = -0.04$, $P = .035$), and macular CVV ($\beta = -0.007$, $P = .022$) (Table 3).

We also assessed the influence of potential confounders in these outcomes. Our results revealed that sex, spherical equivalent, and intraocular pressure were not significant predictors of average CVD, average macular CVD, and macular CVV (Table 3). Increasing age was significantly associated with a reduction in our choroidal vascular indices.

A prior history of anti-vascular endothelial growth factor (anti-VEGF) injections was not a statistically significant predictor of overall average CVD, average macular CVD, or macular CVV ($P > .7$) in patients with diabetes. In the subgroup of patients with PDR, we also compared choroidal vessel indices among patients that had undergone PRP to those who had not. Our results revealed that there was no statistically significant difference in overall average CVD ($\beta = -0.02$, $P = .123$), average macular CVD ($\beta = 0.03$, $P = .227$), and macular CVV ($\beta = 0.002$, $P = .323$).

• **MULTIVARIATE ANALYSES:** Results of multivariate analyses of overall average CVD, average macular CVD, and macular CVV comparing diabetic eyes to control eyes are summarized in Table 4. As detailed, we included age in the multivariate models owing to its significance on univariate analysis. Despite not presenting a significant association in our univariate assessments, we also included spherical equivalent owing to the well-established influence of this parameter in choroidal vasculature.³²

Multivariate analysis revealed that increasing severity of diabetic retinopathy was significantly associated with decreased choroidal vascular indices. Specifically, the presence of NPDR was significantly associated with decreased overall average CVD ($\beta = -0.01$, $P = .041$). Eyes with NPDR + DME presented decreased overall average CVD ($\beta = -0.02$, $P = .009$) and average macular CVD ($\beta = -0.03$, $P = .023$). Finally, eyes with PDR showed decreased overall average CVD ($\beta = -0.02$, $P = .005$), average macular CVD ($\beta = -0.04$, $P = .011$), and macular CVV ($\beta = -0.007$, $P = .011$).

DISCUSSION

WE PRESENT A MULTICENTER CROSS-SECTIONAL STUDY USING SS-OCT en face images to analyze choroidal vascularity in different stages of DR. Building upon previous CT work, we introduce CVD and CVV as new quantitative parameters of the choroidal vasculature. Our results revealed that, even after accounting for confounding factors, diabetic eyes with DME or PDR demonstrated a reduced CVD, as compared to controls. Additionally, eyes with PDR also demonstrated reduced CVV. These findings are in line with the notion that vascular abnormalities accumulate with the severity of DR, which is reflected in the choroidal vascular indices that we assessed.

Our study adds to the growing body of literature on diabetic choroidopathy³⁶ and, to the best of our knowledge, is the first to examine CVD in different stages of DR. Much of the previous literature has focused on CT, with most studies showing some degree of choroidal thinning in

TABLE 3. Univariate Multilevel Mixed Linear Regression Analysis Comparing Choroidal Vascular Indices in Control Eyes to Eyes With Different Stages of Diabetic Retinopathy

	Average CVD			Average Macular CVD			Macular CVV		
	β Coefficient	95% CI	<i>P</i> Value	β Coefficient	95% CI	<i>P</i> Value	β Coefficient	95% CI	<i>P</i> Value
Age	-0.001	-0.002 to -0.001	<.0005*	-0.003	-0.004 to -0.002	<.0005*	-0.0005	-0.0006 to -0.0003	<.0005*
Sex	-0.0007	-0.01 to 0.01	.936	-0.007	-0.03 to 0.02	.543	-0.001	-0.005 to 0.003	.549
SE	-0.0004	-0.003 to 0.002	.762	0.003	-0.003 to 0.009	.308	0.0005	-0.0004 to 0.001	.286
IOP	-0.00003	-0.001 to 0.001	.962	0.002	-0.001 to 0.004	.181	0.0001	-0.0002 to 0.0005	.461
Left eye	-0.003	-0.007 to 0.001	.205	-0.002	-0.001 to 0.006	.650	-0.00003	-0.001 to 0.001	.951
Years with DM	-0.0001	-0.0008 to 0.0006	.756	-0.0007	-0.002 to 0.0007	.351	-0.00007	-0.0003 to 0.00001	.524
HbA1c	0.0008	-0.003 to 0.004	.656	0.002	-0.005 to 0.009	.537	0.0004	-0.0006 to 0.001	.410
DM med type	-0.0009	-0.007 to 0.006	.780	-0.009	-.02 to 0.004	.170	0.0004	-0.002 to 0.002	.721
Level of DR (as compared to controls)									
No DR	-0.002	-0.01 to 0.02	.817	0.01	-0.02 to 0.05	.375	-0.0005	-0.005 to 0.005	.859
NPDR	-0.01	-0.03 to -0.001	.034*	-0.02	-0.05 to 0.002	.073	-0.003	-0.007 to 0.001	.157
NPDR + DME	-0.02	-0.03 to 0.005	.007*	-0.03	-0.05 to -0.002	.033*	-0.003	-0.008 to 0.0007	.108
PDR	-0.03	-0.05 to 0.008	.005*	-0.04	-0.08 to -0.003	.035*	-0.007	-0.01 to -0.001	.022*

