Perfusion Deficits in Diabetes Without Retinopathy Localize to the Perivenular Deep Capillaries Near the Fovea on OCT Angiography

Peter L. Nesper, MD, MS, Amani A. Fawzi, MD

Purpose: To localize early capillary perfusion deficits in patients with diabetes mellitus (DM) without clinical diabetic retinopathy (DR) using averaged OCT angiography (OCTA).

Design: Retrospective cross-sectional study.

Participants: Patients with DM without DR and healthy controls.

Methods: We measured perfusion deficits in the full retina, superficial capillary plexus (SCP), and deep capillary plexus (DCP) on averaged 3 × 3-mm OCTA images. Perfusion deficits were defined as the percentage of retinal tissue located >30 μm from blood vessels, excluding the foveal avascular zone (FAZ). One eye from each patient was selected based on image quality. We measured deficits in the parafoveal region, the 300 μm surrounding the FAZ, and 300 to 1000 μm surrounding the FAZ. If a capillary layer within one of these regions was significantly different in DM without DR compared with controls, we further characterized the location of perfusion deficit as periarteriolar, perivenular, or the capillaries between these 2 zones.

Main Outcome Measures: Location of increased perfusion deficits in patients with DM without DR compared with controls.

Results: Sixteen eyes from 16 healthy controls were compared with 16 eyes from 16 patients with DM without DR (age 45.1 ± 10.7 and 47.4 ± 15.2 years respectively, $P = 0.64$). Foveal avascular zone area and perfusion deficits in the entire parafovea and the 300 to 1000-μm ring around the FAZ were not significantly different between groups ($P > 0.05$ for all). Perfusion deficits in 300 μm around the FAZ were significantly increased in patients with DM without DR in full retinal thickness, SCP, and DCP ($P < 0.05$ for all). When analyzing the perivenular, periarteriolar, and capillary zones, only the perivenular DCP perfusion deficits were significantly increased (5.03 ± 2.92% in DM without DR and 2.73 ± 1.97% in controls, $P = 0.014$).

Conclusions: Macular perfusion deficits in patients with DM without DR were significantly increased in the region nearest the FAZ, mainly at the perivenular deep capillaries. Further research on these early changes may improve our understanding of the capillaries most susceptible to vascular injury and disruption during diabetes.

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Diabetic retinopathy (DR) is a leading global cause of visual impairment.[1] Microvascular alterations are a central feature of DR, characterized by the degeneration of pericytes and endothelial cells, leading to microaneurysms, vascular occlusion, and ischemia.[2,3] Progression of ischemia leads to neovascularization, a cause for significant vision loss.[4] The use of retinal imaging has allowed clinicians to detect early pathological changes in the vasculature, even before the onset of clinical disease. OCT angiography (OCTA) has been used extensively to study the capillary changes that occur in patients with diabetes mellitus (DM) without clinical retinopathy.[5-8] Some studies suggest that early changes preferentially occur in the deep capillaries,[6,8] while others have found a larger increase in perfusion deficits in the superficial capillaries, making this an interesting area for further study.[9,10]

Hwang et al[11] were first to describe an automated method for detecting the avascular area in patients with DR. This group subsequently improved the algorithm for avascular area detection and found extrafoveal avascular area to be an important biomarker in DR, more sensitive than vessel density.[7,12] Geometric perfusion deficit (GPD) percentage is a similar parameter for measuring these avascular areas that was later introduced by Chen et al,[13] who found that this parameter was significantly more repeatable than vessel density in most of their comparisons. The GPD parameter takes into account that oxygen diffuses mainly transversely in the inner retina during light-adapted

conditions[14,15] as well as the estimated physiologic intercapillary spacing in the healthy macula based on prior imaging studies.[16-19] Our group has shown that GPD percentage was more sensitive in detecting clinically referable eyes (moderate nonproliferative DR [NPDR] or worse retinopathy or diabetic macular edema) than standard vessel density parameters and performed well in predicting future complications in DR.[20,21] Thus, the 2 parameters, extrafoveal avascular area and GPD, measure similar perfusion deficits and could be more sensitive in detecting microvascular changes than vessel density.

A recent study showed that macular capillary GPD within DR severity groups (DM without DR, and mild, moderate, and severe NPDR) was higher in the perivenular region compared to the periarteriolar region, suggesting macular perfusion deficits may occur preferentially near venules during DR.[22] In contrast, Ishibazawa et al[23] found nonperfusion in 12 × 12 mm and widefield OCTA was present to a greater extent on the arterial side compared with the venous side in more severe stages of the disease, including moderate NPDR, severe NPDR, and proliferative DR. Neither study assessed the extent of perfusion deficits with respect to arterioles and venules in patients with DM without DR compared to healthy controls. In the current study, we used averaged OCTA scans to localize early capillary nonperfusion in patients with DM without DR by quantifying GPD location based on capillary plexus, proximity in arterioles or venules, and eccentricity from the foveal avascular zone (FAZ).

Methods

This was a retrospective cross-sectional study of healthy subjects and patients with DM examined at the Department of Ophthalmology at Northwestern University, Chicago, Illinois between October 2018 and June 2022. The study was approved by the Institutional Review Board of Northwestern University, followed the tenets of the Declaration of Helsinki, and was performed in accordance with the regulations set forth by the Health Insurance Portability and Accountability Act. We obtained written informed consent from all participants before imaging.

