

Glycemic Status Modifies Retinal Structure–Function Coupling in Type 2 Diabetes Without Clinically Detectable Diabetic Retinopathy

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Article

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Abstract

Background/Objectives:

To investigate whether glycemic status modifies the association between retinal microvascular density and retinal function in patients with type 2 diabetes mellitus (T2DM) without clinically detectable diabetic retinopathy (DR).

Subjects/Methods:

This cross-sectional study included 103 patients (206 eyes) with T2DM without clinically detectable DR. Optical coherence tomography angiography quantified parafoveal vessel density (PFVD) and foveal avascular zone circularity, while retinal function was assessed using the RETeval-derived DR score. Multivariable generalized estimating equation models evaluated the associations of HbA1c and PFVD with retinal function and their interaction, accounting for inter-eye correlation. Sensitivity analyses examined HbA1c thresholds of 7.0%, 7.5%, 8.0%, and 8.5%.

Results

Higher HbA1c was associated with a higher DR score ($\beta = 0.67$, 95% CI, 0.28–1.06; $P < 0.001$), whereas higher PFVD was associated with a lower DR score ($\beta = -0.29$, 95% CI, -0.49 to -0.09 ; $P = 0.0027$). A significant HbA1c $\times$ PFVD interaction was observed ($\beta = 0.13$, 95% CI, 0.03–0.23; $P = 0.0048$), indicating attenuation of the inverse PFVD–retinal function association with increasing HbA1c. At the 8.5% threshold, lower PFVD was associated with higher DR scores in patients with HbA1c $< 8.5\%$ ($\beta = -0.44 \pm 0.10$, $P < 0.001$), but not in those with HbA1c $\geq 8.5\%$ ($\beta = 0.02 \pm 0.27$, $P = 0.96$; P for interaction = 0.019).

Conclusions

Glycemic status modifies the relationship between retinal microvascular density and retinal function before clinically detectable DR. Combining quantitative vascular imaging with objective functional assessment may help identify early diabetic retinal dysfunction.

Introduction

Diabetic retinopathy (DR) has traditionally been regarded as a microvascular complication of diabetes mellitus [1, 2]. Increasing evidence, however, indicates that retinal neurodegeneration and neurovascular dysfunction develop before clinically detectable retinal vascular lesions become apparent [3–5]. These early abnormalities involve disruption of the retinal neurovascular unit, consisting of retinal neurons, glial cells, endothelial cells, and pericytes, suggesting that functional impairment may precede overt structural manifestations of diabetic retinopathy [6, 7].

Optical coherence tomography angiography (OCTA) has enabled the identification of subtle retinal microvascular alterations in diabetic patients without clinically visible diabetic retinopathy [8–10]. Reduced parafoveal vessel density (PFVD) and abnormalities of the foveal avascular zone have consistently been reported during this preclinical stage [9–11]. Likewise, electrophysiological studies using RETeval have demonstrated delayed implicit times, reduced amplitudes, and elevated DR scores before funduscopically detectable retinopathy develops [12–14]. These observations indicate that both structural and functional biomarkers are capable of detecting early retinal injury.

Despite these advances, previous studies have largely investigated retinal microvascular abnormalities and retinal dysfunction independently. Consequently, an important question remains unanswered: how retinal structural alterations are translated into functional impairment during the earliest stage of diabetic retinal disease. More importantly, whether this structure-function relationship is influenced by chronic glycemic status has not been systematically investigated.

Experimental studies have demonstrated that chronic hyperglycemia induces oxidative stress, mitochondrial dysfunction, endothelial injury, inflammation, and impairment of neurovascular coupling [15–17]. These pathological changes may alter the retina's ability to compensate for microvascular insufficiency, thereby modifying the relationship between retinal capillary loss and electrophysiological dysfunction. We therefore hypothesized that hyperglycemia does not merely worsen retinal damage, but fundamentally modifies the relationship between retinal microvascular integrity and retinal function.

Accordingly, this study investigated the interaction between parafoveal vessel density measured by OCTA and retinal functional impairment assessed by the RETeval-derived DR score in diabetic patients without clinically visible diabetic retinopathy. We further examined whether this interaction remained robust across multiple clinically relevant HbA1c thresholds using sensitivity and stratified analyses. We therefore hypothesized that glycemic status would modify the relationship between retinal microvascular integrity and retinal function during the preclinical stage of diabetic retinal disease. Specifically, we sought to determine whether the association between OCTA-derived PFVD and RETeval-derived retinal dysfunction differed across levels of glycemic burden.

