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Retina

Association of Male Sex and Microvascular Alterations on Optical Coherence Tomography Angiography in Diabetes

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Purpose: Epidemiologically, men have a higher incidence, severity, and progression of diabetic retinopathy (DR) than women. We investigated microvascular differences between men and women with diabetes on optical coherence tomography angiography (OCTA).

Methods: Three × 3 mm OCTA macula scans of non-diabetic and patients with diabetes were obtained. Vascular parameters included parafoveal vessel density (VD), vessel length density (VLD), and flow index (FI) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) as well as foveal avascular zone (FAZ) area and perimeter. Multivariable linear regression was used for statistical analysis.

Results: There were 1809 patients with diabetes and 217 non-diabetic participants that were included in this study. Diabetic individuals included those with no DR (n = 1356), mild non-proliferative DR (NPDR; n = 286), moderate NPDR (n = 126), and severe NPDR/proliferative DR (PDR; n = 41). Male sex was significantly associated with smaller FAZ area/perimeter and lower DCP VLD in both non-diabetic subjects and patients with diabetes. Male sex in the diabetic group was additionally associated with lower SCP VD/VLD and DCP VD. Addition of an interaction between male sex and diabetes status in the interaction analysis showed that being male and diabetic conferred increased reduction in DCP VD and VLD compared to sex-based changes in non-diabetics. Larger FAZ perimeter, lower SCP VD/VLD, and lower DCP VLD were associated with poorer visual acuity in diabetics.

Conclusions: On OCTA, male patients with diabetes may have more severe microvascular disease especially in the DCP compared to women.

Translational Evidence: Sex-based alterations in diabetic microvascular disease has the potential to influence future basic and clinical studies as well as the implementation of OCTA disease markers.

Introduction

Epidemiological studies have found evidence of sex-based risk factors for development of systemic as well as ocular diseases, such as age-related macular degeneration, primary open angle glaucoma, and diabetic retinopathy (DR).[1,2] In the diabetic population, studies indicate that women with diabetes have an elevated excess risk of cardiovascular disease, whereas men may have higher incidence of developing microvascular complications, such as retinopathy, microalbuminuria, and neuropathy.[3–5] Additionally, the male sex may be associated with increased prevalence and severity of DR at time of diabetes diagnosis and with increased risk of DR progression.[1,6–8] Some studies have postulated differences in sex hormones and inflammatory markers as contributing to these differences.[2,9]

DR is characterized by microvascular alterations, such as endothelial basement membrane thickening, pericyte loss, and increased vascular permeability.[10] On noninvasive retinal imaging with optical coherence

tomography angiography (OCTA), the consequences of these processes can be detected as microvascular changes that are present even before signs of clinical retinopathy.[11,12] Some alterations on OCTA include enlargement of the foveal avascular zone (FAZ), decreased vessel and vessel length densities, and increased avascular area in the macula of patients with diabetes that progress with increased disease severity.[13–15]

Using OCTA, investigations of sex-related alterations in non-diabetic healthy individuals have found an association between female sex and larger FAZ size.[16–18] A few studies have also suggested a relatively higher vessel density (VD) in the deep capillary plexus (DCP) of non-diabetic female subjects, with mixed results in the superficial layer.[16,19] However, there have been limited studies examining similar questions in patients with diabetes. The objective of this retrospective case-control study was to investigate the hypothesis that male patients with diabetes will exhibit more severe vascular alterations compared to female patients with diabetes on OCTA.

Methods

This retrospective observational study included a review of records in the Department of Ophthalmology at Zuckerberg San Francisco General Hospital and Trauma center (ZSFG) from March 2018 to March 2022. Study approval was obtained from the Human Research Protection Program at the University of California – San Francisco (UCSF), and from the San Francisco Department of Public Health. Our study was conducted in accordance with the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act regulations.

Study Sample

Study sample included patients without diabetes and patients with diabetes of varying severity: no DR, mild nonproliferative DR (NPDR), moderate NPDR, severe NPDR, and proliferative DR (PDR). Patients with diabetes were referred by their primary care physicians to the telemedicine-based DR screening program, which included visual acuity testing and retinal imaging with fundus photography and OCTA. Subsequently, the department's reading center graded DR severity based on the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria.[20] Patients without diabetes were recruited from the general optometry clinic where they initially presented for comprehensive eye examination for conditions including but not limited to dry eye, refractive error, and glaucoma suspect evaluation. Non-diabetic patients without comorbid retinal diseases were subsequently offered to participate in further retinal imaging with fundus photography and OCTA.

All patients underwent best corrected distance visual acuity (VA) testing using the Snellen chart, which was converted to logMAR VA.[21] Retinal imaging was performed as described below. Demographic and clinical information, such as age, sex, race, most recent hemoglobin A1c (HbA1c), and presence of comorbid hypertension were obtained by subjective report at the time imaging and verified by electronic health records when possible. Sex was recorded as binary biological sexes of male or female. Race and ethnicity data were categorized according to the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics (NCHS) guidelines.[22] Axial length was obtained from the Zeiss IOL Master 500 (Carl Zeiss Meditec, Dublin, CA, USA). Patients with diabetes with DR severity less than PDR who received prior laser photocoagulation or intravitreal injections were excluded. Other exclusion criteria for participants included any history of ocular injury or trauma, any posterior segment ocular disease, such as retinal vascular occlusion, macular degeneration, macular edema, and glaucoma, as well as incomplete demographic, clinical, or imaging information.