Study Sample

Inclusion criteria were patients diagnosed with DM with no clinical signs of DR. Ultrawidefield pseudocolor scanning laser ophthalmoscopy (Optomap Panoramic 200; Optos PLC) images were available for confirmation of the clinical diagnosis in 11 (69%) of the 16 eyes with DM without DR. We also included a cohort of healthy patients with no signs of ocular pathology. Exclusion criteria included eyes with any sign of retinal pathology, including clinical DR, macular edema, and glaucoma. We excluded eyes with a refractive error of < −6 diopters and eyes with previous ocular surgery. We also excluded eyes with significant cataract to minimize artifactual OCT signal attenuation. We obtained patient demographics through a chart review.

OCTA

We imaged patients using the RTVue-XR Avanti system (Optovue Inc), which incorporates the split-spectrum amplitude-decorrelation angiography algorithm[24] and projection artifact removal software.[25] The details of the device have been described extensively elsewhere.[12,26] In short, OCTA quantifies and maps decorrelation signal between 2 consecutive B-scans at each location in the retina, resulting in a noninvasive method for visualizing the retinal vasculature. All patients underwent repeated 3 × 3-mm OCTA macular scans centered at the fovea during a single session. We only included scans with minimal motion or signal artifacts, a scan quality (Q-score) of ≥ 6, and a signal strength index of ≥ 50. We obtained 5 to 10 high quality scans per eye depending on patient comfort.

Image Analysis

We segmented the retinal microvasculature into the superficial capillary plexus (SCP) and deep capillary plexus (DCP) as well as the full retinal thickness slab using the automated settings of the AngioVue Analytics software (version 2018.1.1.63). The SCP was segmented from the inner limiting membrane to 10 μm above the inner plexiform layer. The DCP was segmented from 10 μm above the inner plexiform layer to 10 μm below the outer plexiform layer. The full thickness slab was segmented from the inner limiting membrane to 10 μm below the outer plexiform layer. We exported the images into Fiji, a software distribution of ImageJ (National Institutes of Health).[27] We first registered and averaged each layer as previously described.[28] Averaging multiple angiograms has been shown to improve image quality, reduce noise, and increase vessel connectivity.[29] We then manually delineated the FAZ using the Polygon selections tool in Fiji. We used the Max Entropy plugin and manually identified arterioles and venules by identifying the periarteriolar capillary-free zone on OCTA and the vessel depth on cross section to obtain a map for each vessel type.[16,30,31] We saved the FAZ, arteriole, and venule measurements as regions of interest for the automated GPD calculations.

We used an automated macro in Fiji to identify GPD percentage, which was defined as the percentage of retinal area located > 30 μm from the nearest blood vessel. The GPD parameter is based on the idea that oxygen mainly diffuses laterally in the inner retinal under photopic conditions as well as the estimated normal intercapillary spacing.[13-19] We used the Max Entropy plugin to binarize the large vessels and Huang2 thresholding followed by skeletonization for the capillaries.[30,32] The large vessels were then overlayed onto the skeletonized capillaries before measuring the GPD. We performed a binarized large vessel overlay instead of skeletonizing large vessels in order to avoid artifactual inclusion of physiologic avascular regions surrounding these larger vessels as GPD. The GPD percentage was then automatically calculated by the macro by selecting the region of interest to be the vessels (skeletonized and large vessels) and then enlarging the region by 30 μm, inverting the selection, and measuring the remaining area. This eliminates regions < 30 μm from the nearest vessel from the measurement.

We measured percent area of GPD in the parafoveal region for the SCP, DCP, and full retinal thickness slabs. The parafovea was defined as annulus from the FAZ outline to the 1-mm ring surrounding the FAZ (Fig 1D−F). We then measured the GPD in the 300-μm region surrounding the FAZ (Fig 1J−L) as well as the region from 300 μm to 1000 μm outside the FAZ (Fig 1M−O).

