



Optical coherence tomography angiography analysis of the choriocapillary layer in treatment-naïve diabetic eyes

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Abstract

Purpose To evaluate the capillary flow density (CFD) of choriocapillary (CC) microvasculature using optical coherence tomography angiography (OCT-A) in diabetic eyes and the association of CFD and systemic and metabolic factors.

Methods Cross-sectional study. This study enrolled 282 eyes of 146 subjects, including 43 healthy control eyes, 56 diabetic eyes without diabetic retinopathy (DR), 43 eyes with mild nonproliferative DR (NPDR), 54 eyes with moderate NPDR, 38 eyes with severe NPDR, and 48 eyes with proliferative DR (PDR). CFD was measured in the CC layer. Clinical data were collected. Multiple linear regression analyses were performed to identify associated clinical variables.

Results CFD in the CC layer presented a downward trend with DR progression. Comparisons of CFD in the CC layer between adjacent stages of DR revealed significant differences between severe NPDR and PDR using both 3-mm and 6-mm scan patterns ($P = 0.003$, $P = 0.001$). CFD in the CC layer in DR with diabetic macular edema (DME) was less than that in DR without DME using both 3-mm and 6-mm scan patterns ($P < 0.001$, $P < 0.001$). Coronary artery disease and atherosclerosis in other locations, estimated glomerular filtration rate, and increased HbA1c were associated with CFD in the CC layer using both 3-mm and 6-mm scan patterns (all P values < 0.05).

Conclusions OCT-A revealed decreased CFD in the CC layer in the PDR stage and the presence of DME. Diabetic patients with apparently decreased CFD should be assessed carefully under general conditions.

Keywords Diabetes · Diabetic choroidopathy · Diabetic retinopathy · Optical coherence tomography · Optical coherence tomography angiography · Flow area · Flow density

Introduction

Diabetic retinopathy (DR) is a leading cause of blindness in the active population, affecting almost 35% of all diabetic patients worldwide [1]. The progression of diabetes mellitus (DM) can lead to microvascular damage, vessel occlusion, increased permeability, retinal ischemia, macular edema, and neovascularization [2]. Retinal damage, especially macular dysfunction, contributes to visual impairment. The choroid is responsible for the blood supply to the outer retina, including the retinal pigmented epithelium (RPE) and photoreceptors, and is the only source of

metabolic exchange for the avascular fovea [3]. Choriocapillary (CC) dropout, luminal narrowing and obstruction, and microaneurysms have been documented in the human diabetic choroids [4, 5]. Therefore, diabetic choroidopathy (DC) is associated with macular dysfunction. Indocyanine green angiography (ICGA) and laser Doppler flowmetry can image choroidal vascular abnormalities [6], but cannot provide any information concerning depth-resolved images of the choroid.

Optical coherence tomography (OCT) angiography (OCT-A) has recently enabled dye-less in vivo depth-resolved visualization of microvasculature. Compared with fluorescein angiography (FA) and ICGA, OCT-A allows ophthalmologists to analyze various layers of retinal and choroidal capillary networks with more accuracy and higher resolution [7, 8]. This newly developed imaging method enables more precise, quantitative evaluation of retinal and choroidal vasculature. Since the advent of OCT-A, several studies have reported changes in the microvasculature in patients with DM [9–12].

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Compared with FA, OCT-A can provide quantitative analysis of enlargement of the foveal avascular area and retinal capillary drop-off in a detailed manner. However, most of these studies had small sample sizes and did not consider the effect of previous treatment on microvascular density obtained via OCT-A. In addition, the correlation between systemic conditions and CC has not been investigated.

The primary purpose of this study was to assess CC capillary flow density (CFD) changes in patients with diabetes and healthy subjects using OCT-A. The secondary purpose was to investigate the correlation between CFD and systematic conditions.

Methods

This observational, cross-sectional study was approved by the Peking Union Medical College Hospital Institutional Review Board (S-K631) and was conducted in accordance with the Declaration of Helsinki. All participants provided signed informed consent. This study enrolled 282 eyes of 146 subjects who presented in the Department of Ophthalmology, Peking Union Medical College Hospital, Beijing, China from August 1, 2017 to May 1, 2018. All enrolled diabetic eyes were untreated.