Image Acquisition

All participants underwent imaging with ultra-widefield fundus photography (Optos Daytona, Optos PLC, Dunfermline, UK) and Cirrus HD-OCT 5000 with AngioPlex OCT Angiography (Carl Zeiss Meditec, Dublin, CA, USA). The OCT instrument utilized an optical microangiopathy algorithm (OMAG) to analyze amplitude and phase differences between consecutive B scans.[23] We obtained 3 × 3 mm^2 scans centered on the fovea which comprised of 4 repeated B-scans at consecutive positions along the Y-axis for a total of 245 B-scans and 245 A-scans per B-scan along the X-axis. Motion tracking with real-time retinal-tracking reduced motion artifacts.[24]

In addition to the signal strength index (SSI) provided by the machine, all images were manually reviewed for quality and potential confounding ocular diseases before inclusion. We only included images with SSI of 8 or above, without motion or shadow artifacts, and without significant decentration of >20 microns from the foveal center.

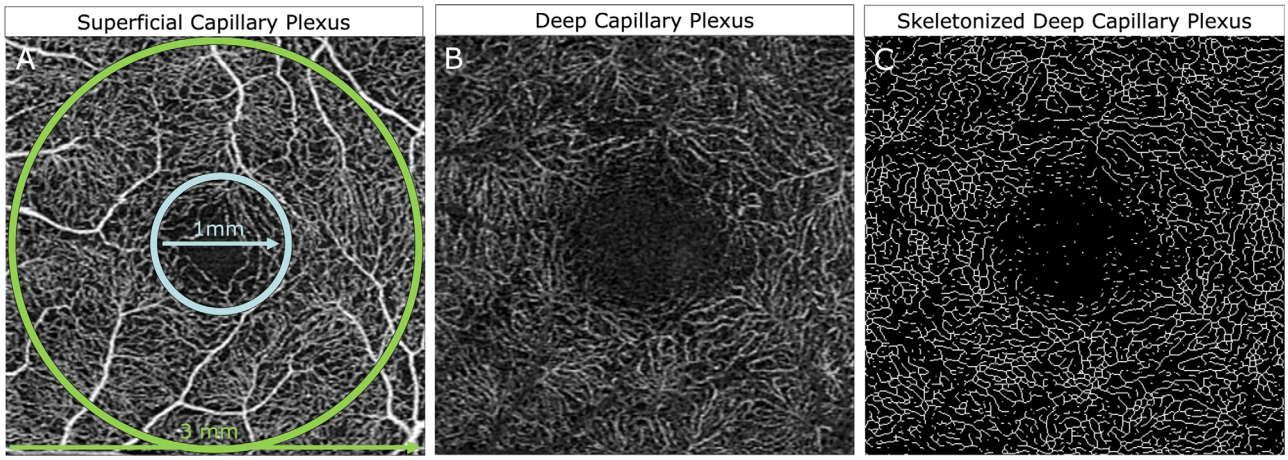

Figure 1. Optical coherence tomography angiography en face images of the superficial and deep capillary plexuses. (**A**) En face image of the superficial capillary plexus delineating the parafoveal region between annular rings of 1 mm and 3 mm in diameter. (**B**) Enface image of the deep capillary plexus. (**C**) Skeletonized angiography scan showing all vessels represented as 1 mm in diameter, which is used to calculate vessel length density.

Image Analysis

We obtained eight vascular parameters: FAZ area and perimeter as well as parafoveal VD, vessel length density (VLD), and flow index (FI) of the superficial capillary plexuses (SCP) and DCP. The parafoveal area was defined as the annulus centered on the fovea with inner and outer ring diameters of 1 mm and 3 mm, respectively (Fig. 1A). The OCTA machine segmented the SCP as the inner 70% and the DCP as the outer 30% of the inner retina, which is defined between the internal limiting membrane and 110 µm anterior to the retinal pigment epithelium.[25]

The FAZ area and perimeter are measures of the size and length of the FAZ contour, respectively. These parameters were calculated on ImageJ[26] by manually delineating the FAZ on whole retina angiograms that included both the SCP and DCP. Correction for ocular magnification using the methods described previously by Linderman et al. were applied to all FAZ parameters.[27] Manual analysis was performed by two separate graders (authors A.T.T. and Y.S.Z.) for 10% of the images to confirm intergrader reliability and completed by a single grader for all images (author A.T.T.). Graders were masked to the sex of the participant.

Vessel density was defined as the percentage of the parafoveal area occupied by blood vessels or the percentage of the parafoveal area with blood flow. VLD represented the total length of blood vessels as percentage of the total parafoveal area. In VLD, all vessels are skeletonized to be 1-pixel wide, which eliminates the disproportionate influence of larger vessels on VD and makes VLD more sensitive to changes in smaller vessels. Flow index is a measure of the average flow in the parafoveal blood vessels.