If any of these regions (parafoveal, FAZ to 300 μm, or 300−1000 μm outside the FAZ) in any capillary layer studied were found to have significantly increased GPD in patients with DM without DR, we further partitioned the region to study whether the GPD was located in the periarteriolar zone, perivenular zone, or capillary zone (capillaries located between the periarteriolar and perivenular zones) (Fig 2). The periarteriolar zone was defined as the 100 μm surrounding the arteriole mask (Fig 2D−F). The perivenular zone was defined as the 100 μm surrounding the venule mask (Fig 2G−I). The capillary zone was defined as the
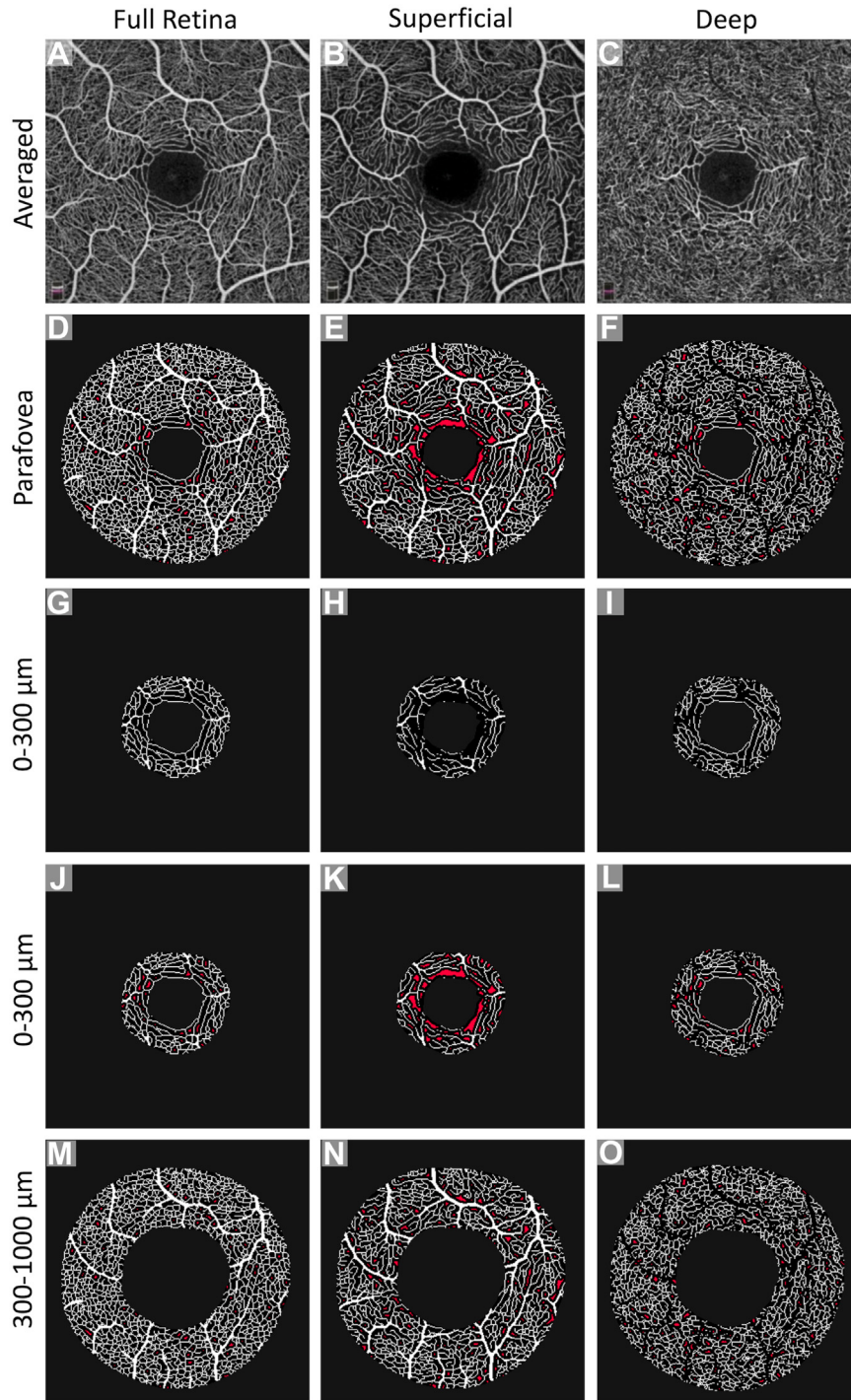
Figure 1. Regions of geometric perfusion deficit (GPD) measurements. OCT angiogram of a healthy subject. Figure is separated into full retinal thickness (first column), superficial capillary plexus (SCP) (second column), and deep capillary plexus (DCP) segmentations (third column). First row (**A–C**) shows the averaged angiograms. The second row (**D–F**) shows GPD (red) in the parafovea. The third row (**G–I**) shows the 300-μm annulus surrounding the foveal avascular zone (FAZ) before the addition of GPD (red) in the fourth row (**J–L**). The fifth row (**M–O**) shows GPD (red) in the region from 300 to 1000 μm surrounding the FAZ. To reduce shadowing artifacts in the GPD measurement, large SCP vessels were overlaid onto the DCP to eliminate these regions from the calculation.

