The human retina has a high oxygen demand that is supplied by 2 nonoverlapping vascular layers. While the inner retina is exclusively supplied by the retinal circulation, the outer retina, which is avascular, obtains its oxygen supply primarily via diffusion from the choroidal circulation and only minimally from the retinal circulation. Retinal vascular supply in the macula consists of 3 capillary plexuses. The superficial capillary plexus lies in the retinal nerve fiber layer or ganglion cell layer, while the middle capillary plexus and deep capillary plexus (DCP) are located on the inner and outer borders of the inner nuclear layer, respectively. It has been reported that the DCP contributes 10% to 15% of the oxygen supply to the photoreceptor cells, especially during dark adaptation.3

Because the foveola and perifoveal macula are completely avascular, their oxygen supply depends mainly on the choriocapillaris, although lateral diffusion of oxygen from the surrounding retinal capillaries may also contribute. Indeed, this capillary-free zone is termed the foveal avascular zone (FAZ) on fluorescein angiography (FA) and can vary in size significantly even in healthy individuals.5,6

Macular ischemia and enlargement of the FAZ can occur in a variety of retinal vascular diseases, including diabetic retinopathy (DR), hypertensive retinopathy, retinal vein...
Diabetic Macular Nonperfusion and Outer Retinal Disruption

At a Glance

- In 9 eyes diagnosed as having either nonproliferative or proliferative diabetic retinopathy, a possible association is shown between macular areas of capillary nonperfusion on fluorescein angiography and outer retinal disruption on spectral-domain optical coherence tomography.
- In this setting, the retinal deep capillary plexus may play an important role in these photoreceptor abnormalities.
- In diabetic retinopathy, areas of retinal nonperfusion can be associated with photoreceptor structural abnormalities.
- The finding of outer retinal disruption in diabetic retinopathy on spectral-domain optical coherence tomography might predict overlying areas of capillary nonperfusion.

occlusion, and sickle cell disease,\textsuperscript{5,7-12} leading to decreased perifoveal capillary blood flow and in turn causing chronic ischemia of the retinal tissue.\textsuperscript{5} Several studies in the 1970s used FA to define the functional importance of macular ischemia,\textsuperscript{5,9,10} which is now considered an important predictor of poor functional outcome in patients with diabetes mellitus (DM).\textsuperscript{13}

Recently, spectral-domain optical coherence tomography (SD-OCT) has allowed more direct in vivo assessment of the retinal microstructure. Several OCT studies have shown thinning of the inner retinal layers, including the ganglion cell layer and retinal nerve fiber layer, in patients with DM.\textsuperscript{14-21}

We hypothesized that macular ischemia and enlargement of the FAZ may contribute to outer retinal and photoreceptor disruption on SD-OCT in diabetic patients in the absence of diabetic macular edema (DME), as a possible consequence of ischemia at the level of the DCP. To investigate our hypothesis, we performed detailed analysis of 13 eyes of 9 diabetic patients, focusing on 9 eyes with various degrees of macular ischemia and macular capillary nonperfusion without DME.

Methods

We performed a retrospective analysis of the imaging database to identify all patients with diabetic macular nonperfusion who underwent FA and SD-OCT examinations in the Department of Ophthalmology, Northwestern University, Chicago, Illinois, from July 8, 2014, to December 1, 2014. This analysis was conducted between December 2, 2014, and January 31, 2015. The institutional review board at Northwestern University approved this research protocol. Informed consent was not required owing to the retrospective nature of this study.

We included patients with enlargement of the FAZ or capillary nonperfusion within the macula in the setting of either nonproliferative DR (NPDR) or proliferative DR (PDR). Fellow eyes with a normal FAZ were included as controls.

We excluded eyes with DME and eyes with other associated retinal diseases, such as age-related macular degeneration or vitreomacular traction. We also excluded eyes that had undergone surgical retinal repair or intravitreal anti-vascular endothelial growth factor or steroid injection and patients who received laser treatment in the preceding 5 years and/or any laser treatment in the nonperfused area in the macula as revealed on FA. Patients with significant cataracts graded higher than NO3 or NC3 were excluded to avoid an optical artifact that may compromise SD-OCT image quality.

The FA images were evaluated by the agreement of 2 independent masked graders (L.M.J. and A.A.F.) to define enlargement of the FAZ and area of nonperfusion and to stage the DR using protocols and standard photographs from the Early Treatment Diabetic Retinopathy Study report number II.\textsuperscript{22-28} Based on these criteria, diabetic macular ischemia was identified as absent or present. In situations of disagreement between graders, consensus was reached by an open discussion. Medical records were reviewed to gather information about visual acuity, data regarding slitlamp biomicroscopy, and history of retinal laser treatment.