Superficial VD and VLD were calculated by the built-in CIRRUS version 11.0 software. SCP FI and DCP VD, VLD, and FI were analyzed in an automated fashion using enface angiograms in ImageJ. The ImageJ analysis started with binarization of the image with Li auto threshold to define vessel signal from background noise.[28] Auto threshold was chosen due to its repeatability and the Li method previously demonstrated high reproducibility especially in the DCP. [29] VD was calculated as per the definition above. To determine VLD, the vessels were skeletonized to be 1-pixel wide using the AnalyzeSkeleton plugin (Fig. 1C).[30] We calculated FI as the average pixel intensity above the binarization threshold in the parafoveal area of the respective capillary plexuses. The average pixel intensity represents the average decorrelation value, which is an indicator of blood velocity based on motion contrast.[31]

Statistical Analysis

Statistical analysis was performed using R version 4.[32] A two-way random effects intraclass correlation (ICC) was used to assess intergrader reliability for FAZ area and FAZ perimeter calculations. Only one eye of each patient was included in the analysis—the right eye by default unless quality metrics were not met. Student's *t*-test was performed to evaluate differences in characteristics between groups. Multivariable linear regression analyses were used to investigate the

relationship between OCTA parameters and sex as the primary analysis and secondarily between OCTA parameters and VA. An interaction term between sex and diabetes was introduced in the analysis to determine whether diabetes altered the relationship of sex and OCTA parameters. All multivariable linear regressions were adjusted for covariates of age, race/ethnicity, SSI, axial length, and hypertensive status in all patients with the addition of HbA1c, duration of disease, and retinopathy severity when appropriate in the diabetic analyses. All *P* values shown have been adjusted for multiple comparisons and controlled for false discovery rates using the Benjamini-Hochberg method.[33] A post-adjustment *P* value of < 0.05 was considered statistically significant for all analyses.

Results

Patient characteristics and demographic information are shown in Table 1 for both diabetic and non-diabetic participants. In total, 1809 patients with diabetes and 217 non-diabetic patients were included in the study. Of all the patients, 50.9% of the patients with diabetes and 46.5% of the non-diabetics were male patients. Most diabetic participants had either no DR (78%) or mild NPDR (15.6%). Overall, the quality of the images was excellent with >80% of diabetic and non-diabetic images achieving an SSI of 10. Absolute agreement ICC for grading of FAZ parameters between the two graders were >0.9 for both area and perimeter.

Graphical box-plot representations are shown in Figures 2 and 3, illustrating the median parameter values in non-diabetics and patients with diabetes of varying DR severity. In general, for both sexes, the FAZ area and perimeter increased whereas superficial and deep VD and VLD decreased with progressive retinopathy severity.

Table 2 summarizes the multivariable linear regression analysis of the relationship between male sex and OCTA parameters after adjusting for covariates of age, race/ethnicity, SSI, hypertensive status, and axial length

Table 1. Baseline Clinical Characteristics of Diabetic and Non-Diabetic Patients

	Diabetic			Non-Diabetic		
Characteristic	F ($n = 887$)	M ($n = 922$)	*P* Value[†]	F ($n = 116$)	M ($n = 101$)	*P* Value[‡]
Age, y (SD)[*]	56.6 (11.3)	54.1 (10.8)	<0.001	52.4 (14.5)	52.7 (13.5)	0.77
Race/ethnicity, *n* (%)						
NH White	43 (4.8)	107 (11.6)		17 (14.7)	31 (30.7)	
Hispanic	397 (44.8)	379 (41.1)		42 (36.2)	37 (36.6)	
NH Asian	246 (27.7)	208 (22.6)		34 (29.3)	17 (16.8)	
NH Black	49 (5.5)	74 (8.0)		8 (6.9)	6 (5.9)	
Other	152 (17.1)	154 (16.7)		15 (12.9)	10 (9.9)	
Hypertension, *n* (%)	520 (58.6)	561 (60.8)		37 (33.0)	39 (38.6)	
Signal strength, mean (SD)[*]	9.77 (0.52)	9.72 (0.57)	0.093	9.79 (0.52)	9.73 (0.60)	0.47
LogMar VA, mean (SD)[*]	0.102 (0.13)	0.079 (0.11)	<0.001	0.040 (0.068)	0.030 (0.064)	0.26
Axial length, mm (SD)[*]	23.15 (0.90)	23.79 (0.95)	<0.001	23.24 (1.02)	24.01 (1.06)	<0.001
Severity, *n* (%)						
No DR	692 (78.0)	664 (72.0)				
Mild NPDR	138 (15.6)	148 (16.1)				
Moderate NPDR	47 (5.3)	79 (8.6)				
Severe NPDR/PDR	10 (1.1)	31 (3.4)				
Disease duration, y (SD)[*]	7.83 (6.79)	7.04 (6.61)	0.012			
HbA1c, mean % (SD)[*]	7.86 (1.88)	8.40 (2.31)	<0.001			

DR, diabetic retinopathy; F, female; HbA1c, hemoglobin A1c; M, male; n, number of individuals; NH, non-Hispanic; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SD, standard deviation; VA, visual acuity.

[*]Values reported as mean (standard deviation).

[†]*P* values of Student's *t*-test between male and female patients with diabetes.

[‡]*P* values of Student's *t*-test between male and female patients without diabetes.