area > 100 μm from the nearest arteriole or venule (Fig 2J–L). We chose 100 μm after we performed an initial analysis in 6 eyes at 300 μm outside of the FAZ and found that enlarging the large vessel mask by 100 μm resulted in similar sized vascular compartments for the periarteriolar, perivenular, and capillary spaces. The Max Entropy large vessel mask was overlayed onto the DCP to identify the 3 vascular spaces and to reduce the likelihood of shadowing artifacts on the deep vessels.
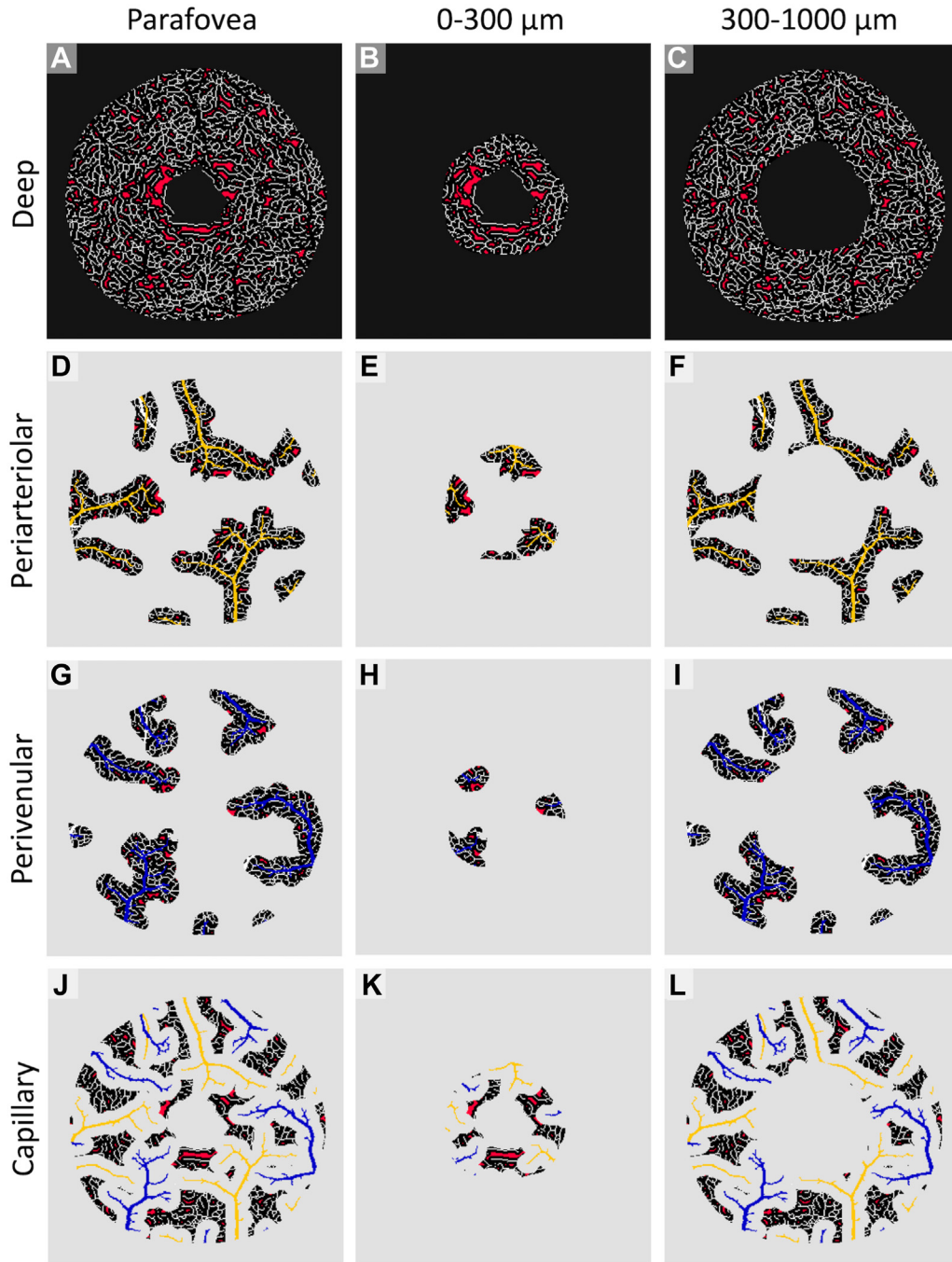
Figure 2. Zones of geometric perfusion deficit (GPD) measurements. Processed deep capillary plexus (DCP) OCT angiogram of a patient with diabetes without retinopathy. Figure is separated into parafoveal region (first column), the 300 μm surrounding the foveal avascular zone (FAZ) (second column), and the region from 300 to 1000 μm surrounding the FAZ (third column). First row (**A–C**) shows the entire respective region of the DCP. The second row (**D–F**) shows GPD (red) in the periarteriolar zone, within 100 μm of arterioles. The third row (**G–I**) shows GPD (red) in the perivenular zone, within 100 μm of venules. The fourth row (**J–L**) shows GPD (red) in the capillary zone, > 100 μm from arterioles or venules.

We also measured the adjusted flow index, which is an indirect and relative measure of blood flow velocity.[9] Pixel intensity on OCTA is based on the decorrelation value of the voxel, which is correlated linearly with flow velocity within a limited range.[33] We first measured the adjusted flow index of the large arterioles and venules by taking the average pixel intensity of the area obtained from the Max Entropy arteriole and venule mask outlines, and then compared them between healthy and DM without DR groups. We then assessed for differences in adjusted flow index for each region (parafoveal, FAZ to 300 μm, or 300–1000 μm outside the FAZ) in each capillary layer using mask outlines obtained with Huang2 thresholding.

Statistics

Statistical analyses were performed using SPSS version 29 (IBM SPSS Statistics; IBM Corporation). Shapiro−Wilk tests were used to test if the data were normally distributed. Independent-samples *t* tests were used to test for differences between healthy controls and DM without DR groups for normally distributed data. Mann−Whitney U tests were used for data with a skewed distribution. To reduce the likelihood of type 1 errors, we performed statistical comparisons in a stepwise fashion and only compared periarteriolar, perivenular, and capillary zones if the overall region was found to be significant. Shapiro−Wilk tests for percentage of glycated hemoglobin and duration of DM were significant, so we assessed the correlation between these variables and GPD using nonparametric Spearman Rank correlation coefficients. For each of these tests, a *P* value of < 0.05 was considered statistically significant.