Methods

Study Design and Participants

This single-center cross-sectional observational study was approved by the Research Ethics Committee of Taipei City Hospital, Taiwan (TCHIRB-11304001), and followed the tenets of the Declaration of Helsinki. We consecutively enrolled patients with type 2 diabetes mellitus from the ophthalmology outpatient clinic of Taipei City Hospital Zhongxiao Branch between January 1, 2025 and April 30, 2026. Written informed consent was obtained from each patient. All participants underwent comprehensive ophthalmic examinations, including best-corrected visual acuity, slit-lamp biomicroscopy, intraocular pressure measurement, dilated fundus examination, optical coherence tomography angiography (OCTA), and RETeval electroretinography.

Patients with any clinically visible diabetic retinopathy were excluded after independent grading by two retinal specialists according to the International Clinical Diabetic Retinopathy Disease Severity Scale. Additional exclusion criteria included previous retinal laser treatment, intravitreal injection, vitreoretinal surgery, glaucoma, retinal vascular diseases, age-related macular degeneration, high myopia (spherical equivalent < -6.0 D), significant media opacity, poor-quality OCTA images (signal strength < 7), and severe systemic diseases that could affect retinal or vascular function.

Ocular and Systemic Assessments

Retinal Microvascular Assessment via OCTA

Retinal microvascular parameters were evaluated using a spectral-domain OCTA device (Cirrus HD-OCT 6000; Carl Zeiss Meditec, Dublin, CA, USA) utilizing an optical microangiography (OMAG) algorithm. Imaging was performed using a 3×3 mm scan pattern centered on the fovea, consisting of 350×350 A-scans. Parafoveal vessel density (PFVD) and foveal avascular zone (FAZ) circularity were automatically quantified within the superficial capillary plexus (SCP) using the integrated Zeiss AngioPlex, version 11.5 software. The SCP segmentation boundary was automatically defined from the internal limiting membrane (ILM) to the inner plexiform layer (IPL). The "parafoveal" region was defined according to the standard Early Treatment Diabetic Retinopathy Study (ETDRS) grid, encompassing the annular zone located between the inner 1-mm and outer 3-mm concentric rings centered on the fovea. To ensure reproducibility, all examinations were performed by a single experienced technician under dilated conditions. To minimize potential structural-functional cross-talk or light-stress bias, OCTA imaging was consistently performed after the RETeval electroretinography examination on the same day. Strict quality control was enforced for all OCTA scans. Images with a signal strength < 7 , significant motion artifacts (e.g., vessel misalignment or banding), segmentation errors, or decentration were excluded from the analysis. Two independent retinal specialists reviewed all segmentation profiles, and any missegmentations were manually corrected or excluded. In our center, the intra-session repeatability of PFVD measurements using this protocol demonstrated an intraclass correlation coefficient (ICC) of 0.92, 95% CI: 0.88–0.95.

Retinal function was evaluated under non-mydriatic conditions using the handheld RETeval system (LKC Technologies, Gaithersburg, MD, USA) with skin-adhesive Sensor Strip electrodes. The primary functional outcome was the RETeval-derived diabetic retinopathy (DR) score. Individual photopic and scotopic electroretinographic parameters were also analyzed according to the ISCEV extended protocols [18].

Systemic cardiometabolic, vascular, and autonomic assessments were performed on the same day as the ophthalmic examinations. Glycemic status was determined by glycated hemoglobin (HbA1c, %) measured using high-performance liquid chromatography (Bio-Rad Laboratories, Inc.). Renal function was assessed by the estimated glomerular filtration rate (eGFR), calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation.

Systemic vascular function was evaluated after 10 minutes of supine rest using a digital vascular screening system (VS-1500AE; Fukuda Denshi Co., Ltd., Tokyo, Japan), which automatically measured the Cardio-Ankle Vascular Index (CAVI) and Ankle-Brachial Index (ABI). Cardiac autonomic function was assessed using a 5-minute heart rate variability (HRV) recording (WE-MDANSA-01; Wegene Technologies Inc., Taiwan), from which time-domain (SDNN and RRIV) and frequency-domain (LF, HF, and LF/HF ratio) parameters were derived.

Statistical Analysis

Continuous variables were summarized as mean $\pm$ standard deviation (SD), and categorical variables as counts and percentages. Patient-level continuous variables were compared using independent-samples t-tests, and categorical variables using Pearson's χ^2 tests. For eye-level outcomes, generalized estimating equations (GEEs) with an exchangeable working correlation structure were used to account for within-participant correlation between fellow eyes. The RETeval-derived diabetic retinopathy (DR) score served as the primary outcome variable. Model 1 evaluated the independent associations of age, sex, HbA1c, parafoveal vessel density (PFVD), and FAZ circularity with DR score. Model 2 additionally incorporated an interaction term (HbA1c $\times$ PFVD) to determine whether glycemic status modified the relationship between retinal microvascular integrity and retinal function. To assess the robustness of the interaction, sensitivity analyses were performed by dichotomizing HbA1c at four clinically relevant thresholds (7.0%, 7.5%, 8.0%, and 8.5%). Separate stratified GEE models were subsequently constructed within

each glycemic subgroup to evaluate whether the association between PFVD and retinal function differed according to metabolic status. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Two-sided P values < 0.05 were considered statistically significant.