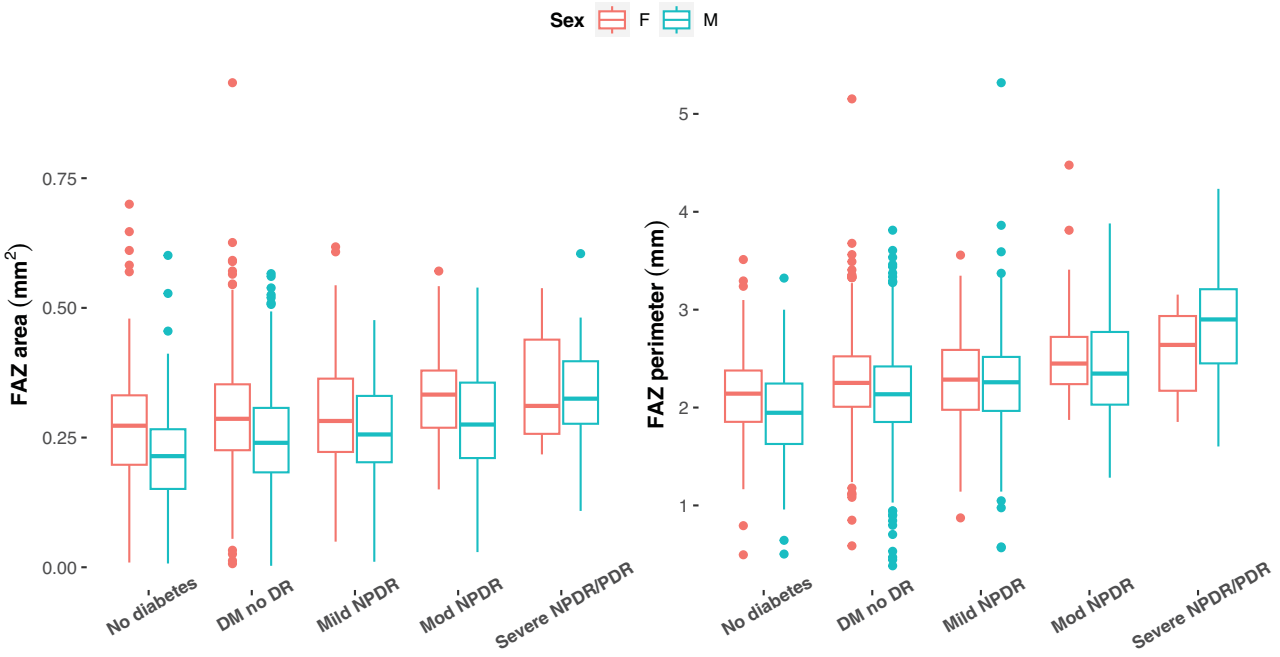

Figure 2. Boxplots of foveal avascular zone parameters across non-diabetic and diabetic individuals with varying retinopathy severity. Line inside box illustrates median. Lower and upper box boundaries show 25th and 75th quartiles, respectively. Female patients are shown in *red* and male patients are shown in *blue* for each group. DM no DR, diabetes mellitus without diabetic retinopathy; F, female; FAZ, foveal avascular zone; M, male; Mod, moderate; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

for all participants in addition to HbA1c, duration of disease, and retinopathy severity in the diabetic group. In both non-diabetic and diabetic participants, our analysis showed that men had a significantly smaller FAZ area ($B = -0.051$, $B = -0.039$, $P < 0.01$, and $P < 0.001$, respectively) and FAZ perimeter ($B = -0.18$, $B = -0.079$, $P = 0.026$, and $P < 0.01$, respectively). Male patients in both groups also demonstrated greater SCP FI ($B = 0.018$, $B = 0.011$, $P < 0.01$, and $P < 0.001$, respectively). All P values were adjusted for Benjamini-Hochberg correction.

Vessel density analysis in Table 2 showed that among non-diabetics, male sex was only associated with reduced DCP VLD ($B = -0.20$, $P = 0.036$). Among patients with diabetes, male sex was associated with reduced DCP VD and VLD ($B = -1.20$, $B = -0.48$, and $P < 0.001$, respectively) as well as SCP VD and VLD ($B = -0.41$, $B = -0.36$, and $P < 0.001$, respectively). To explore whether the impact of sex was different among diabetic versus non-diabetic patients, we then combined the two study populations and ran a multivariable analysis that included a sex by diabetes interaction term in addition to adjusting for age, race/ethnicity, hypertensive status, SSI, axial length, and DR severity (Table 3). Our analysis revealed that the effects of male sex on DCP vessel density alterations in the diabetic and non-diabetic groups were qualified by a significant interaction between male sex and diabetes (VD $B_{\text{male x diabetes}} = -0.67$, $P = 0.041$ and VLD $B_{\text{male x diabetes}} = -0.24$, $P = 0.029$) meaning that male patients with diabetes had reduced DCP VD and VLD values that were not explained by sex or diabetic status alone.

Further subgroup analysis based on DR severity found that the no DR group had similar sex-based differences as the combined diabetic group above, the mild NPDR group showed increased superficial FI and decreased deep VD and VLD, whereas the moderate NPDR group only showed decreased superficial and deep VLD (Table 4). No significant differences were found between male and female patients in the severe NPDR/PDR group.

We found that larger FAZ perimeter ($B = 0.013$, $P = 0.04$), lower SCP VD and VLD ($B = -0.003$, $B = -0.006$, $P = 0.03$, and $P < 0.001$, respectively), and lower DCP VLD ($B = -0.009$, $P = 0.04$) were correlated with higher logMAR visual acuity (worse vision) in a multivariable regression of patients with diabetes after adjusting for age, race, sex, SSI, hypertensive status, axial length, and HbA1c (Table 5). Although women had statistically significant higher logMAR vision (worse vision) than men on average, this difference was less than 5 ETDRS letters.

