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Association of Retinal Oximetry with Peripheral Diabetic Retinopathy Lesions and Nonperfusion on Ultra-widefield Angiography

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Purpose: To evaluate the association of retinal ischemia measured using retinal oximetry with retinal nonperfusion and predominantly peripheral lesions on ultra-widefield (UWF) fluorescein angiography (FA PPL).

Design: Prospective single-center, image evaluation study.

Participants: Images from 42 eyes from 21 participants with diabetes.

Methods: Ultra-widefield images were evaluated to determine diabetic retinopathy (DR) severity. Ultra-widefield FA images were used to measure nonperfusion area (NPA, mm^2) and nonperfusion index (NPI) and FA PPL presence. Retinal oximetry was performed to measure venous oxygen saturation (VO_2, %) and arteriovenous difference (A-V, %) within a 2-disc diameter ring centered on the optic disc.

Main Outcome Measures: Nonperfusion area, NPI, and presence of FA PPL.

Results: Mean age was 40.7 ± 10.4 years, diabetes duration 21.4 ± 10.0 years, hemoglobin A1c (HbA1c) 7.7 ± 1.0, 33.3% (14) were female, and 76.2% (32) had type 1. Distribution of DR on UWF color imaging was no-DR 9.5% (4); mild 45.2% (19), moderate 21.4% (9), and severe 9.5% (4) nonproliferative DR; and proliferative DR 14.3 (6) with FA PPL present in 25 (59.5%). Mean NPA/NPI was associated with increasing DR severity ($P = 0.0014/0.0018$), even after correction for diabetes duration and HbA1c ($P = 0.0029/0.0025$). In multivariate analysis adjusting for diabetes duration, HbA1c, and DR severity, the presence of FA PPL was associated with increasing VO_2 and decreasing A-V (VO_2; $P = 0.03$, A-V; $P = 0.009$).

Conclusions: Past studies have established an increased risk of DR progression with the presence of FA PPL. These data show that FA PPL presence is associated with retinal oximetry measures consistent with the presence of venous shunting or reduced retinal oxygen consumption, possibly indicative of greater areas of retinal ischemia. These findings highlight the value of retinal oximetry as a noninvasive measure of retinal ischemia and as a potential marker for increased risk of DR worsening.

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Diabetic retinopathy (DR) is characterized by alterations in the retinal blood vessels and blood flow, leading to retinal nonperfusion (NP) and ischemia.[1–4] Diabetes-induced NP and ischemia[5–8] typically affect the retinal capillaries and cause loss of these vessels in both the central and peripheral retina.[1] Fluorescein angiography (FA) is an invasive imaging technique that uses the intravenous administration of fluorescein dye to visualize areas of NP and capillary loss in the retina.[9] Retinal oximetry directly measures retinal oxygenation and ischemia based on the differential light absorption of oxygenated hemoglobin and deoxygenated hemoglobin allowing for a noninvasive measurement of oxygen saturation in retinal vessels.[10–12] These imaging techniques provide important additional information on the extent and severity of disease that is not captured by current DR severity scales.

Ultra-widefield (UWF) retinal photography, which images areas beyond the ETDRS 7-standard fields, has shown that predominantly peripheral lesions (PPL) are present in ~50% of eyes.[5,13–18] Predominantly peripheral lesions are defined as DR lesions that are more extensive or severe outside the ETDRS 7-standard fields. The presence of PPL is based on either UWF color or UWF FA imaging and indicates a more severe level of DR compared to lesions located solely within the ETDRS fields.[7,19] The baseline presence of PPL on UWF-FA (FA PPL) in an eye suggests a nearly twofold increased risk of future DR worsening and the development of vision-threatening retinopathy over the next 4 years.[5] Additionally, eyes with FA PPL show larger areas of retinal NP compared with eyes without FA PPL. These findings suggest that FA PPL can identify eyes within the same DR severity level that are at

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increased risk for DR progression. The use of UWF-FA provides the opportunity to visualize retinal perfusion across the vast majority of the retina using a single image and allows a more accurate quantification of the extent of retinal NP than previously possible. However, the widespread routine use of UWF-FA in the clinical setting to quantitate NP may not be feasible due to its invasive nature and associated risks. Further research is needed to establish noninvasive, safe, and cost-effective methods for assessing NP and ischemia.

Retinal oximetry, a noninvasive imaging technique, provides information about metabolic activity and directly quantifies retinal hypoxia by measuring oxygen levels within the retinal blood vessels. The use of retinal oximetry may offer a noninvasive measure of retinal ischemia, similar to the insights provided by FA PPL, and help identify eyes at increased risk for DR worsening. In this study, we investigate the relationship between retinal NP and retinal oxygenation to identify noninvasive retinal markers that are associated with established UWF-FA–derived features associated with DR worsening.