Fluorescein angiography was performed according to the standard procedure (TRC-50DX; Topcon Corp). It was also used to identify angiographic macular edema defined as dye leakage within a 500-µm radius from the fovea or a 1500-µm radius in cases with hard exudates in this area. An early-phase FA (20-30 seconds) and a late-frame FA (3-5 minutes) were selected for this analysis.

Diabetic macular edema was diagnosed as either clinical or with SD-OCT or angiographic evidence of macular edema, and these eyes were excluded from this study.

The SD-OCT images were obtained using the Spectralis HRA+OCT imaging device (Heidelberg Engineering). The SD-OCT imaging protocol comprised 31 horizontal B-scans per volume scan of 20° × 20° in the macula, and each B-scan included an average of 30 frames. Retinal volume was measured using the incorporated software of the SD-OCT. The tracking feature of the Heidelberg Spectralis HRA+OCT was used. This feature allows every OCT B-scan to be registered to its exact location on the infrared image, which was superimposed onto the relevant FA frames, hence facilitating point-to-point correlations of the retinal imaging findings between the FA and SD-OCT.

Two observers (L.M.J. and A.A.F.), masked to any associated information and FA findings, analyzed the pathologic changes on the SD-OCT scans independently. For each patient, the scans were qualitatively evaluated as normal or with the presence of thinning of the inner retina and/or outer nuclear layer (ONL) or disruption of the external limiting membrane, inner segment–outer segment (IS/OS) junction, and OS-retinal pigment epithelium junction. In situations of disagreement between readers, consensus was reached by an open discussion.

Results

This study included a total of 13 eyes of 9 diabetic patients aged 34 to 58 years. The mean duration of DM was 14.5 years. Nine eyes showed either an enlargement of the FAZ and/or capillary nonperfusion in the macula, which tightly colocalized with
outer retinal disruption on the SD-OCT. We did not see generalized outer retinal disruption in these patients, but rather a very close spatial correspondence between the outer retinal disruption and the region of FAZ enlargement and/or macular nonperfusion, suggesting a possible causal relationship.

Four fellow eyes with a normal FAZ and macular perfusion served as controls and did not show any retinal abnormalities on SD-OCT. Five fellow eyes that did not meet the inclusion criteria were excluded. The demographic characteristics and ocular findings are summarized in the Table.

### Imaging Findings

The imaging findings in eyes with capillary nonperfusion are shown in Figure 1, Figure 2, and Figure 3. Figure 4 shows an example of a nonischemic control fellow eye (case 2).

### Case Summaries

**Case 1**

A man in his early 50s had a history of hypertension, hyperlipidemia, and type 2 DM treated with insulin and oral medications (pioglitazone hydrochloride and glipizide). The right eye, included in the study, had undergone macular laser treatment inferotemporal to the fovea 5 years prior. The macular laser treatment was outside the nonperfused area revealed by FA, and there was no evidence of DME either clinically or angiographically (Figure 1A and B). The left eye was excluded because of DME.

**Case 2**

A woman in her mid-30s had a history of hypertension and type 2 DM treated with insulin. Her DM was recently controlled with a rapid decrease in glycated hemoglobin from 11.3% to 8.1% of total hemoglobin (to convert to proportion of total hemoglobin, multiply by 0.01). Both eyes were included in the study. The left eye had severe NPDR and macular capillary nonperfusion (Figure 1C and D). The right eye served as a control because it showed normal macular perfusion (Figure 4).

**Case 3**

A man in his late 50s had a history of hypertension and type 2 DM treated with oral medications (nateglinide, sitagliptin phosphate, and glipizide). Both eyes were included in the study. In the right eye, FA showed an enlargement of the FAZ, a large area of nonperfusion, and the presence of fine retinal neovascularization temporal to the macula (Figure 1E and F). The left eye had a normal FAZ and no retinal abnormalities on SD-OCT scans and served as a control.

**Case 4**

A woman in her late 50s had a history of hypertension, hyperlipidemia, and type 2 DM treated with insulin. The right eye was excluded because of the presence of DME and previous anti-vascular endothelial growth factor injections. The left eye, included in the study, had undergone macular laser treatment superior to the fovea 5 years prior. The macular laser treatment was outside the area of macular nonperfusion on FA and the eye did not show any evidence of angiographic or clinical DME (Figure 2A and B).