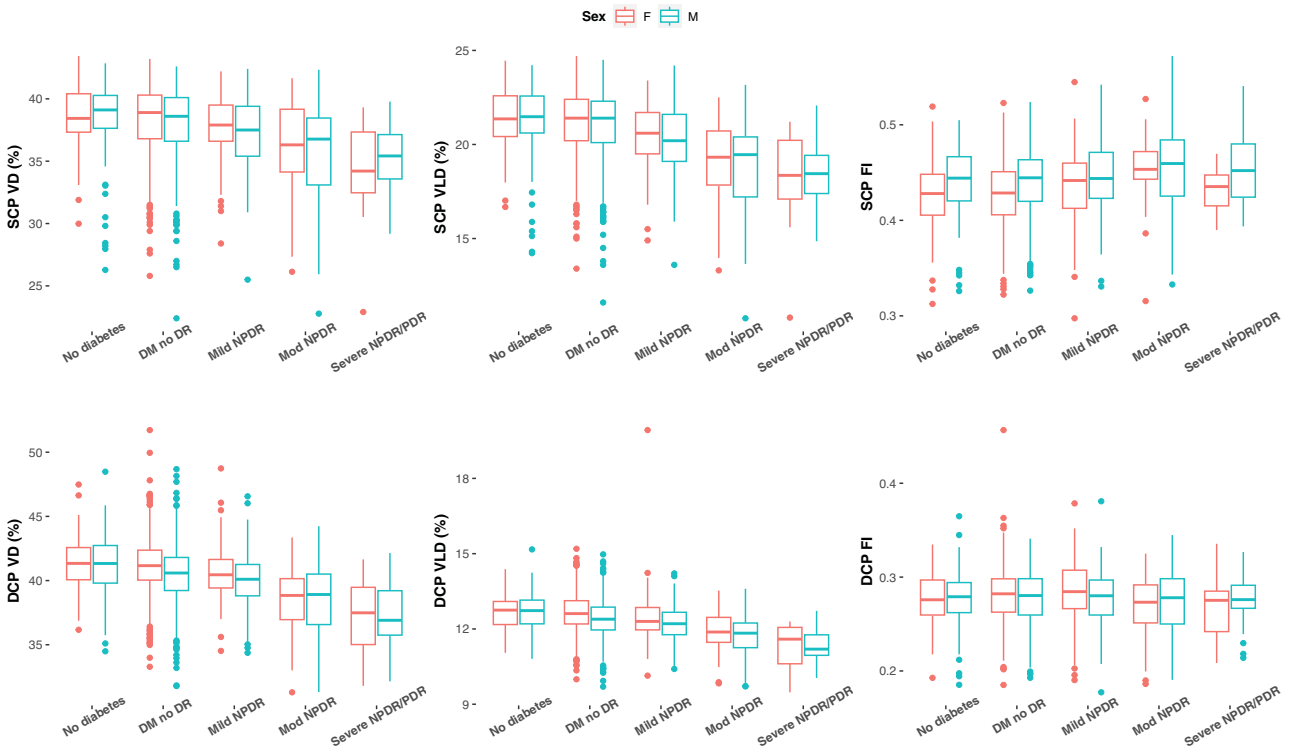

Figure 3. **Boxplots of vascular parameters across non-diabetic and diabetic individuals with varying retinopathy severity.** Line inside box illustrates median. Lower and upper box boundaries show 25th and 75th quartiles, respectively. Female patients are shown in *red* and male patients are shown in *blue* for each group. DCP, deep capillary plexus; DM no DR, diabetes mellitus without diabetic retinopathy; F, female; FI, flow index; M, male; Mod, moderate; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SCP, superficial capillary plexus; VD, vessel density; VLD, vessel length density.

Table 2. Multivariable Regression Analysis of the Association Between Male Sex and Optical Coherence Tomography Angiography (OCTA) Parameters in Diabetic and Non-Diabetic Participants

	Non-Diabetic			Diabetic		
OCTA Parameter	Estimate	95% CI	*P* Value[†]	Estimate	95% CI	*P* Value[‡]
FAZ area (mm^2)	−0.051	(−0.084 to −0.021)	**<0.01**[*]	−0.039	(−0.051 to −0.031)	**<0.001**[*]
FAZ perimeter (mm)	−0.18	(−0.31 to −0.047)	**0.03**[*]	−0.079	(−0.13 to −0.035)	**<0.01**[*]
SCP VD (%)	−0.039	(−0.70 to 0.62)	0.95	−0.41	(−0.64 to −0.18)	**<0.001**[*]
SCP VLD (%)	−0.15	(−0.55 to 0.26)	0.59	−0.36	(−0.51 to −0.22)	**<0.001**[*]
SCP FI	0.018	(0.008 to 0.028)	**<0.01**[*]	0.011	(0.008 to 0.014)	**<0.001**[*]
DCP VD (%)	−0.44	(−0.99, 0.12)	0.20	−1.20	(−1.39 to −1.00)	**<0.001**[*]
DCP VLD (%)	−0.20	(−0.37 to −0.041)	**0.04**[*]	−0.48	(−0.55 to −0.42)	**<0.001**[*]
DCP AFI	0.005	(−0.003 to 0.012)	0.35	−0.002	(−0.005 to 0.001)	0.21

CI, confidence interval; DCP, deep capillary plexus; F, female; FAZ, foveal avascular zone; FI, flow index; M, male; mm, millimeter; SCP, superficial capillary plexus; VD, vessel density; VLD, vessel length density.

[*]Statistical significance at $P < 0.05$ after Benjamini-Hochberg multiple comparison adjustment.

[†]Multivariable regression analysis adjusted for covariates of age, race/ethnicity, hypertension, signal strength, and axial length.

[‡]Multivariable regression analysis adjusted for covariates of age, race/ethnicity, hypertension, signal strength, axial length, hemoglobin A1c, duration of disease, and diabetic retinopathy severity.

Estimates of regression analyses are reported as men relative to women with negative signs indicating relatively smaller parameter means in men compared to women.