Results

Baseline Characteristics

A total of 103 patients with type 2 diabetes mellitus without clinically visible diabetic retinopathy were included in this study. Participants were stratified according to an HbA1c threshold of 7.5%, resulting in 69 patients with good glycemic control and 34 patients with poor glycemic control (Table 1). The two groups were comparable with respect to age, sex distribution, body mass index, blood pressure, duration of diabetes and serum cholesterol levels. Patients with $HbA1c \geq 7.5\%$ had a significantly higher triglyceride concentrations, and higher serum creatinine levels than those with $HbA1c < 7.5\%$.

Table 1
Baseline Characteristics of the Study Participants Stratified by Glycemic Status (HbA1c < 7.5% vs. ≥7.5%)

Variable	Total (n) Mean ± SD	HbA1c < 7.5% (n) Mean ± SD	HbA1c ≥ 7.5% (n) Mean ± SD	p value
Participants	103	69	34	—
Clinical Characteristics				
Male, n (%)	55 (53.4)	36 (52.2)	19 (55.9)	0.72
Age (years)	65.29 ± 10.26	64.71 ± 10.80	66.47 ± 9.04	0.42
DM duration (years)	13.67 ± 7.40	13.03 ± 5.89	14.91 ± 8.96	0.07
BMI (kg/m ²)	25.67 ± 4.60	25.68 ± 4.91	25.66 ± 4.07	0.99
SBP (mmHg)	130.62 ± 19.06	129.18 ± 19.68	133.41 ± 17.71	0.31
DBP (mmHg)	76.52 ± 9.62	76.34 ± 9.84	76.88 ± 9.39	0.80
Spherical Equivalent (D)	-1.48 ± 3.13	-1.76 ± 2.33	-0.91 ± 2.04	0.069
Serum Biomarkers				
HbA1c (%)	7.26 ± 1.37	6.54 ± 0.42	8.71 ± 1.34	< 0.001**
Total cholesterol (mg/dL)	148.42 ± 32.25	148.56 ± 26.55	148.14 ± 41.52	0.96
Triglycerides (mg/dL)	105.68 ± 55.06	95.86 ± 40.27	122.56 ± 71.56	0.03*
Creatinine (mg/dL)	0.93 ± 0.27	0.88 ± 0.15	1.03 ± 0.39	0.04*
eGFR (mL/min/1.73m ²)	77.57 ± 19.38	80.13 ± 11.55	73.18 ± 18.31	0.046*
Urinary Biomarkers				
Creatinine (mg/dL)	91.28 ± 18.04	90.17 ± 18.17	93.32 ± 18.55	0.57
Microalbumin (mg/L)	62.29 ± 15.33	60.44 ± 10.22	65.71 ± 13.40	0.39
Retinal Parameters				
Parafoveal VD (PFVD)	16.10 ± 3.12	16.43 ± 1.99	15.45 ± 2.57	0.035*
FAV Circularity	0.62 ± 0.12	0.63 ± 0.09	0.61 ± 0.09	0.38
DR Score	20.26 ± 3.83	19.46 ± 2.49	21.88 ± 2.97	< 0.001**
Macrovascular Parameters				
CAVI	8.29 ± 1.41	8.45 ± 1.18	7.94 ± 1.57	0.25
ABI	1.07 ± 0.16	1.09 ± 0.10	1.03 ± 0.16	0.03*
Autonomic Function				
Sympathetic RRIV	0.17 ± 0.095	0.16 ± 0.07	0.172 ± 0.11	0.596
SDNN (ms)	23.16 ± 2.43	22.85 ± 2.21	24.90 ± 3.37	0.587
Balance	1.49 ± 1.40	1.41 ± 1.08	1.60 ± 1.46	0.496
Sudomotor Feet (%)	52.86 ± 17.14	53.88 ± 14.37	50.82 ± 13.82	0.304
Data are presented as mean ± standard deviation (SD) or number (percentage), as appropriate. Patient-level continuous variables were compared using independent-samples t-tests, while categorical variables were analyzed with the chi-square test. Eye-level retinal parameters were compared using generalized estimating equations to account for inter-eye correlation; FAZ, foveal avascular zone; ABI, ankle-brachial index; BMI, body mass index; CAVI, cardio-ankle vascular index; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; RRIV, R-R interval variation; SBP, systolic blood pressure; SDNN, standard deviation of normal-to-normal intervals; VD, vessel density. *p < 0.05 **p < 0.01				

Despite the absence of clinically detectable diabetic retinopathy, patients with poorer glycemic control already demonstrated evidence of subclinical target-organ dysfunction. Specifically, they exhibited significantly lower parafoveal vessel density, lower ankle-brachial index, reduced estimated glomerular filtration rate, and substantially higher RETeval-derived DR scores than patients with better glycemic control (Table 1), suggesting that chronic hyperglycemia is associated with simultaneous retinal, vascular, and renal impairment before clinically apparent diabetic retinopathy develops.