Examinations, medical history, and biochemical markers

All treatment-naïve eyes were included in the study if there was no apparent media opacity or hard exudates affecting slit-lamp examination and OCT capture. The exclusion criteria included pregnancy, any other previous disease or treatment involving the retina or choroid, glaucoma, and other diseases that might affect CFD. Poor-quality OCT-A images with an image quality index lower than 7 were also excluded [13]. All patients underwent a comprehensive ophthalmic examination, including assessment of best-corrected visual acuity which was converted to logarithm of the minimal angle of resolution (logMAR), slit-lamp biomicroscopy, axial length measurement using IOLMaster (Version 4.08, Carl Zeiss Meditec AG, Jena, Germany), and OCT-A. The severity of DR was categorized into healthy without DM, DM without DR, mild nonproliferative DR (NPDR), moderate NPDR, severe NPDR, and proliferative DR (PDR). Similarly, the presence of diabetic macular edema (DME) was classified based on the same scale.

Clinical information, including a history of hypertension, dyslipidemia, coronary arterial diseases, and atherosclerosis in other locations, which were documented or diagnosed by physicians in Peking Union Medical College Hospital, was obtained. Hypertension was defined as a blood pressure of 140/90 mmHg or more on more than three occasions after sufficient rest on different days or anti-hypertensive treatment.

Dyslipidemia was defined as total cholesterol ≥ 200 mg/dl, triglycerides ≥ 150 mg/dl, low-density lipoprotein cholesterol ≥ 130 mg/dl, or high-density cholesterol ≤ 40 mg/dl (AU5821 automatic biochemical analyzer, Beckman Coulter K.K., Japan). Coronary arterial diseases and atherosclerosis in other locations included a positive result obtained by ultrasound, computed tomography, or magnetic resonance imaging; a history of relevant symptoms or revascularization, such as coronary artery bypass surgery or angioplasty. The laboratory data were reviewed within a time window of 1 week from the acquisition of OCT-A images. After overnight fasting, blood samples were taken, and hemoglobin A1c (HbA1c) (AU5800 automatic biochemical analyzer, Beckman Coulter K.K., Japan) and creatinine (AU5821 automatic biochemical analyzer, Beckman Coulter K.K., Japan) were measured. The value of estimated glomerular filtration rate (eGFR) value was calculated using the equation utilized in the Chronic Kidney Disease Epidemiology Collaboration [14].

OCT-A image acquisition

Eyes were imaged with an Optovue Avanti RTVue XR SD-OCT (Optovue, Fremont, California, USA). Both Angio Retina 3.0 mm and HD Angio Retina 6.0 mm, centered on fovea, were acquired in each patient. Patients also underwent OCT scans using the raster and cross-line pattern for structural information. The Avanti system employs motion correction technology and 3D projection artifact reduction. The built-in software calculates the CFD of the retinal superficial capillary plexus, retinal deep capillary plexus, and CC layer by computing the percentage area occupied by detected OCT-A-vasculature. Manual corrections were made if any scan errors were identified.

Two trained graders (JY and SX) were masked to all clinical information; these graders diagnosed and categorized DR as well as reviewed OCT-A images independently. In cases with disagreement, a third retinal specialist (YC) made the final decision.

Statistical analysis

Continuous variables are presented as the mean (standard deviation, SD). Categorical variables are given as numbers or percentages. Differences in CFD among the stages of DR and DME were evaluated with ANOVA. Pairwise comparisons were investigated with the LSD test as the post hoc tests. Pearson's correlation coefficient (r) was calculated for correlation analysis. Interscan pattern reliability was evaluated with the intraclass correlation coefficient (ICC) and 95% confidence interval (CI) based on a single rating, concordance, two-way mixed-effect model. ICC values between 0 and 0.5 indicate poor reliability, moderate reliability between 0.5 and 0.75, good reliability between 0.75 and 0.9, and excellent

reliability above 0.9. Multiple linear regression analysis was performed on all of the clinical variables (history of hypertension, dyslipidemia, coronary arterial diseases, and atherosclerosis in other locations as independent variables), and CFD in the CC layer from the right eyes of subjects was used as the dependent variable using the stepwise model with the threshold P value = 0.05 for entry and 0.10 for removal. Statistical analyses were performed using SPSS software version 25.0 (IBM-SPSS, Chicago, IL, USA). Results with $P < 0.05$ were considered statistically significant.