**Case 5**

A man in his early 40s had a history of hypertension, chronic kidney disease, and type 2 DM treated with insulin. The right eye, included in the study, showed evidence of macular non-perfusion on the SD-OCT. We did not see generalized outer retinal disruption in these patients, but rather a very close spatial correspondence between the outer retinal disruption and the region of FAZ enlargement and/or macular nonperfusion, suggesting a possible causal relationship.

### Table. Demographic and Clinical Findings of Study Population

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age, y</th>
<th>DM Type</th>
<th>Duration of DM, y</th>
<th>Glycated Hb, % of Total Hb</th>
<th>HTN</th>
<th>HLD</th>
<th>KD</th>
<th>Study Eye</th>
<th>BCVA</th>
<th>DR Stage</th>
<th>Laser Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/early 50s</td>
<td>2</td>
<td>12</td>
<td>12.3</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Right</td>
<td>20/25</td>
<td>PDR</td>
<td>Macular and PRP</td>
</tr>
<tr>
<td>2</td>
<td>F/mid-30s</td>
<td>2</td>
<td>16</td>
<td>8.1</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Right (control)</td>
<td>20/25</td>
<td>Severe NPDR</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>M/late 50s</td>
<td>2</td>
<td>6</td>
<td>NA</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Right</td>
<td>20/30</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>4</td>
<td>F/late 50s</td>
<td>2</td>
<td>20</td>
<td>9.2</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Left</td>
<td>20/30</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>5</td>
<td>M/early 40s</td>
<td>2</td>
<td>15</td>
<td>5.9</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Right</td>
<td>20/125</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>6</td>
<td>M/early 50s</td>
<td>2</td>
<td>12</td>
<td>NA</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Left</td>
<td>20/50</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>7</td>
<td>M/late 30s</td>
<td>1</td>
<td>25</td>
<td>6.4</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Left</td>
<td>20/30</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>8</td>
<td>M/mid-50s</td>
<td>2</td>
<td>10</td>
<td>8.4</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Right</td>
<td>20/20</td>
<td>PDR</td>
<td>PRP</td>
</tr>
<tr>
<td>9</td>
<td>M/early 40s</td>
<td>2</td>
<td>0.25</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Right (control)</td>
<td>20/20</td>
<td>Moderate NPDR</td>
<td>None</td>
</tr>
</tbody>
</table>

### Abbreviations:

- BCVA, best-corrected visual acuity
- DM, diabetes mellitus
- DR, diabetic retinopathy
- Hb, hemoglobin
- HLD, hyperlipidemia
- HTN, hypertension
- KD, kidney disease
- NA, not available
- NPDR, nonproliferative DR
- PDR, proliferative DR
- PRP, panretinal photocoagulation

### SI conversion factor:

To convert glycated Hb to proportion of total Hb, multiply by 0.01.

### Notes:

- Case 2: The patient had undergone pancreas and kidney transplantation 4 years prior to presentation.

---

### References

1. Diabetic Macular Nonperfusion and Outer Retinal Disruption
2. JAMA Ophthalmology
3. September 2015
4. Volume 133, Number 9
5. jamaophthalmology.com

---

**Copyright 2015 American Medical Association. All rights reserved.**

---

Downloaded From: by a Non-Human Traffic (NHT) User on 11/16/2018
perfusion (Figure 2C and D). The left eye had undergone surgery for traction retinal detachment and was excluded.

**Case 6**
A man in his early 50s had a history of hypertension and type 2 DM treated with an oral medication (glipizide) and insulin. The right eye was excluded because of the presence of DME and previous anti-vascular endothelial growth factor injections. The left eye, included in the study, had macular ischemia in the setting of inactive PDR (Figure 2E and F).

**Case 7**
A man in his late 30s had a history of hypertension and type 1 DM and had undergone pancreas and kidney transplantation 4 years prior to presentation. The right eye was excluded because of the presence of a vitreous hemorrhage. The left eye, included in the study, had macular capillary nonperfusion in the setting of inactive PDR and a history of cataract extraction (Figure 3A and B).