Table 3. Multivariable Regression Analysis for the Association Between Male Sex and Optical Coherence Tomography Angiography (OCTA) Parameters as Modified by the Presence of Diabetes in Patients With and Without Diabetes

	Male Sex X Diabetes Status		
OCTA Parameter	Estimate	95% CI	*P* Value
FAZ area (mm^2)	0.004	(−0.093 to 0.173)	0.84
FAZ perimeter (mm)	0.048	(−0.093 to 0.17)	0.60
SCP VD (%)	−0.171	(−0.82 to 0.48)	0.67
SCP VLD (%)	−0.071	(−0.48 to 0.34)	0.79
SCP AFI	−0.003	(−0.012 to 0.006)	0.62
DCP VD (%)	−0.669	(−1.23 to −0.11)	**0.04**[*]
DCP VLD (%)	−0.236	(−0.42 to −0.055)	**0.03**[*]
DCP FI	−0.004	(−0.011 to 0.003)	0.39

CI, confidence interval; DCP, deep capillary plexus; FAZ, foveal avascular zone; FI, flow index; mm, millimeter; SCP, superficial capillary plexus; VD, vessel density; VLD, vessel length density.

[*]Statistical significance at $P < 0.05$ after Benjamini-Hochberg multiple comparison adjustment.

All analyses were adjusted for covariates of age, race/ethnicity, hypertension, signal strength, axial length, and diabetic retinopathy severity.

Table 4. Multivariable Regression Analysis of the Association Between Male Sex and Optical Coherence Tomography Angiography (OCTA) Parameters in Diabetics of Varying Retinopathy Severity

	NoDR (*N* = 1356)		MildNPDR (*N* = 286)		ModerateNPDR (*N* = 126)		SevereNPDR/PDR (*N* = 41)	
OCTA Parameter	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
FAZ area (mm^2)	**−0.041**[*]	(−0.055 to −0.032)	−0.028	(−0.057 to 0.003)	−0.029	(−0.077 to 0.011)	−0.050	(−0.16 to 0.042)
FAZ perimeter (mm)	**−0.095**[*]	(−0.15 to −0.046)	−0.023	(−0.16 to 0.11)	−0.080	(−0.31 to 0.13)	0.48	(−0.025 to 0.98)
SCP VD (%)	**−0.38**[*]	(−0.64 to −0.13)	−0.37	(−0.93 to 0.20)	−1.32	(−2.58 to 0.073)	0.046	(−3.15 to 3.24)
SCP VLD (%)	**−0.34**[*]	(−0.49 to −0.18)	−0.30	(−0.66 to 0.0.064)	**−0.95**[†]	(−1.68 to −0.21)	−0.524	(−2.50 to 1.45)
SCP FI	**0.012**[*]	(0.009 to 0.016)	**0.011**[†]	(0.003 to 0.02)	0.006	(−0.023 to 0.011)	0.026	(−0.009 to 0.06)
DCP VD (%)	**−1.31**[*]	(−1.53 to −1.09)	**−0.84**[*]	(−1.28 to −0.39)	−0.83	(−1.86 to 0.20)	−1.48	(−4.10 to 1.14)
DCP VLD (%)	**−0.51**[*]	(−0.58 to −0.44)	**−0.39**[*]	(−0.59 to −0.21)	**−0.38**[†]	(−0.70 to −0.067)	−0.53	(−1.27 to −0.22)
DCP FI	−0.001	(−0.004 to 0.001)	−0.007	(−0.014 to 0)	0	(−0.011 to 0.011)	−0.009	(−0.039 to 0.021)

CI, confidence interval; DCP, deep capillary plexus; DR, diabetic retinopathy; FAZ, foveal avascular zone; FI, flow index; mm, millimeter; N, number of individuals; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SCP, superficial capillary plexus; VD, vessel density; VLD, vessel length density.

Statistical significance at [*]$P < 0.001$ and [†]$P < 0.05$ after Benjamini-Hochberg multiple comparison adjustment.

95% confidence interval reported in (xxx).

Estimates reported as men relative to women with negative signs indicating relatively smaller parameter values in men compared to women.

All analyses adjusted for covariates of age, race/ethnicity, hypertension, signal strength, axial length, hemoglobin A1c, duration of disease, and diabetic retinopathy severity.

Discussion

Using OCTA, we revealed significant microvascular alterations affecting non-diabetic and diabetic male patients, with diabetic male patients showing more pronounced alterations. Our study found sex-based microvascular differences, such as smaller FAZ, higher superficial flow, and lower deep VLD in non-diabetic male patients that also existed in male patients with early diabetes. However, diabetic male patients, specifically those who were early in their disease course, showed further alterations in the vascular density parameters of superficial and deep retinal capillary plexuses, suggesting potentially more severe microvascular disease in male patients with diabetes. Additionally, there may be visual acuity differences related to microvascular differences in both male and female

Table 5. Association Between logMAR Visual Acuity and Optical Coherence Tomography Angiography (OCTA) Parameters

	Diabetics		
OCTA Parameter	Estimate	95% CI	*P* Value
FAZ area (mm^2)	0.040	(−0.018 to 0.082)	0.26
FAZ perimeter (mm)	0.013	(0.003 to 0.024)	**0.04***
SCP VD (%)	−0.003	(−0.005 to −0.001)	**0.03***
SCP VLD (%)	−0.006	(−0.01 to −0.003)	**<0.001***
SCP AFI	0.067	(−0.095 to 0.228)	0.53
DCP VD (%)	−0.003	(−0.005to 0)	0.05
DCP VLD (%)	−0.009	(−0.017 to −0.002)	**0.04***
DCP FI	−0.222	(−0.42 to −0.025)	0.06

CI, confidence interval; DCP, deep capillary plexus; FAZ, foveal avascular zone; FI, flow index; mm, millimeter; SCP, superficial capillary plexus; VD, vessel density; VLD, vessel length density.