CI = confidence interval; CVD = choroidal vascular density; CVV = choroidal vascular volume; DM = diabetes mellitus; DME = diabetic macular edema; DR = diabetic retinopathy; HbA1c = hemoglobin A1c; IOP = intraocular pressure; Med = medication (insulin, oral antidiabetics, or both); NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SE = spherical equivalent.

Significant *P* values (<.05) are indicated by asterisk (*).

TABLE 4. Multilevel Mixed Multivariate Linear Regression Analysis Comparing Choroidal Vascular Indices in Control Eyes to Eyes With Different Stages of Diabetic Retinopathy

	Average CVD			Average Macular CVD			Macular CVV		
	β Coefficient	95% CI	<i>P</i> Value	β Coefficient	95% CI	<i>P</i> Value	β Coefficient	95% CI	<i>P</i> Value
Age	-0.002	-0.002 to -0.001	<.0005*	-0.002	-0.004 to -0.002	<.0005*	-0.0005	-0.0007 to -0.0004	<.0005*
SE	0.001	-0.002 to 0.003	.569	0.005	0 to 0.011	.048*	0.001	0.0002 to 0.002	.011*
Level of DR (as compared to controls)									
No DR	0.007	-0.007 to 0.02	.344	0.03	-0.004 to 0.06	.087	0.001	-0.003 to 0.005	.654
NPDR	-0.01	-0.02 to -0.0005	.041*	-0.02	-0.05 to 0.002	.078	-0.003	-0.006 to 0.009	.143
NPDR + DME	-0.02	-0.03 to -0.004	.009*	-0.03	-0.05 to -0.004	.023*	-0.002	-0.006 to 0.001	.175
PDR	-0.02	-0.04 to -0.007	.005*	-0.04	-0.08 to -0.01	.011*	-0.007	-0.01 to -0.002	.011*

CI = confidence interval; CVD = choroidal vascular density; CVV = choroidal vascular volume; DME = diabetic macular edema; DR = diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SE = spherical equivalent.

Significant *P* values (<.05) are indicated by asterisk (*).

eyes with diabetes, especially with more severe diabetic retinopathy.^{8-17,19} The exact pathophysiology of diabetic choroidopathy, as well as its relation with diabetic retinopathy, remains to be established. Diabetic choroidopathy may occur as a primary manifestation of systemic diabetic disease independent of diabetic retinopathy. Alternatively, it can be secondary to diabetic changes in the retina or treatment interventions, either ocular or systemic. Notably, we observed that in diabetic patients without diabetic retinopathy, there were no significant differences in

choroidal vascular indices when compared to controls. This suggests the possibility that diabetic choroidopathy may occur simultaneously with or as a result of diabetic retinopathy, rather than preceding it, though further study of this particular subset of patients is needed. Elucidating the relationship between diabetic choroidopathy and retinopathy is essential for a more complete understanding of diabetic eye disease.