Results

In total, 282 eyes of 146 subjects were reviewed. The mean (SD) age of the 146 patients was 54.27 (12.59). The clinical characteristics of the diabetic patients are summarized in Table 1. For the DR severity level, 56 (19.86%) eyes had no DR, while 43 (15.25%), 54 (19.15%), 38 (13.48%), and 48 (17.02%) eyes had mild NPDR, moderate NPDR, severe NPDR, and PDR, respectively. Of those eyes, 96 (34.04%) eyes had DME. In addition, 43 (15.25%) eyes were nondiabetic control eyes. Ten eyes were excluded owing to poor OCT-A image quality.

As shown in Fig. 1, the mean (SD) CFD in the CC layer using the 3-mm scan pattern for the control, no DR, mild NPDR, moderate NPDR, severe NPDR, and PDR was 65.66 (0.50), 63.50 (0.61), 62.07 (0.67), 58.13 (1.04), 57.31 (1.13), and 53.79 (0.67), respectively (Fig. 1). The pairwise

comparisons of CFD using the 3-mm scan pattern between mild NPDR and moderate NPDR and between severe NPDR and PDR showed significant differences ($P = 0.001$, $P = 0.003$), while the other comparisons between adjacent stages showed no significant differences. Moreover, using the 6-mm scan pattern, the mean (SD) CFD values in the CC layer were 68.33 (0.40), 67.46 (0.54), 66.48 (0.54), 62.59 (0.84), 61.42 (0.80), and 58.19 (0.60) for the control, no DR, mild NPDR, moderate NPDR, severe NPDR, and PDR, respectively (Figs. 1 and 2). The pairwise comparisons of CFD using the 6-mm scan pattern between mild NPDR and moderate NPDR and between severe NPDR and PDR showed significant differences ($P < 0.001$, $P = 0.001$), while the other comparisons between adjacent stages showed no significant differences. With the exception of the PDR stage, the Pearson correlation test revealed a significant correlation between 3-mm and 6-mm scan patterns in the CC layer in each DR stage. The ICC (95% CI) of the 3-mm scan pattern and the 6-mm scan pattern in the CC layer was 0.889 (0.864–0.991), which presents good reliability.

CFD with and without DME in the CC layer at various stages of DR is shown in Table 2. Using the 3-mm scan pattern, the mean (SD) CFD values for DR without DME and DR with DME group were 62.39 (0.58) and 53.98 (0.60) in the CC layer, respectively. The pairwise comparisons of CFD using the 3-mm scan pattern between the DR without DME group and DR with DME group showed significant differences ($P < 0.001$), while the other comparisons between adjacent groups showed no significant differences. In addition, using the 6-mm scan pattern, the mean (SD) CFD values for DR without DME and DR with DME group were 66.19 (0.49) and 58.80 (0.48) in the CC layer, respectively (Fig. 1). The pairwise comparisons of CFD using the 6-mm scan pattern between the DR without DME group and DR with DME group showed a significant difference ($P < 0.001$), while the other comparisons between adjacent groups showed no significant differences.

In the multiple linear regression analysis results after controlling for age, gender, severity of DR, and axial length of eyes, the CFD in the CC layer using both 3-mm and 6-mm scan patterns was significantly associated with the axial length of eyes ($P < 0.001$, $P < 0.001$), coronary artery disease ($P = 0.015$, $P = 0.001$), atherosclerosis in other locations ($P = 0.006$, $P < 0.001$), eGFR ($P < 0.001$, $P < 0.001$), and HbA1c ($P = 0.001$, $P < 0.001$) (Table 3). CFD in the CC layer using 6-mm scan patterns was also significantly associated with dyslipidemia ($P = 0.042$).