**Case 8**
A man in his mid-50s had a history of hypertension and type 2 DM treated with an oral medication (glipizide) and insulin. Both eyes were included in the study. The right eye had no active PDR. Optical coherence tomography showed disruption of the outer retinal layers that colocalized with an enlargement of the FAZ (Figure 3C and D). The left eye had inactive PDR and served as a control because it showed normal macular perfusion.
Case 9
A man in his early 40s had a history of hypertension, hyperlipidemia, and recently diagnosed uncontrolled type 2 DM that was treated with oral medication (metformin hydrochloride). Both eyes were included in the study. The right eye had NPDR and served as a control because it showed a normal FAZ. The left eye had macular capillary nonperfusion in the setting of moderate NPDR (Figure 3E and F).

Discussion
The use of multimodal imaging in this series of 13 eyes of 9 diabetic patients highlights a possible contribution of macular nonperfusion to photoreceptor disruption in DR. In contrast with previous reports, our analysis illustrated that enlargement of the FAZ and capillary nonperfusion in the macula not only leads to thinning of the inner retina but can also be associated with outer retinal changes, including disruption of the external limiting membrane and the IS/OS junction as well as thinning of the ONL and photoreceptors, in diabetic patients. By confining our studies to eyes without DME, we excluded the potential confounding effects of chronic macular edema on the outer retina. In our series, we found no eyes in which the outer retina was completely normal on SD-OCT when there was an enlargement of the FAZ. The size of the FAZ is determined by the most central, deep capillaries; hence, as the FAZ expands, it is likely that the DCP is affected. The correlation we observed therefore suggests that deep capillary ischemia in the setting of DR might lead to disruption of the outer retina in diabetic patients with macular nonperfusion.

Wangsa-Wirawan and Linsenmeier reviewed the evidence that the photoreceptor ISs are the most important consumers of choroidal oxygen. The outer retinal oxygen gradient shows a steep decline with the nadir at the IS. Also, studies of cat retinas have highlighted that oxygen tension in the retina...
is highest at the choroid, rapidly decreases at the level of the ONL/ISs, and then increases again in the inner retinal layers.\(^2\),\(^27\),\(^28\) In the dark, the watershed retinal layers, where both the choroidal and retinal circulations might provide oxygen support, are where the oxygen tension decreases to its lowest level.\(^3\) The photoreceptor axon terminals reside in the outer plexiform layer (OPL) and are replete with mitochondria to supply their energy.\(^29\) The DCP is located at the inner aspect of the OPL and is unquestionably important for nutrition of photoreceptor synapses. However, the retinal circulation is also responsible for about 15% of the metabolic demand of the ISs, especially in darkness. Because of this, the photoreceptor and the OPL in this watershed zone may be particularly susceptible to ischemic insults affecting the choroid and/or DCP.

Currently, FA is the most widely used clinical tool to evaluate abnormalities of the smaller vessels and capillaries.\(^30\),\(^31\) Little is known about the influence of the size of the FAZ on photoreceptor layer integrity.

Using Adaptive Optics scanning laser ophthalmoscopy, Pinhas et al\(^32\) recently showed that healthy young individuals could have occult nonperfused retinal capillaries adjacent to the FAZ. They suggested these might represent normal variation and/or an early sign of pathology, more extensive in the vasculopathic eyes.\(^32\) It is important to note that FA (includ-
Compatible with prior reports,\textsuperscript{32, 37, 38} we additionally reveal the disruption. Eight of these eyes showed thinning of the inner retina.\textsuperscript{9} Eyes of 9 patients showed characteristics of photoreceptor genesis.\textsuperscript{34} Thus, it is not surprising that previous studies could be recognized as an important player in retinal pathogenesis.\textsuperscript{34} This is supported by the finding that outer retinal changes in the setting of capillary nonperfusion are likely specific to the metabolic derangements associated with DR. Indeed, cases of the most severe retinal capillary ischemia, namely central retinal artery occlusion, maintain a robust A-wave\textsuperscript{44} and an intact outer retinal capillary network, indicating Adaptive Optics scanning laser ophthalmoscopy-based FA\textsuperscript{44} does not reveal features of the DCP,\textsuperscript{33} which has only recently been recognized as an important player in retinal pathogenesis.\textsuperscript{34} Thus, it is not surprising that previous studies could not reach definitive conclusions regarding the effect of angiographic capillary nonperfusion or macular ischemia on retinal sublayers in diabetic patients.\textsuperscript{35-38}