Retinal Electrophysiological Function

Under photopic flicker stimulation, both 16- and 32-Td/s responses demonstrated prolonged implicit times together with significantly reduced amplitudes, indicating impaired cone-mediated retinal function. Light-adapted recordings further showed delayed a-wave and b-wave implicit times, whereas baseline pupil diameter was significantly smaller in patients with HbA1c $\geq 7.5\%$.

Scotopic recordings revealed similar functional deterioration. Patients with poorer glycemic control demonstrated reduced dark-adapted b-wave amplitudes together with prolonged dark-adapted a-wave latency, suggesting that retinal dysfunction involved both rod- and cone-mediated pathways. Collectively, these findings indicate widespread retinal functional impairment despite the absence of clinically detectable diabetic retinopathy (Supplementary Table 1).

Correlation Between Glycemic Status, Retinal Structure, and Function

Correlation analyses demonstrated that higher HbA1c levels were associated with higher DR scores ($r = 0.30$, $p < 0.01$) and lower parafoveal vessel density ($r = -0.23$, $p < 0.01$), whereas lower parafoveal vessel density was associated with higher DR scores ($r = -0.17$, $p < 0.05$). These findings support a close relationship between chronic hyperglycemia, retinal microvascular rarefaction, and early retinal dysfunction before clinically detectable diabetic retinopathy develops.

Hyperglycemia Modifies the Structure-Function Relationship

Multivariable GEE regression analysis demonstrated that both HbA1c and PFVD were independently associated with the RETeval-derived DR score after adjustment for age, sex, and FAZ circularity (Table 2). Higher HbA1c was associated with a higher DR score ($\beta = 0.67$, $P < 0.001$), whereas higher PFVD was associated with a lower DR score ($\beta = -0.29$, $P = 0.0027$). Importantly, the HbA1c $\times$ PFVD interaction was statistically significant ($\beta = 0.13$, $P = 0.0048$), indicating that the inverse association between PFVD and DR score became weaker with increasing HbA1c.

Table 2
Generalized Estimating Equation Regression Models Analyzing the Independent and Interactive Effects of HbA1c and Parafoveal Vessel Density on DR Score.

Variable	Model 1			Model 2		
	Main Effects Model			Interaction Model		
	Beta	SE	P value	Beta	SE	P value
Constant	28.22	3.09	< 0.001**	43.63	6.25	< 0.001**
Sex (Male vs. Female)	0.12	0.52	0.82	0.10	0.51	0.85
Age	-0.13	0.02	< 0.001**	-0.13	0.02	< 0.001**
HbA1c	0.67	0.20	< 0.001**	-1.32	0.73	0.07
PFVD	-0.29	0.10	0.0027**	-1.33	0.38	0.0047**
FAZ Circularity	0.45	2.16	0.84	1.25	2.14	0.56
PFVD $\times$ HbA1c	--	--	--	0.13	0.05	0.0048**
Regression coefficients (Beta) and standard errors (SE) were estimated using generalized estimating equations (GEE) to account for the inter-eye correlation within individual participants. Female is the reference group for the Sex variable. Abbreviations: FAZ, foveal avascular zone; DR Score, diabetic retinopathy score; GEE, generalized estimating equation; HbA1c, glycated hemoglobin; PFVD, parafoveal vessel density; SE, standard error. **p < 0.01						

Sensitivity analyses using HbA1c thresholds of 7.0%, 7.5%, 8.0%, and 8.5% demonstrated that dichotomized HbA1c status remained independently associated with the DR score across all four thresholds (Table 3). The largest effect estimate was observed at the HbA1c $\geq 7.5\%$ threshold ($\beta = 2.10 \pm 0.53$, $P < 0.001$), with statistically significant associations also observed at the 7.0%, 8.0%, and 8.5% thresholds. PFVD remained inversely associated with DR score across these models.

Table 3

Impact of Binary HbA1c Categorization on DR Score Prediction: Sensitivity Analysis Across Various Cut-off Points Using Generalized Estimating Equation Regression.