Discussion

In the present study, we described the application of OCT-A to study diabetic eyes in different stages and evaluated the relationship between CC flow density and a variety of measures

Table 1 Clinical characteristics of diabetic patients

	Diabetic participants
Number of patients	103
Female, n (%)	63 (61.17)
Age, years (SD)	54.27 (12.59)
Axial length, mm (SD)	23.24 (1.02)
Choroidal thickness, μm (SD)	284.47 (84.71)
Dyslipidemia, n (%)	66 (64.08)
Coronary artery disease, n (%)	20 (19.41)
Atherosclerosis in other locations, n (%)	50 (48.54)
Hypertension, n (%)	49 (47.57)
eGFR, ml/min/1.73 m ² (SD)	90.45 (25.52)
Hemoglobin A1c, %	8.09 (1.76)
Diabetes treatment	
Oral hypoglycemic agent, n (%)	63 (61.17)
Insulin, n (%)	5 (4.85)
Insulin and oral hypoglycemic agent, n (%)	25 (24.27)
Use of anti-hypertensive medication, n (%)	45 (43.69)
Use of anti-coagulant medication, n (%)	38 (36.89)
Use of lipid-lowering medication, n (%)	46 (44.66)

eGFR, estimated glomerular filtration rate; SD, standard deviation

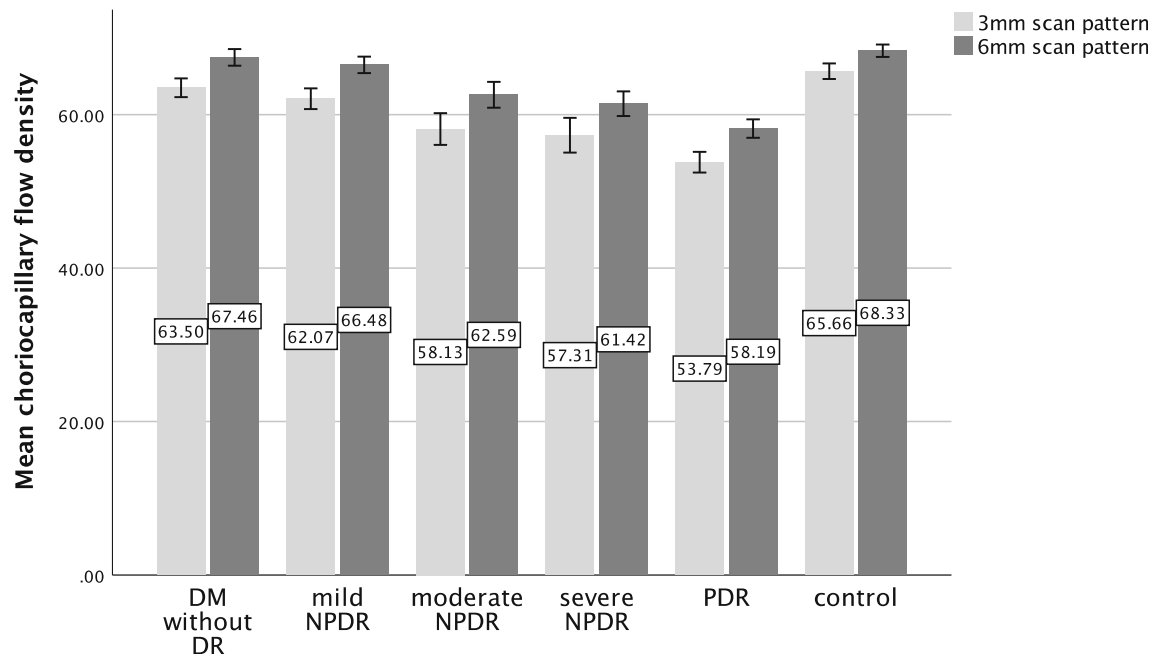


Fig. 1 Choriocapillary flow density in the choriocapillary layer. Bar graph of mean choriocapillary flow density in the choriocapillary layer with 95% confidence intervals. The mean choriocapillary flow density

shows a decreasing trend with the progression of diabetic retinopathy. The mean choriocapillary flow density in the 6-mm scan pattern is greater than that in the 3-mm scan pattern

with systemic and metabolic vascular factors. Our study suggested that OCT-A is a quantitative, noninvasive tool to evaluate the CC microvasculature in diabetic eyes.