Results

We included 16 eyes of 16 healthy subjects and 16 eyes of 16 patients with DM without DR (age 45.1 ± 10.7 and 47.4 ± 15.2 years respectively, $P = 0.64$). There were no significant differences in demographic data between groups, as shown in Table 1. A mean of 6.3 ± 1.2 images per eye were averaged to obtain the final averaged OCTA image and the number of images used was not significantly different between groups (Table 1). Foveal avascular zone area was not significantly different between healthy controls and DM without DR (0.302 ± 0.09 mm^2 and 0.249 ± 0.09 mm^2 respectively, $P = 0.093$). The percentage of GPD was not significantly different in patients with DM without DR compared with healthy controls in any capillary layer within the parafoveal region and the 300 to 1000 μm surrounding the FAZ (Table 2). The percentage of GPD was significantly increased in all capillary layers (full, SCP, and DCP) within the 300 μm region surrounding the FAZ (Table 2). When partitioned further, the DCP perivenular zone within the 300-μm region surrounding the FAZ was the only area with significantly greater GPD in patients with DM without DR

($5.03 \pm 2.92\%$ DM without DR and $2.73 \pm 1.97\%$ healthy controls, $P = 0.014$; Figs 3 and 4). All other comparisons were not significant (Table 2).

Adjusted flow index was not significantly different between groups ($P > 0.05$ for all). Correlations between deep perivenular GPD in the 300 μm surrounding the FAZ and either percentage of glycated hemoglobin or DM duration were not significant ($P > 0.05$ for both). When separating the DM group into type 1 and type 2, we found FAZ area was significantly larger in type 2 DM compared with type 1 (0.203 ± 0.058 mm^2 for type 1 and 0.294 ± 0.087 mm^2 for type 2, $P = 0.027$). While there was no significant difference in DM duration between groups (13.5 ± 5.9 years duration for type 1 DM and 14.3 ± 12.6 for type 2, $P = 0.635$), there was a significant difference in age (36.1 ± 10.7 years for type 1 DM and 58.7 ± 9.5 for type 2, $P < 0.001$). We compared the FAZ between type 1 and type 2 further by measuring the acircularity index[34] and found they were not significantly different (1.34 ± 0.17 mm^2 and 1.33 ± 0.19 mm^2 respectively, $P = 0.916$). We also found no significant difference in GPD parameters between type 1 and type 2 DM ($P > 0.05$ for all).

Discussion

In the current study, we assessed the location of capillary perfusion deficits in patients with DM without DR compared with healthy, age-matched subjects using averaged OCTA and found GPD was significantly increased in the 300 μm surrounding the FAZ in the full retina, SCP, and DCP. When we further partitioned this region to assess the proximity of these perfusion deficits to arterioles and venules, we found that only the 100 μm perivenular region in the DCP had significantly increased GPD compared with healthy controls ($P = 0.014$), suggesting that the deep venous capillaries nearest the FAZ may be particularly susceptible to early capillary closure and flow abnormalities in DM. Interestingly, even though the capillaries near the FAZ were affected, we found no difference in FAZ size

Table 1. Demographic Data for Healthy Control Subjects and Patients With DM Without Clinical DR

Parameter	Healthy Subjects	DM Without DR	P Value
Number of subjects	16	16	-
Age (years)	45.1 ± 10.7	47.4 ± 15.2	0.635
Sex (female/male)	11/5	11/5	1.000
Refractive error (diopters)	-0.86 ± 1.82	-1.30 ± 1.91	0.506
Intraocular length (IOL, mm)	24.05 ± 1.37	24.25 ± 1.04	0.702
No. missing IOL data (%)	6 (38)	3 (19)	-
No. scans for averaging	6.5 ± 1.3	6.0 ± 1.2	0.188 (†)
HbA1c (%)	-	7.1 ± 1.2	-
Duration of DM (years)	-	13.9 ± 9.5	-
Type of DM (1/2)	-	8/8	-

DM = diabetes mellitus; DR = diabetic retinopathy; HbA1c = percentage of glycated hemoglobin.
All continuous variables are reported as the average ± standard deviation. Demographics were compared using independent-samples *t* tests, unless noted by (†), which indicates a nonparametric Mann−Whitney U comparison.

Table 2. Perfusion Deficits Localize to the Perivenular Deep Capillaries Near the Fovea

Region	Segmentation	Zone	Healthy Control GPD	DM Without DR GPD	P Value
Parafoveal	Full	Entire	0.84 ± 0.28	1.11 ± 0.46	0.050 (†)
	SCP	Entire	3.67 ± 1.33	4.78 ± 2.11	0.087
	DCP	Entire	2.33 ± 1.42	3.08 ± 1.74	0.189
0—300 μm surrounding FAZ	Full	Entire	1.95 ± 0.60	3.01 ± 1.65	0.029* (†)
	Full	Periarteriolar	3.17 ± 1.14	4.14 ± 2.80	0.651 (†)
	Full	Perivenular	1.73 ± 0.99	2.50 ± 2.36	0.418 (†)
	Full	Capillary	2.25 ± 1.30	2.81 ± 1.42	0.256
	SCP	Entire	9.62 ± 3.58	13.25 ± 5.54	0.036*
	SCP	Periarteriolar	14.07 ± 5.64	16.41 ± 7.60	0.330
	SCP	Perivenular	12.06 ± 5.70	15.11 ± 8.28	0.234
	SCP	Capillary	11.97 ± 5.44	14.47 ± 7.28	0.279
	DCP	Entire	4.23 ± 2.81	7.09 ± 4.26	0.046* (†)
	DCP	Periarteriolar	4.95 ± 2.48	8.02 ± 5.74	0.122 (†)
	DCP	Perivenular	2.73 ± 1.97	5.03 ± 2.92	0.014*
	DCP	Capillary	4.48 ± 4.15	5.81 ± 3.68	0.274 (†)
300—1000 μm surrounding FAZ	Full	Entire	0.59 ± 0.22	0.73 ± 0.30	0.137
	SCP	Entire	2.37 ± 1.01	2.96 ± 1.46	0.193
	DCP	Entire	1.90 ± 1.14	2.20 ± 1.29	0.429 (†)