Qualitatively, changes in the choroidal vascular architecture have been observed in histologic studies, including basement membrane thickening of small choroidal blood

vessels, luminal narrowing of capillaries, microaneurysms, dropout of the choriocapillaris, and choroidal neovascularization.^{3,36} Angiographic studies using fluorescein angiography and indocyanine green angiography have also shown irregular filling of the choriocapillaris, early hypofluorescent spots representing filling delays, and late hyperfluorescent spots representing intrachoroidal microvascular abnormalities.^{37,38}

Other studies have devised different strategies to quantitatively characterize the choroidal vasculature from OCT images in DR. For instance, one group investigated the largest hyporeflective lumen on EDI SD-OCT B-scans as a proxy for choroidal vascularity.³⁹ The authors described that the mean diameter of the largest hyporeflective lumen was reduced in patients with diabetes. However, though this was a large study, there is no mention of stratifying patients into different stages of DR. Similarly, a second group also found a reduced CVI in DR using EDI SD-OCT images, but DR stage was not specified.²³

In our study, in contrast to the prior literature, we used SS-OCT to define CVD. SS-OCT offers several advantages, particularly higher-resolution imaging of the choroid, deeper signal penetration by its longer wavelength, and automatic choroidal thickness measurements. Thus, we assert that the CVD is preferable to the CVI and largest hyporeflective lumen as an index for choroidal vascularity. We are also not aware of recent studies measuring CVD from en face SS-OCT images in the context of DR. Previous authors have used SS-OCT to qualitatively describe, in eyes with diabetes, the presence of focal narrowing of choroidal blood vessels in the Haller layer, as well as the presence of vessels ending prematurely in the superficial or middle portion of the Haller layer (referred to as “vascular stumps”).²⁸ These authors did note a statistically significant trend toward increased proportions of “vascular stumps” in eyes with more advanced stages of diabetic retinopathy.

As the CVV is derived from both the CVD and CT, it is likely that statistically significant reductions in CT contributed to significant reductions in CVV in PDR. For NPDR and NPDR + DME, the differences in CT were not statistically significant, which may be owing to a true lack of differences, or to our study design, considering the heterogeneity of these groups (subjects were not further subclassified into mild, moderate, and severe NPDR). In NPDR without DME, overall CVD was significantly reduced but macular CVD was not, suggesting that notable submacular choroidal pathology only becomes present in later stages of DR, whereas diffuse choroidal changes may already be present in early stages of DR.

Our study has several strengths. It was a multicenter study, where we prospectively recruited a relatively large number of patients; patients with DR were subdivided by stage of DR; our data were derived from high-resolution SS-OCT imaging as opposed to SD-OCT; and we

performed multivariate analysis, accounting for confounding factors, such as age and spherical equivalent. Additionally, we devised a masking procedure in the image-processing algorithm to eliminate the optic disc and retinal vessels from the binarized images. This allowed for quantification of a larger area of the choroid than what had previously been reported. Our results were consistent among different vascular indices, which serves to strengthen the internal validity of our study. Furthermore, based on our experience, en face SS-OCT may actually be superior to OCT angiography for choroidal vessel analysis, given inherent artifacts and limitations of the current OCT angiography technology.⁴⁰

Our study does have some limitations. Though there was a small number of patients with type 1 diabetes in our study, univariate and multivariate regression analyses did not reveal a statistically significant association between diabetes type and CVD or CVV indices. Despite baseline differences in some clinical characteristics, such as sex, medication type, and years with diabetes, these variables were analyzed in our regression models and were not found to have statistically significant associations with our outcomes (CVD and CVV). We did include patients who had previously undergone PRP and intravitreal anti-VEGF injections, which is known to affect CT.^{41–44} To account for this, we excluded eyes that had PRP or anti-VEGF injections 3 months prior to study inclusion, to minimize any acute changes associated with treatment, but this might have affected the results. Reassuringly, we found no statistically significant difference in the choroidal vascular indices of eyes with PDR that had undergone PRP in comparison to those who had not had received this treatment. We also found that history of anti-VEGF injection was not statistically associated with our CVD or CVV indices. It is possible that the inclusion of patients with a history of hypertension could have affected our results for CVD and CVV, which may represent a limitation of this study. In evaluation of the associations between choroidal vascular indices and stages of diabetic retinopathy, multiple tests were completed that could have led to false-positive results. Finally, our study was not adequately powered to detect statistically significant differences in the subgroup of patients with PDR with and without DME. Additional large prospective studies examining CVD and CVV through diabetic retinopathy should help us better understand the choroid's role in diabetic eye disease.

In conclusion, we used swept-source OCT en face imaging to identify reductions in choroidal vessel density and volume in diabetic eyes; increasing reductions were observed with increasing diabetic retinopathy severity. Additional work with CVV and CVD in diabetic eye disease may contribute to improved understanding of pathogenesis, prognosis, and treatment of diabetic retinopathy.

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