We reported several significant findings that might advance our understanding of DC. First, this study showed that the CFD in the CC layer significantly decreased with worsening

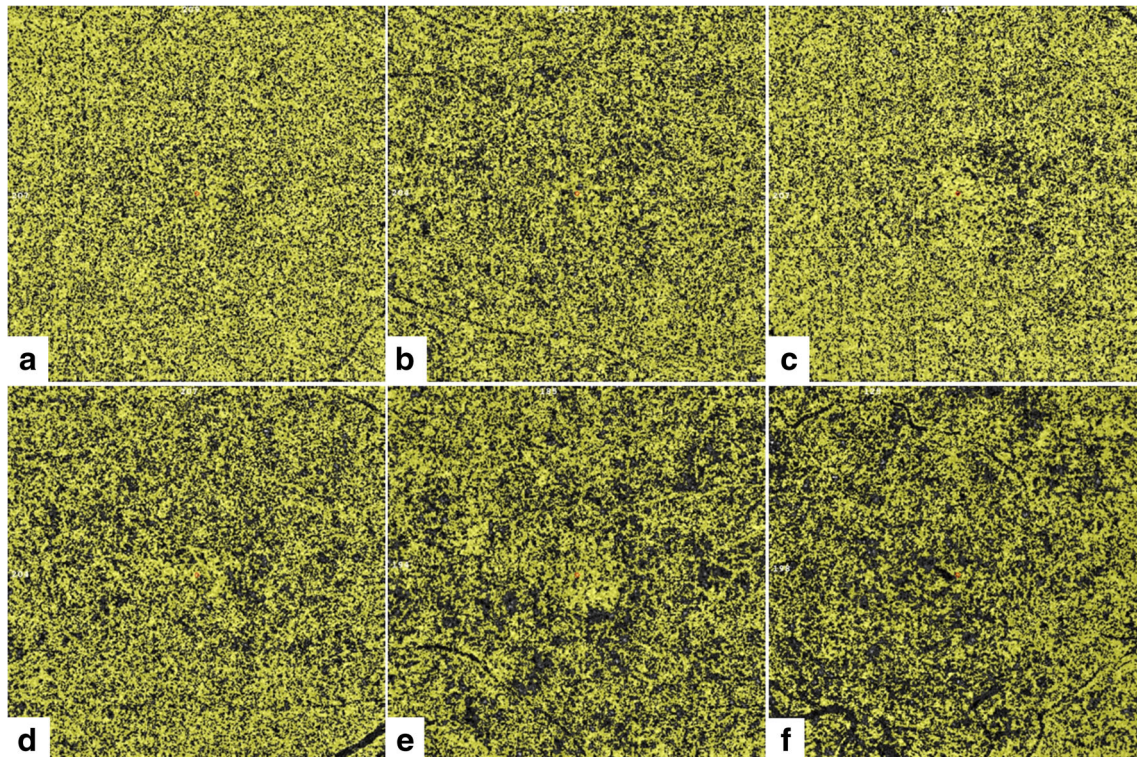


Fig. 2 Nonsegmented OCT-A images at CC layer. Representative OCT-A images in the CC layer of a healthy eye (a), a diabetic eye without DR (b), an eye with mild NPDR (c), an eye with moderate NPDR (d), an eye

with severe NPDR (e), and an eye with PDR (f). Areas of choriocapillary flow are shown here in yellow. Reduced flow areas are present in eyes with severe NPDR and PDR

Table 2 Choriocapillary flow density with and without diabetic macular edema in the choriocapillary layer at various stages of diabetic retinopathy, mean (standard deviation)