DCP = deep capillary plexus; DM = diabetes mellitus; DR = diabetic retinopathy; FAZ = foveal avascular zone; GPD = geometric perfusion deficit; SCP = superficial capillary plexus.
Geometric perfusion deficit was reported as an average percentile ± standard deviation. The GPD measurements were compared between study groups using independent-samples t tests, unless noted by (†), which indicates a nonparametric Mann—Whitney U comparison.
*Statistical significance ($P < 0.05$).

($P = 0.093$). Therefore, GPD could be a more sensitive marker than FAZ area for early vascular changes before the onset of DR. It is also possible that these DCP perivenular defects precede breakdown of the FAZ and could predict regions of future FAZ expansion, which deserves further longitudinal study.

Our finding of deep capillary changes in patients with DM without DR is consistent with previous reports of microvascular alterations occurring in this stage of preclinical DR.[6,8,35] Deep capillary alterations have been shown to be associated with decreased central visual function in subjects with DR without retinopathy.[6] This could be consistent with neuronal alterations somewhere along the visual pathway before the onset of DR. A possible metabolic link between these early DCP alterations and visual dysfunction could be related to reactive oxygen species (ROS) from metabolically deranged photoreceptors, which have been shown to be a major producer of ROS in the retina.[36–38] The increased ROS production from the photoreceptors could impart further stress on the pericytes and endothelial cells already in a critical state from inflammation and hyperglycemia in the setting of diabetes. Deep capillary closure and flow dysregulation would, in turn, make the photoceptors more ischemic, causing further increase in ROS and creating a positive feedback loop.[39,40]

We also found the 300 μm surrounding the FAZ to be the most important area of capillary change in our cohort, which is consistent with a recent report by Han et al[41] who found the foveal density-300 to be the most useful biomarker for distinguishing patients with DM without DR from healthy controls. By partitioning this "foveal density-300" region into perivenular, periarteriolar, and capillary zones, we were able to distinguish the central perivenular deep capillary zone as the most significant biomarker for distinguishing patients with DM without DR. It is worth highlighting that this 300-μm ring is also the region with the greatest density of cone photoreceptors. Therefore, this foveal region of perivenular deep capillaries may be particularly susceptible to vascular damage. In diabetes, this region combines the high density of ROS-producing cone photoreceptors with slower flowing venous vasculature farther from the highly oxygenated arterioles, which together would result in a lower oxygen tension and higher tendency for leukostasis and leukocyte-mediated endothelial cell injury.