Variable	Model 1 (Cut-off $\geq 8.5\%$)			Model 2 (Cut-off $\geq 8.0\%$)			Model 3 (Cut-off $\geq 7.5\%$)			Model 4 (Cut-off $\geq 7.0\%$)		
	Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	Beta	SE	P value
Constant	33.18	2.61	< 0.001	33.36	2.60	< 0.001	33.01	2.54	< 0.001	33.51	2.62	< 0.001
Sex (Male vs Female)	0.53	0.71	0.45	0.53	0.86	0.54	0.51	0.94	0.59	0.53	0.96	0.58
Age	-0.13	0.03	< 0.001**	-0.13	0.03	< 0.001**	-0.14	0.02	< 0.001**	-0.13	0.03	< 0.001**
PFVD	-0.32	0.10	0.0086**	-0.33	0.10	0.0058**	-0.32	0.09	0.0064**	-0.35	0.10	0.0030**
FAZ Circularity	0.69	2.19	0.75	0.83	2.18	0.70	1.21	2.13	0.57	0.86	2.20	0.70
HbA1c Categorization												
$\geq 8.5\%$ vs. < 8.5%	1.95	0.81	0.02*									
$\geq 8.0\%$ vs. < 8.0%				1.57	0.61	0.01*						
$\geq 7.5\%$ vs. < 7.5%							2.10	0.53	< 0.001**			
$\geq 7.0\%$ vs. < 7.0%										1.01	0.51	0.049*
Regression coefficients (Beta) and standard errors (SE) were estimated using generalized estimating equations (GEE) to account for the inter-eye correlation within individual participants. Female is the reference group for the Sex variable. P values less than 0.05 indicate statistical significance. Abbreviations: DR Score, diabetic retinopathy score; GEE, generalized estimating equation; HbA1c, FAZ, foveal avascular zone; glycated hemoglobin; PFVD, parafoveal vessel density; SE, standard error. *p < 0.05 **p < 0.01												

Stratified GEE analyses further evaluated the association between PFVD and DR score across prespecified HbA1c thresholds (Table 4). At the HbA1c threshold of 8.5%, lower PFVD was strongly associated with higher DR scores among participants with HbA1c < 8.5% ($\beta = -0.44 \pm 0.10$, $P < 0.001$), whereas no significant association was observed among those with HbA1c $\geq 8.5\%$ ($\beta = 0.02 \pm 0.27$, $P = 0.96$). The difference between the two PFVD slopes was statistically significant (β for interaction = 0.54, $P = 0.019$). At the HbA1c threshold of 8.0%, PFVD remained inversely associated with DR score among participants with HbA1c < 8.0% ($\beta = -0.35 \pm 0.12$, $P = 0.003$), whereas the association did not reach statistical significance among those with HbA1c $\geq 8.0\%$ ($\beta = -0.26 \pm 0.17$, $P = 0.14$); the interaction between HbA1c status and PFVD was not statistically significant ($\beta = 0.097$, $P = 0.74$). At the 7.5% threshold, PFVD was inversely associated with DR score in the HbA1c < 7.5% subgroup ($\beta = -0.38 \pm 0.12$, $P = 0.002$), but not in the HbA1c $\geq 7.5\%$ subgroup ($\beta = -0.24 \pm 0.16$, $P = 0.14$), with no significant interaction ($\beta = 0.13$, $P = 0.61$). At the 7.0% threshold, PFVD was inversely associated with DR score in both HbA1c < 7.0% ($\beta = -0.39 \pm 0.14$, $P = 0.007$) and HbA1c $\geq 7.0\%$ ($\beta = -0.28 \pm 0.13$, $P = 0.04$) subgroups, and the interaction was not statistically significant ($\beta = 0.062$, $P = 0.11$). Overall, statistically significant effect modification was demonstrated only at the HbA1c 8.5% threshold, whereas the other prespecified thresholds did not show significant differences between PFVD slopes.

Table 4
Stratified GEE Analysis of the Association Between PFVD and DR Score Across HbA1c Thresholds