*Statistical significance at $P < 0.05$ after Benjamini-Hochberg multiple comparison adjustment.

All analyses were adjusted for covariates of age, sex, race/ethnicity, hypertension, signal strength, axial length, and hemoglobin A1c.

patients as shown by the correlation between worse vision and larger FAZ perimeter, lower SCP VD and VLD, and lower DCP VLD.

We show reduction in DCP VD and VLD in our male patients with diabetes that are beyond sex differences in non-diabetic men. Previously, Lavia et al. found in 148 eyes of 84 healthy individuals that men had lower VD in the intermediate and deep retinal layers,[16] a trend supported by the lower DCP VD and VLD in our non-diabetic male patients with only DCP VLD reaching statistical significance after adjustment of covariates and multiple comparisons (see Table 2). There may be modest vessel density differences between the sexes in healthy controls, but our interaction analysis of male sex and diabetes status suggests that male patients with diabetes have significantly disproportionate reduction in DCP VD and VLD — the diagnosis of diabetes amplified the deep VD and VLD loss in male patients beyond sex differences that may exist in healthy controls (see Table 3). Further subgroup analysis revealed that the greatest sex-related reductions in DCP VD and VLD are likely attributed to early diabetics with no DR or mild NPDR (see Table 4). However, this phenomenon may be explained by our relatively smaller sample sizes of patients with diabetes with more severe DR or by a true elimination of sex differences with progressive microvascular damage in later disease stages. A prior study investigating sex-based differences in aqueous humor inflammatory markers in patients with diabetes had also found elevated markers in male subjects with no DR that did not exist in more severe disease, which seems to support our latter explanation.[9]

In the SCP, male patients with diabetes showed increased flow, decreased VD, and decreased VLD compared to female patients with diabetes, which was similarly only significant in early disease on subgroup analysis. Our finding of increased superficial FI with decreased SCP VD and VLD in male patients may seem contradictory, but in the early course of diabetes, the relationship between vessel density measures and flow may be inversely related. Previously, Nesper et al. had shown initial increase of SCP FI with decrease of SCP VD in those with diabetes without DR compared to controls before both the FI and VD decreased in more severe DR, leading to a proposed early compensatory mechanism of flow in response to decreased vessel density.[14] Results from our non-diabetic controls who also demonstrated higher SCP FI seem to suggest that there may be differences in superficial flow between healthy male and female subjects that also exist in early diabetes. In terms of SCP VD and VLD, although our control male subjects did not show significant alterations, You et al. revealed decreased superficial VD in healthy male subjects compared to female subjects on 6 × 6 mm OCTA scans, so there may exist some pre-existing sex-based VD differences in the SCP of healthy individuals that we did not detect due to our different scan sizes, race/ethnic populations, and adjustment of covariates.[34] Thus, it is possible that the SCP VD and VLD reduction in our male patients with diabetes are influenced by both diabetes and potential pre-existing sex-related differences that are present in healthy individuals as well. Ultimately, our findings seem to suggest a stronger correlation between male sex and DCP alterations in diabetics as compared to

SCP changes, which is supported by findings in healthy individuals that suggest DCP rather than SCP as the site of more profound sex-related vascular density differences on OCTA.[35]

Taken together, our results suggest that male patients with diabetes may have more severe microvascular disease especially in the DCP layer compared to female patients, and these differences may present early before any detectable clinical retinopathy, diminishing in later stages of disease. Our findings correspond to prior epidemiological studies indicating male sex as a risk factor for the presence, severity, and progression of DR.[1,6–8] Singh et al. further showed that male patients with diabetes are more likely to develop microvascular complications involving multiple organs including DR.[5] Sex-based retinal vascular alterations in our male patients with diabetes could be a manifestation or contributor to the increased microvascular risk that male patients with diabetes face. In patients with diabetes but no DR, alterations in DCP vasculature on OCTA may occur early as a subclinical finding. [36,37] Furthermore, DCP VD and VLD have been shown to correlate with retinopathy severity as well as predict incidence and progression of DR.[13,14,38] Thus, reduced DCP VD and VLD in male patients with diabetes may both be a manifestation of more severe microvascular disease and may portend elevated risk for disease progression, corresponding to prior epidemiological studies.

In our patients with diabetes, larger FAZ perimeter, lower SCP VD and VLD, and lower DCP VLD were correlated with worse vision (higher logMar visual acuity), which may be a potential clinically important implication of FAZ, SCP, and DCP microvascular alterations. The fovea has the highest concentration of photoreceptors in the macula and it is possible that as choroidal supply may be affected by retinal vascular diseases, the FAZ area which is devoid of the retinal circulation may be an area where photoreceptors are vulnerable.[39] Reduction in SCP vessel densities especially in VLD signifies the loss of capillaries that supply the inner retina because VLD is more sensitive to changes to smaller caliber vessels. The DCP spans the areas of inner nuclear layer and outer plexiform layer, which contains interneurons that are critical for photoreceptor survival, and may contribute with the choroidal circulation to photoceptor perfusion.[40–42] Therefore, reduced flow in the DCP may be associated with worse vision due to photoreceptor alterations. Our findings are consistent with prior reports of the correlation between reduced VA and microvascular damage on OCTA, such as FAZ enlargement, as well as SCP and DCP vessel density reduction.[39,43,44] These prior reports have focused mostly on patients with diabetes with more advanced disease, whereas we show that in our population skewed toward early disease, correlations between visual acuity and OCTA parameters may still exist. However, our analysis is limited by the lack of consideration of lens status in our population as well as by the lack of clear conclusion regarding the impact of these parameters on VA as related to sex because women have larger FAZ perimeter whereas men have lower vessel density related metrics. Additionally, further investigations are needed to determine which and to what extent these parameters may predict vision loss.