Our results of perivenular as opposed to periarteriolar capillary changes are consistent with a recent study that found larger perivenular GPD than periarteriolar GPD within DM without DR and NPDR groups.[22] These same findings appear to conflict with those of Ishibazawa et al[23] who found arterial-adjacent nonperfusion occurred to a greater extent than venous-adjacent nonperfusion. We believe there are a number of possible reasons for the observed differences, including methodology differences in the assignment of nonperfused areas to arterioles or venules, the segmentation of the retinal layers, image averaging, and the use of a cutoff size for nonperfusion. Importantly, these latter authors also studied more severely diseased eyes, including moderate NPDR, severe NPDR, and proliferative DR, while our study only compared healthy patients with patients with DM without DR. We know from previous studies that the correlation of OCTA parameters with DR severity is not constant throughout the course of the disease.[9,42] Ishibazawa et al also used 12 × 12-mm scans and widefield montages, while we used averaged 3 × 3-mm scans focusing mainly on the central 300 μm surrounding
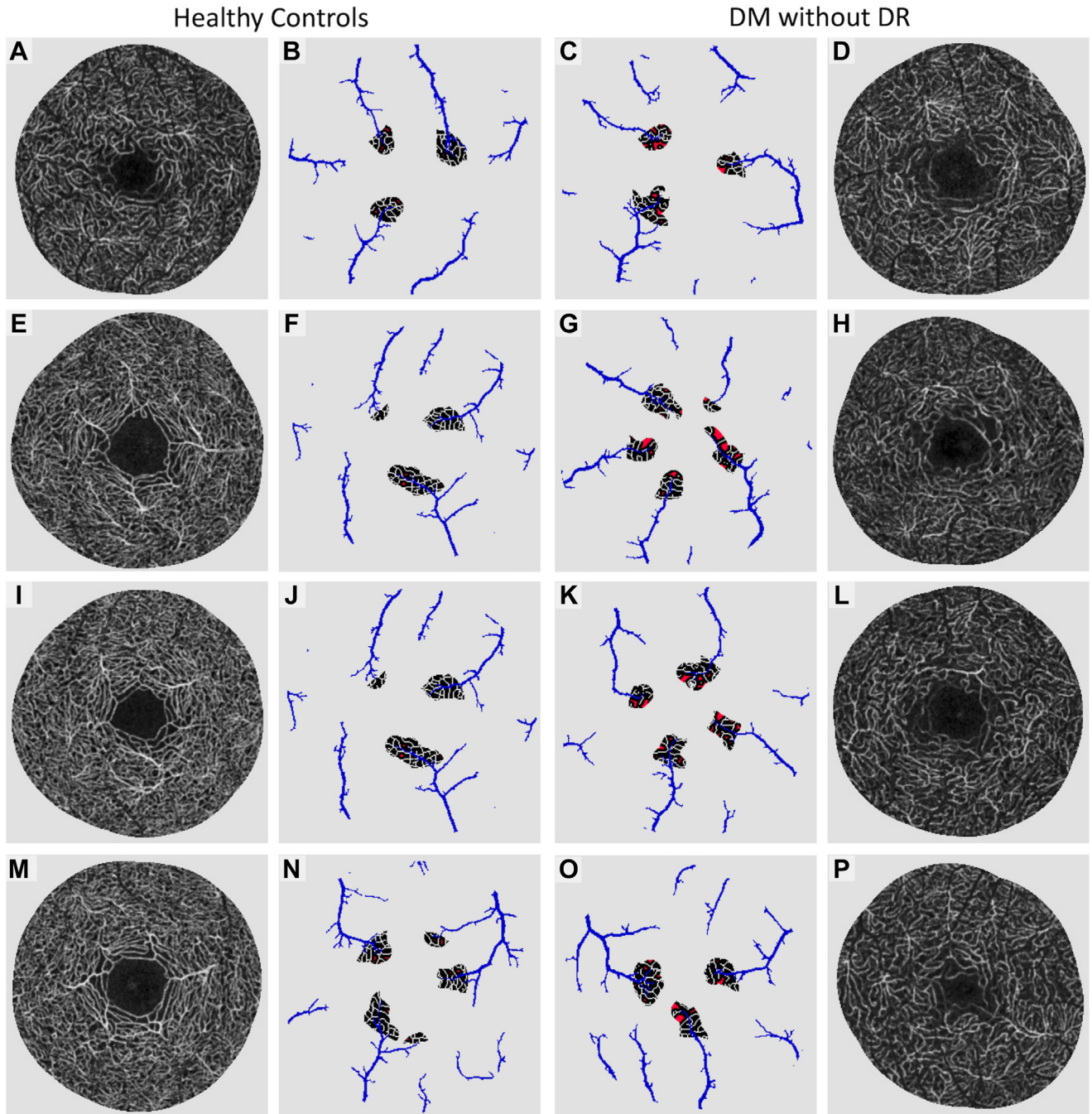
Figure 3. Perfusion deficits are increased in the perivenular deep capillary plexus (DCP) near the foveal avascular zone (FAZ) in eyes with diabetes without retinopathy. Averaged DCP vessels are shown in columns 1 (**A**, **E**, **I**, and **M**) and 4 (**D**, **H**, **L**, and **P**). The perivenular zone DCP within the 0 to 300 μm surrounding the FAZ is shown with geometric perfusion deficit (GPD) (red) and the location of venules (blue) in columns 2 (**B**, **F**, **J**, and **N**) and 3 (**C**, **G**, **K**, and **O**). Columns 1 and 2 are from 4 healthy subjects and columns 3 and 4 are from 4 patients with diabetes mellitus (DM) without diabetic retinopathy (DR). Red GPD in this zone is increased in patients with diabetes ($P = 0.014$).

the fovea. The microvascular disease process in the retinal periphery where rods predominate is likely different from the central macula with its high density of ROS-producing cone photoreceptors.[36] Furthermore, image averaging has been shown to decrease noise and increase vessel connectivity, though previous studies offer conflicting results regarding the effect of averaging on quantitative OCTA parameters.[29,43,44] Therefore, we acknowledge a difference in methodology that precludes direct comparison between the 2 studies and also suggest that the pathophysiology of DR is likely unique in the macula due to the high density of cone photoreceptors.