HbA1c 8.5% Threshold Subgroups				
	HbA1c < 8.5%		HbA1c ≥ 8.5%	
Predictor	Beta ± SE	P value	Beta ± SE	P value
Sex (Male vs. Female)	0.50 ± 0.53	0.34	-2.77 ± 2.26	0.22
Age (years)	-0.12 ± 0.03	< 0.001	-0.26 ± 0.11	0.02
PFVD (%)	-0.44 ± 0.10	< 0.001	0.02 ± 0.27	0.96
FAZ Circularity	1.46 ± 2.17	0.50	-10.36 ± 12.02	0.39
HbA1c × PFVD Interaction Beta = 0.54 P value = 0.019*				
HbA1c 8.0% Threshold Subgroups				
	HbA1c < 8.0%		HbA1c ≥ 8.0%	
Predictor	Beta ± SE	P value	Beta ± SE	P value
Sex (Male vs. Female)	0.55 ± 0.59	0.35	-2.67 ± 1.25	0.033
Age (years)	-0.13 ± 0.03	< 0.001	-0.16 ± 0.06	0.007
PFVD (%)	-0.35 ± 0.12	0.003	-0.26 ± 0.17	0.14
FAZ Circularity	0.14 ± 2.48	0.95	0.30 ± 5.09	0.95
HbA1c × PFVD Interaction Beta = 0.097 P value = 0.74				
HbA1c 7.5% Threshold Subgroups				
	HbA1c < 7.5%		HbA1c ≥ 7.5%	
Predictor	Beta ± SE	P value	Beta ± SE	P value
Sex (Male vs. Female)	0.21 ± 0.58	0.72	-0.64 ± 1.10	0.56
Age (years)	-0.14 ± 0.03	< 0.001	-0.13 ± 0.06	0.04
PFVD (%)	-0.38 ± 0.12	0.002	-0.24 ± 0.16	0.14
FAZ Circularity	1.38 ± 2.44	0.57	1.43 ± 4.73	0.76
HbA1c × PFVD Interaction Beta = 0.13 P value = 0.61				
HbA1c 7.0% Threshold Subgroups				
	HbA1c < 7.0%		HbA1c ≥ 7.0%	
Predictor	Beta ± SE	P value	Beta ± SE	P value
Sex (Male vs. Female)	0.17 ± 0.74	0.81	-0.19 ± 0.78	0.81
Age (years)	-0.17 ± 0.03	< 0.001	-0.08 ± 0.04	0.03
PFVD (%)	-0.39 ± 0.14	0.007	-0.28 ± 0.13	0.04
FAZ Circularity	0.69 ± 3.09	0.82	1.48 ± 3.22	0.65
HbA1c × PFVD Interaction Beta = 0.062 P value = 0.11				
Data are presented as regression coefficients (Beta) ± standard errors (SE). All regression coefficients were estimated using generalized estimating equations (GEE) accounting for within-participant inter-eye correlation. P for interaction represents the statistical difference between the PFVD slopes in the two HbA1c strata and was estimated using an interaction term in the full cohort. Female was used as the reference category for sex. Abbreviations: DR Score, diabetic retinopathy score; GEE, generalized estimating equation; HbA1c, glycated hemoglobin; FAZ, foveal avascular zone; PFVD, parafoveal vessel density; SE, standard error. * p < 0.05				

Figure 1 illustrates the adjusted relationship between parafoveal vessel density and REtEval-derived DR score according to HbA1c status. Among eyes from participants with HbA1c < 8.5%, lower parafoveal vessel density was significantly associated with higher DR scores (adjusted $\beta = -0.44$, SE = 0.10, P < 0.001). In contrast, no significant association was observed among those with HbA1c ≥ 8.5% (adjusted $\beta = 0.02$, SE = 0.27, P = 0.956). These findings are consistent with effect modification of the PFVD–DR score association by glycemic status.

Discussion

The principal finding of this study was that glycemic status modified the association between retinal microvascular density and retinal function in patients with type 2 diabetes without clinically detectable diabetic retinopathy. Higher HbA1c was independently associated with greater retinal functional impairment, whereas the inverse association between PFVD and DR score became weaker as HbA1c increased. In particular, at the HbA1c threshold of 8.5%, lower PFVD was strongly associated with higher DR scores in participants with HbA1c < 8.5%, whereas this association was not observed in those with HbA1c ≥ 8.5%. These findings suggest that the relationship between retinal microvascular integrity and retinal function is not uniform across levels of glycemic burden.

Our findings extend previous observations that retinal microvascular, neural structural, and functional abnormalities may emerge with different patterns during the early stages of diabetes. Multimodal imaging studies have demonstrated retinal vascular abnormalities in patients with diabetes without clinically detectable diabetic retinopathy, while structural studies have identified early neuroretinal changes in the absence of visible vascular lesions [19–22]. Importantly, multimodal OCT and OCTA assessment in patients with type 2 diabetes without diabetic retinopathy has shown that macular vascular parameters are associated with selected measures of visual function and multifocal electroretinographic responses, although these structure–function relationships are not uniform across retinal parameters [9]. Together, these findings suggest that retinal microvascular and functional abnormalities do not necessarily evolve in parallel and that their relationship may vary during the preclinical stage of diabetic retinopathy. Our results extend this concept by demonstrating that glycemic status itself modifies the association between retinal microvascular density and retinal functional impairment.