	Without diabetic macular edema			With diabetic macular edema		
	Eye, <i>n</i>	3-mm scan pattern	6-mm scan pattern	Eye, <i>n</i>	3-mm scan pattern	6-mm scan pattern
Mild NPDR	43	62.07 (4.41)	66.48 (3.54)	0	N/A	N/A
Moderate NPDR	36	61.09 (5.65)	64.53 (5.02)	18	52.20 (7.68)	58.71 (6.63)
Severe NPDR	10	63.88 (2.87)	66.63 (2.17)	28	54.97 (6.49)	59.56 (4.27)
PDR	0	N/A	N/A	48	53.79 (4.67)	58.19 (4.17)

severity of DR at the stage of moderate NPDR and PDR and with the presence of DME, which suggested that the severity of DC did not entirely agree with the severity of DR. Second, more importantly, we showed that a variety of systemic vascular and metabolic vascular factors, including dyslipidemia, eGFR, and HbA1c, were associated with CC CFD, especially using the 6-mm scan pattern. Third, although the CFD in the CC layer using a 6-mm scan pattern was greater than that using a 3-mm scan pattern, the two scan areas showed good reliability and correlation. The differences in CFD values between the two scan patterns might result from differences in resolution and distinguishability. However, one of the advantages of the 6-mm scan pattern was that the CFD was associated with more systemic and metabolic factors.

Compared with previous studies, the present study used a built-in software to assess the CFD in the CC layer in treatment-naïve diabetic eyes of a larger sample size in an automated manner with 3D projection artifact reduction. In contrast, in a cross-sectional study of 44 eyes, Nesper et al. used OCT-A without 3D projection artifact reduction to compare the nonflow area in the CC layer in diabetic eyes and similarly found an increased nonflow area in diabetic eyes [11]. A projection artifact reduction algorithm is more likely to provide reliable results [15]. Choi et al. also reported CC flow impairment qualitatively using a swept-source OCT-A prototype, and motion errors were not corrected [9]. Our study provided CC flow density quantitative results, and motion errors were corrected using the built-in algorithm, which made

measurements more reliable. In addition, Conti et al. reported a decreased trend of CFD in the CC layer in 99 diabetic eyes among which some had been treated [10]. Since the effect of treatment including photocoagulation and anti-vascular endothelial growth factor (anti-VEGF) injection on OCT angiograms of the CC layer have not been thoroughly studied and interpreted, we enrolled diabetic eyes with no previous treatment. However, compared with the aforementioned studies, the present study came to a generally similar conclusion as follows: decreased CFD in the CC layer in diabetic eyes. Alternatively, Carnevali et al. used OCT-A to assess CC in only 25 diabetic eyes without DR and found no significant difference in the vessel density of CC between diabetic patients and healthy subjects [12]. In contrast, our study revealed a downward trend of CFD in the CC layer with worsening DR severity.

Histopathological studies of diabetic eyes reported degeneration of the CC layer with loss of endothelial cells, obstruction and choroidal aneurysms, and RPE degeneration, ultimately resulting in choroidal neovascularization [16]. As DR progresses, the level of VEGF increases, especially in eyes with diffuse diabetic DME and PDR [17–19]. DME, partially resulting from a relatively high level of VEGF, as well as the stage of PDR, which was related to neovascularization resulting from VEGF, suggests similar significant pathophysiologic alterations [17, 18]. In our study, a significant decrease in the CFD of the CC layer was observed in eyes in the PDR stage and in eyes with DME. Therefore, decreased CFD in the CC layer was associated with the VEGF level. However, Conti

Table 3 Multivariate analysis of age, gender, severity of diabetic retinopathy, and axiallength adjusted factors of systemic conditions associated with choriocapillary flow density in diabetic patients