Previous studies have revealed that macular ischemia in the setting of DME may be associated with photoreceptor OS shortening and disruption of the IS/OS junction.\textsuperscript{36, 39} However, because visibility of the IS/OS junction is highly susceptible to OCT signal strength, the possibility that an indistinct IS/OS junction line could be related to an optical artifact of the overlying cystic changes could not be completely excluded in these studies. Furthermore, it is not clear in these studies whether these eyes had long-standing DME and hence secondary photoreceptor disruption\textsuperscript{39} or whether the thinning was potentially related to prior laser therapy. To this point, another more recent study did not find significant associations between FAZ size and the mean area of the disrupted IS/OS junction or the external limiting membrane in patients with treatment-naive DME.\textsuperscript{38}

Several studies explored the relationship between the nonperfused areas on FA and thinning of the inner retinal layers on OCT in eyes with DR\textsuperscript{41, 40}, as well as in patients with retinal vein occlusion, but none of these studies reported the concurrent presence of outer retinal changes.\textsuperscript{41-43} In our series, all 9 eyes of 9 patients showed characteristics of photoreceptor disruption. Eight of these eyes showed thinning of the inner retinal layers as well. While the inner retinal findings are compatible with prior reports,\textsuperscript{32, 37, 38} we additionally reveal the association of diabetic capillary nonperfusion with outer retinal OCT changes in eyes without DME. Moreover, by overlaying the FA to the OCT, we confirm that these areas of outer retinal disruption on OCT correlated tightly to zones of capillary nonperfusion and showed no angiographic leakage in the late phase.

It is intriguing to speculate about the exact mechanism whereby capillary nonperfusion on FA can explain both the thinning of the inner retina and the photoreceptor changes. We can think of at least 3 additional mechanisms related to capillary nonperfusion in diabetic patients that could contribute to outer retinal changes in DR, including (1) concomitant vascular diseases, (2) acidosis in the outer retina following ischemia, and (3) “diabetic choriodopathy.” Furthermore, we know that FA is only able to show the distribution of the superficial capillary network, leading to a significant lack of information regarding the DCP network.\textsuperscript{32} The missing information about the DCP might be important in understanding the pathological changes in the outer retina on SD-OCT. We believe these outer retinal changes in the setting of capillary nonperfusion are likely specific to the metabolic derangements associated with DR. Indeed, cases of the most severe retinal capillary ischemia, namely central retinal artery occlusion, maintain a robust A-wave\textsuperscript{44} and an intact outer retinal structure on OCT. In cases of central retinal artery occlusion, the normal choroidal blood supply is sufficiently robust to meet the photoreceptor oxygen demand in these situations.

Evaluating the SD-OCT of our patients, we find that the outer retinal findings on OCT appeared very similar to late findings in classic acute macular neuroretinopathy (Figure IC and D, case 2). It has already been suggested\textsuperscript{14} that the initial OPL/ONL hyperreflectivity on SD-OCT in acute macular neuroretinopathy may reflect diffuse edema of photoreceptor cell bodies and their axons because of ischemia of DCP. Over time, these patients develop thinning of the ONL and disruption of the IS/OS and OS/retinal pigment epithelium junctions. We believe that in our series of diabetic patients, several systemic risk factors, such as hypertension, hyperlipidemia, kidney dysfunction, and hyperglycemia, contributed to severe ischemic insult of the retina as shown by the capillary nonperfusion on FA, resulting in ischemia involving all of the retinal capillary layers, particularly the DCP. However, because of the potential of OCT angiography to better visualize the DCP compared with FA, further studies are necessary to validate our findings in diabetic patients.\textsuperscript{45}

Additionally, outer retinal acidosis can occur in the setting of ischemia and contribute to photoreceptor compromise. We cannot exclude its contribution to photoreceptor disruption on OCT in our series.\textsuperscript{46}

Finally, while our study was not designed to examine the choroidal contribution, concurrent outer retinal ischemia related to ischemia of the choroid due to diabetic choriodopathy cannot be excluded.\textsuperscript{47-51}

We acknowledge the inherent limitation of our retrospective series including relatively small numbers of eyes. This was mainly related to the strict exclusion criteria we applied
to limit our study to eyes with macular nonperfusion in the absence of DME.

Conclusions

We believe that ischemic maculopathy with underlying ischemia of the DCP may play an important role in photoreceptor compromise in diabetic patients. Based on our findings on OCT and corresponding FA, we propose that clinicians carefully consider outer retinal structural changes on OCT in the setting of DR because these changes may be harbingers of overlying areas of retinal capillary nonperfusion. Future studies to investigate visual function, including microperimetry, are needed to delineate the functional consequences of photoreceptor compromise in this population.