Chronic hyperglycemia is an established driver of diabetic microvascular complications, and intensive glycemic control has been shown to reduce the risk of diabetic retinopathy and other microvascular outcomes [23–25]. Diabetes also affects the retinal neurovascular unit, which integrates neuronal, glial, and vascular responses to metabolic demand [6, 16–18]. Neurovascular dysfunction and retinal neurodegeneration have been reported before clinically detectable diabetic retinopathy, suggesting that functional and neural abnormalities may accompany or precede overt vascular lesions [19, 21–26]. Impaired retinal neurovascular coupling may further alter the relationship between neural activity and retinal blood flow, potentially contributing to variability in the functional consequences of microvascular impairment [16]. Quantitative OCT and OCTA studies have also demonstrated abnormalities in retinal vascular and neural parameters in patients with diabetes without retinopathy, providing further evidence of early neurovascular unit involvement [27–29]. These observations provide a plausible biological context for our finding that higher HbA1c attenuated the inverse association between PFVD and retinal functional impairment. However, because oxidative stress, mitochondrial dysfunction, endothelial injury, and neurovascular coupling were not directly measured in the present study, these mechanisms should be regarded as plausible explanations rather than established mechanisms underlying the observed associations.

The sensitivity analyses provide additional context for the interaction finding. Although PFVD remained inversely associated with DR score across several glycemic strata, a statistically significant difference between PFVD slopes was observed only at the HbA1c threshold of 8.5%. Therefore, the 8.5% threshold should not be interpreted as a universal biological or clinical cutoff. Rather, it identifies a subgroup in which the divergence between retinal microvascular and functional measures was most apparent in this cohort. The consistency of the primary continuous HbA1c × PFVD interaction supports the presence of effect modification, whereas the threshold-specific findings should be considered exploratory and require validation in larger longitudinal cohorts.

These findings may have clinical implications for the detection of preclinical diabetic retinal dysfunction. Current diabetic retinopathy screening is primarily based on the detection of clinically apparent retinal lesions [25]. However, retinal neurodegenerative and functional abnormalities may occur before clinically apparent retinopathy develops [5–7, 30]. An integrated assessment combining OCTA-derived microvascular biomarkers with objective electrophysiological measures may therefore improve early identification of retinal dysfunction that is not fully captured by conventional funduscopic assessment. This multimodal approach may be particularly informative in patients with higher HbA1c levels, in whom retinal functional impairment may be less closely reflected by microvascular density alone.

Several limitations should be acknowledged. First, the cross-sectional design precludes causal inference regarding the temporal relationship between retinal microvascular compromise and retinal functional impairment. Longitudinal studies are needed to determine whether changes in retinal microvascular density precede, accompany, or follow electrophysiological dysfunction during the preclinical stage of diabetic retinopathy. Second, because only patients without clinically detectable diabetic retinopathy were enrolled, the findings may not be directly applicable to patients with established or more advanced diabetic retinopathy. Third, the relatively small number of participants in the HbA1c ≥ 8.5% subgroup may have reduced the precision and statistical power of subgroup-specific estimates. Fourth, the HbA1c thresholds were prespecified for sensitivity analyses but were not derived from a formal threshold-identification procedure. Therefore, the 8.5% threshold should be interpreted as an exploratory effect-modification threshold rather than a clinically validated cutoff. Importantly, the HbA1c × PFVD interaction was statistically significant in the primary continuous HbA1c model and remained significant in the stratified analysis using the 8.5% threshold; however, interaction terms at the other prespecified HbA1c thresholds did not reach statistical significance. Future multicenter prospective studies with larger and more diverse populations are warranted to validate the observed effect modification and determine whether combined structural, vascular, and functional biomarkers provide incremental value for early retinal risk stratification.

In conclusion, in patients with type 2 diabetes without clinically detectable diabetic retinopathy, glycemic status modified the association between retinal microvascular integrity and retinal function. Higher HbA1c was associated with greater retinal functional impairment, and the relationship between lower parafoveal vessel density and impaired retinal function varied across levels of glycemic control. These findings suggest that retinal microvascular imaging combined with objective functional assessment may provide complementary information for identifying early retinal dysfunction that is not apparent on routine clinical examination. Integrating structural and functional retinal biomarkers may therefore improve early risk stratification of patients with diabetes before clinically detectable diabetic retinopathy develops

Declarations

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Author Contribution Statement: IMF was responsible for data collection and analysis, drafting and writing the manuscript, study conception and design, data analysis and interpretation, and provided supervision and project administration. FGL contributed to data analysis and interpretation. KML and CYY contributed to data collection and data interpretation. All authors read and approved the final manuscript.