	3-mm scan pattern β , (95% CI)	<i>P</i>	6-mm scan pattern β , (95% CI)	<i>P</i>
Dyslipidemia	1.757, (−1.881, 5.395)	0.340	2.729, (0.107, 5.352)	<i>0.042</i>
Coronary artery disease	−3.206, (−5.766, −0.645)	<i>0.015</i>	−3.118, (−4.964, −1.273)	<i>0.001</i>
Atherosclerosis in other locations	−4.158, (−7.086, −1.299)	<i>0.006</i>	−4.716, (−6.827, −2.605)	<i>< 0.001</i>
Hypertension	−1.321, (−3.463, 0.820)	0.223	0.217, (−1.326, 1.761)	0.78
eGFR	0.107, (0.060, 0.153)	<i>< 0.001</i>	0.072, (0.039, 0.106)	<i>< 0.001</i>
Hemoglobin A1c, %	−1.287, (−1.866, −0.708)	<i>< 0.001</i>	−1.199, (−1.616, −0.782)	<i>< 0.001</i>

eGFR, estimated glomerular filtration rate

Significant *P* value (< 0.05) in italic text

et al. reported no significant difference was found in CFD in the CC layer between the NPDR and PDR groups [10]. The present study with a larger sample size and NPDR classification strongly suggested that CFD in the CC layer in eyes with PDR was indeed less than that in eyes with NPDR.

Furthermore, in the multivariate analyses, low CFD in the CC layer was associated with coronary artery disease, atherosclerosis in other locations, and HbA1c and eGFR levels. Previous clinical studies revealed that DR severity was positively associated with TC, TG, and LDL-C and negatively associated with HDL-C [20, 21]. Similarly, in our study, the severity of DC showed a similar correlation with lipid metabolism [22]. Notably, CFD in the CC layer using a 6-mm scan pattern rather than a 3-mm scan pattern was associated with dyslipidemia. Therefore, evaluation of CFD in the CC layer with a larger scan area was recommended because it presented more correlations with systemic conditions. In addition, DM is a disease concerning inflammation [23, 24], and coronary artery disease and atherosclerosis in other locations also involve inflammation [25]. Therefore, we investigated the relationship between artery diseases and DC. We found that DC is associated with coronary artery disease and atherosclerosis in other locations using both 3-mm and 6-mm scan patterns. In addition, a previous study reported that the production of VEGF secondary to renal glomerular injury might increase the VEGF level in systemic circulation, which might lead to the progression of DC [26]. In the present study, decreased CFD in the CC layer was associated with increased eGFR. Moreover, there is no established relationship between DC and the level of HbA1c, and our study indicates a positive association of the severity of DC and the level of HbA1c, which suggests that several hyperglycemia-triggered metabolic pathways lead to abnormalities in the retinal capillary bed as well as in the CC bed [27, 28]. For other systemic factors, hypertension was reportedly positively associated with the prevalence and progression of DR [29]. However, in our study, no clear association between hypertension with DC was established. Different histological structures of the retinal capillaries and CC might contribute to different correlations with hypertension. In summary, diabetic patients with apparently decreased CFD should be assessed carefully under general conditions.

We acknowledge several limitations of this study. First, the lack of follow-up data did not allow us to analyze the role of HbA1c for a longer period. Second, large-sample longitudinal studies are warranted for the evaluation of the relationship between systemic and metabolic factors and DC onset and progression. Third, the OCT-A scan area was limited to the central macula, which does not represent the entire CC layer. Fourth, the relationship between CFD in the CC layer and some factors, including choroidal thickness and smoking history, were not analyzed. Different studies presented conflicting results on the choroidal thickness in diabetic eyes [18],

which made it difficult to explain the relationship between CFD in the CC layer and choroidal thickness. Similarly, the impact of tobacco smoking on choroid is uncertain [30, 31]. And the calculation of cumulative smoking exposure requires exact parameters which are difficult for ophthalmic patients to recall sometimes, while semiquantitative metrics might introduce bias [32]. Therefore, the relationship between smoking history and CFD in the CC layer was not investigated in the present study.

In conclusion, we demonstrated decreased CC flow density associated with DR severity using OCT-A in diabetic patients compared to that in healthy controls. The CC flow density showed a correlation with systemic metabolic and vascular factors. Diabetic patients with lower CFD in the CC layer should be evaluated under general condition. OCT-A has the potential to be used in prospective studies and interventional trials, to deepen our understanding of DC in a noninvasive manner.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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