Both our healthy subjects and male patients with diabetes demonstrated smaller FAZ size and lower FAZ perimeter than women. The sizes of these sex-based FAZ differences are similar in diabetics and controls, whereas diabetes status did not further modify this difference. This is consistent with prior reports of enlarged FAZ in women compared to men across several healthy populations.[16,45–48] It has been postulated that larger FAZ in women and certain ethnic groups may be related to thinner fovea, but the underlying cause and clinical implications of these differences are largely unknown.[16,49] Although enlargement of the FAZ has been associated with DR severity and estimating disease progression,[13,14,38,50] it is unknown whether sex-based FAZ differences in both healthy and diabetic individuals portend greater risks for females or whether capillary density reduction in male patients with diabetes is more impactful for disease progression. Notably, in our diabetic population, enlargement of the FAZ in female patients was seen in the DM without DR group but not in later disease when in fact the FAZ size and perimeter seemed to equalize between the sexes, suggesting that even if protective effects of a smaller FAZ existed for male patients, this effect could be lost with further DR progression. However, the lack of significance in later stages of DR can also be due to our limited sample size in those stages. Further investigations are needed to evaluate whether larger FAZ in healthy subjects and female patients with diabetes have clinical implications.

Currently, the underlying mechanisms of these sex differences are unknown, but several hypotheses have been proposed based on biological sex hormones, inflammation, and lifestyle factors. Prior studies have shown forms of estrogen to prevent endothelial dysfunction, vascular inflammation, and atherosclerosis as well as to promote vasodilatory modulators like nitric oxide, whereas androgens have been shown to be both beneficial and disruptive to vasculature function.[51–53] Additionally, there is increased risk of vascular disease such as stroke in post- versus premenopausal women and increased DR progression

during pregnancy, supporting the potential influence of sex hormones on vascular disease.[54,55] However, sex hormones are likely not solely responsible for the disproportionate microvascular disease seen in male patients with diabetes, as men continue to have elevated vascular risks even compared to women of post-menopausal age.[5] Others have proposed that an additional component of DR is metabolic dysregulation and glial cell activation leading to an inflammatory state.[56] Haq et al. have previously demonstrated higher concentrations of aqueous markers of inflammation in male patients with diabetes, indicating a heightened inflammatory state as another potential cause of more severe DR in men.[9] Finally, lifestyle choices, such as diet, exercise, and adherence to care, may further play a role in sex differences in disease.

Our study highlights the importance of considering sex in future DR study designs whether from bench side or bedside, as diabetes may affect men and women differently. As more OCTA data become available for normative DR databases and for disease prediction, sex needs to be considered as a potential confounding factor. Our results also raise further questions regarding the underlying mechanisms that drive these sex differences, which may lead to further revelations of disease pathophysiology and potential therapeutic targets. The presence of detectable quantitative microvascular changes provides a basis for further study and a noninvasive method to detect and monitor potential changes, which is often not possible in other organs where diabetic microvascular changes also exhibit a sex preference. The lower VD and VLD in men may explain or be a manifestation of the increased risk of men for DR progression from an epidemiological perspective, but whether this warrants changes in clinical practice requires continued investigations.

The strengths of our study include a large sample size that is several-fold larger than many concurrent publications and arises from a diverse urban population. To our knowledge, this is the first study evaluating sex differences in the discussed OCTA parameters in patients with diabetes, which fills a void in our understanding of diabetic microvascular changes as related to sex. We also used stringent quality control metrics to evaluate all images included in the study including both manual review and conservative SSI exclusion criteria of <8. In addition, multiple covariates were considered in our statistical analysis to minimize influence of confounders. Limitations include our study sample consisting mostly of patients with early disease and relatively fewer patients with more severe DR, which could explain the uneven distribution of men versus women in the more advanced DR groups. However, the number of individuals with moderate NPDR and severe NPDR/PDR is still comparable to many concurrent publications. Another limitation to consider is the lack of a universal gold standard method for determining image analysis thresholds and poor inter-method reproducibility between different thresholding methods.[57,58] Therefore, it is important to utilize consistent methods for analysis within the study and across studies when making comparisons. We also recognize that small variations may exist in our ocular magnification corrections of FAZ parameters using the correction formula developed by Linderman et al. for the AngioVue OCT-A system (Optovue, Inc., Fremont, CA, USA). as we used the Cirrus 5000 system in this study.[27] Last, we are cognizant that sex is a complex biological construct that may be intertwined with complicated social factors that we may have not accounted for in our study.

In conclusion, our study outlines sex-based differences in both non-diabetic controls and patients with diabetes with varying degrees of DR severity. Although men who are healthy or with early diabetes have smaller FAZ and higher superficial flow, male patients with diabetes with no DR or mild NPDR also demonstrate reduced superficial vessel density and disproportionately reduced deep vessel density. This suggests that male patients with early diabetic disease may have more significant microvascular damage compared to female patients. Additionally, microvascular alterations present in men and women may be correlated with poorer visual acuity in our population. Therefore, sex should be considered in further studies of DR, and investigating the underlying etiology of these sex differences may enrich our understanding of DR pathophysiology, elucidate potential treatments, and enhance our management and counselling of patients.

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