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Figures

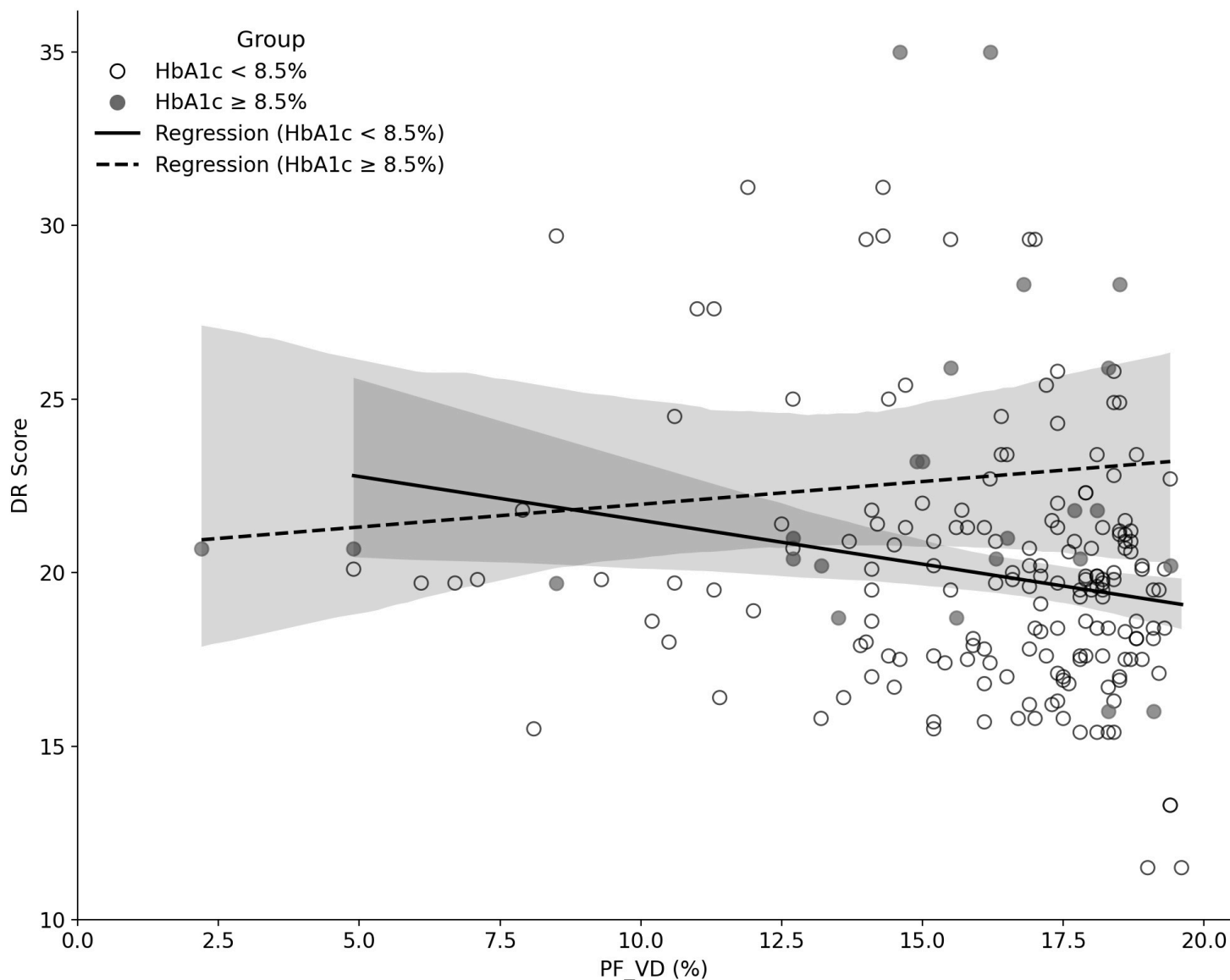


Figure 1

Adjusted association between parafoveal vessel density and RETeval-derived DR score according to HbA1c status.

Scatterplot showing the adjusted association between parafoveal vessel density (PFVD) and the RETeval-derived diabetic retinopathy (DR) score according to HbA1c status. Among eyes from participants with HbA1c <8.5%, lower PFVD was significantly associated with higher DR scores (adjusted $\beta = -0.44$, SE = 0.10, $P < 0.001$). In contrast, no significant association between PFVD and DR score was observed among eyes from participants with HbA1c $\geq 8.5\%$ (adjusted $\beta = 0.02$, SE = 0.27, $P = 0.96$). The difference between the two PFVD slopes was statistically significant (P for interaction = 0.019). These findings indicate that the inverse association between retinal microvascular density and retinal function was attenuated at higher HbA1c levels

Supplementary Files

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