Healthy Controls   DM without DR
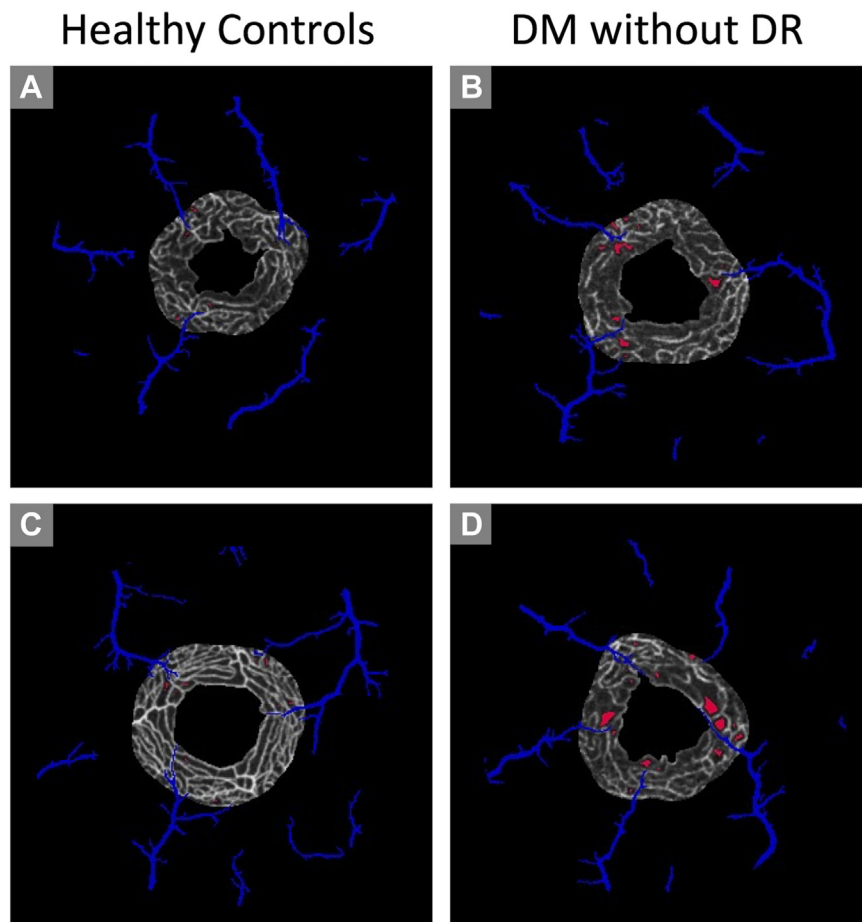
Figure 4. Overlay of geometric perfusion deficit (GPD) areas on original averaged angiogram reveals prevalence of increased perfusion deficits near the venules. The averaged deep capillary plexus (DCP) angiogram of the 0 to 300-μm region around the foveal avascular zone is shown with GPD (red) and venules (blue) overlay. Perivenular DCP GPD is increased in patients with diabetes mellitus (DM) without diabetic retinopathy (DR) (right column, **B** and **D**) compared with healthy controls (left column, **A** and **C**). Only the GPD in the perivenular zone is shown for comparison between groups.

Interestingly, histologic studies in eyes with DR show mural wall thickening that particularly affects the precapillary arterioles, predisposing downstream capillaries to occlusion.[45,46] Our group has also recently shown loss of vascular mural cells at the level of the arterioles in DM without DR, preceding any apparent clinical retinopathy.[47] We hypothesize that these changes would disrupt blood flow regulation at the arterioles, and that the downstream capillaries most susceptible prior to the onset of DR are on the venular end of the capillary bed, a region more susceptible to leukostasis, slow flow, higher ROS, and lower oxygen. A direct comparison of capillary closure on the arteriolar versus venular side of the vasculature in patients with DM without DR using histological methods would offer more insight.

We found no significant difference in FAZ area between the healthy control and DM without DR groups, which is consistent with previous reports.[6,7,42] The substantial variability in FAZ size among healthy subjects has led researchers to search for a better way to define the pathologic FAZ. Acircularity index is a measure of the deviation of the FAZ from a perfect circle and circumvents the physiologic variability in FAZ size.[34] We initially found a significant increase in FAZ area in patients with type 2 compared with type 1 diabetes, which aligns with other studies.[5,10] Yet, upon further analysis, we did not find any difference in acircularity index or other GPD parameters, leading us to revise our interpretation to suggest minimal difference between type 1 and type 2 DM in our study. There were only 8 eyes per group and therefore this comparison was underpowered. The type 1 compared with type 2 subjects were also not age matched in this study. Further analysis of the capillary differences between type 1 and type 2 diabetes in patients without DR in larger cohorts would be necessary and would benefit from the use of averaged OCT angiograms such as in this study.

Limitations of this study include a small number of eyes due to our strict inclusion and quality criteria. Larger studies are needed to confirm and support our results. The study was also limited by not performing Bonferroni correction for multiple comparisons, but we did perform a stepwise

analysis and thereby only further partitioned regions into perivenular or periarteriolar if that region was significant. This reduced the likelihood of type 1 errors in this pilot study. We were also limited to the DM without DR group and future studies expanding this methodology would help elucidate the progression of capillary abnormalities throughout the stages of DR. Future GPD studies may also benefit from the incorporation of additional software advances, such as the iterative watershed segmentation algorithm recently developed by Ong et al[48] for precise evaluation of the 3-dimensional connectivity of capillaries with larger arterioles and venules. We also acknowledge the inherent limitations of OCTA technology, which may potentially limit complete visualization of the deep retinal vasculature.[16] Finally, we only assessed the macula, but future studies using faster scanning and wider field OCTA could provide high quality macular and peripheral vasculature assessment.

In summary, we found that perfusion deficits in patients with DM without DR localized to the perivenular deep capillaries within the 300 μm surrounding the FAZ using averaged OCTA. This compartment may be particularly susceptible to vascular impairment early in diabetes due to its proximity to ROS-generating cone photoreceptors and the slower flow of the smallest venous capillaries at the DCP. The central perivenular deep capillaries should be targeted for further analysis for their potential use as early pathologic biomarkers in preclinical DR, which may further improve our understanding of the earliest vascular changes in this highly prevalent disease.

Footnotes and Disclosures

Correspondence:

Amani A. Fawzi, MD, Department of Ophthalmology, Feinberg School of Medicine, Northwestern University, 645 N. Michigan Avenue, Suite 440, Chicago, IL 60611. E-mail: afawzimd@gmail.com.

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