Methods

This single-site cross-sectional observational study was performed at the Beetham Eye Institute of the Joslin Diabetes Center in Boston, a tertiary referral center for diabetes care. The study protocol and design were consistent with the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board of the Joslin Diabetes Center. Informed consent was obtained from all patients before study participation, and the conduct of the study complied with the Health Insurance Portability and Accountability Act.

Eligible study participants were ≥18 years of age with a history of type 1 or 2 diabetes mellitus, willing to sign the consent form for this study, and able to undergo the study imaging procedures. Exclusion criteria included pupillary miosis or inability to dilate, prior panretinal laser photocoagulation treatment, and media opacities precluding adequate imaging of the retina. Each participant's medical record was reviewed for age, gender, duration of diabetes mellitus, and most recent glycated hemoglobin level (hemoglobin A1c [HbA1c]) preceding the imaging.

Study Procedures/Image Analysis

Certified retinal photographers acquired mydriatic nonsimultaneous stereoscopic, on-axis, nonsteered 200° UWF retinal color images and UWF-FA images using the California UWF device (Optos plc). The UWF color fundus photos were graded to determine ETDRS DR severity level. The UWF-FA images were evaluated for the presence or absence of FA PPL and to measure the extent of retinal NP. All images were evaluated at a centralized reading center under standardized conditions. All UWF images were projected stereographically using proprietary prototype software available from the manufacturer (Optos plc) and registered to ensure that the foveal center was located at the center of each image. A template of the combined 7 ETDRS fields was overlaid digitally on all UWF-FA images based on individual foveal and optic nerve head location to assess the distribution of FA PPL and NP in the periphery. Each lesion was graded separately and considered predominantly peripheral in a specific field if >50% of the lesions were in the retinal periphery compared with inside the ETDRS fields. An eye was considered to have an FA PPL if any lesion graded in any of ultrawide peripheral fields 3 through 7 was predominantly peripheral.[14] Measurement of retinal NP involved selecting UWF-FA frames with peak fluorescence to provide the highest contrast between perfused and nonperfused retina, allowing more accurate assessment of the extent of NP. The measurement of NP followed a standardized protocol that has been previously described.[5] Briefly, this included trained graders (C.M.P.) first delineating the total gradable area on the UWF-FA by drawing a line around the visible retina, as shown in Figure 1; by using the same free-hand tool, the NP areas (NPAs) were drawn over the edge of the perfused area bordering the NPA. The NP index (NPI) for each eye was calculated by dividing NPA by total gradable area. The total NPA and total gradable area for each eye were calculated in square millimeters by summing the size of all pixels that comprise the mask using a proprietary tool that implements DICOM Supplement 17311[20] and was provided by the manufacturer (Optos plc). Using this tool, the size of an individual pixel was defined by its location in the image and was calculated using spherical trigonometry after projecting it back onto a sphere, thus allowing an accurate measurement of retinal area (mm^2) independent of peripheral image distortion.

In this cohort, the retinal oximetry was performed by certified imagers (A.M.T, J.R.) using the Oxymap T1 (Oxymap ehf) after mydriasis. The oximetry equipment and the postprocessing image analysis have been previously described.[21] In brief, the

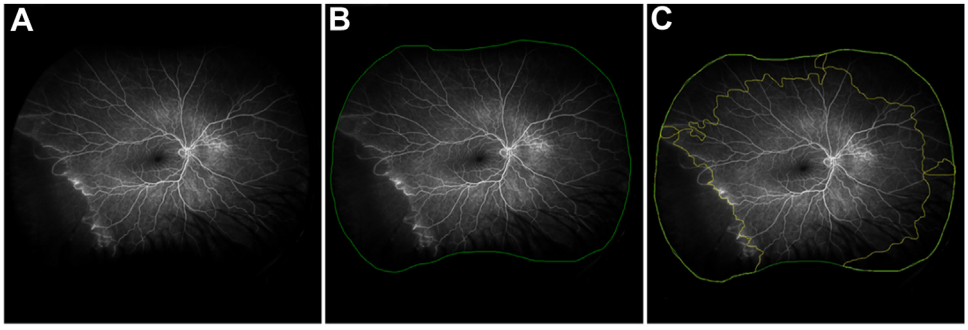

Figure 1. A, Ultra-widefield fluorescein angiography (UFW-FA) image. **B,** Green line indicates the total gradable area within the UWF-FA image. **C,** Yellow line delineates the extension of nonperfusion area as marked from trained grader by using free-hand tool for drawing.

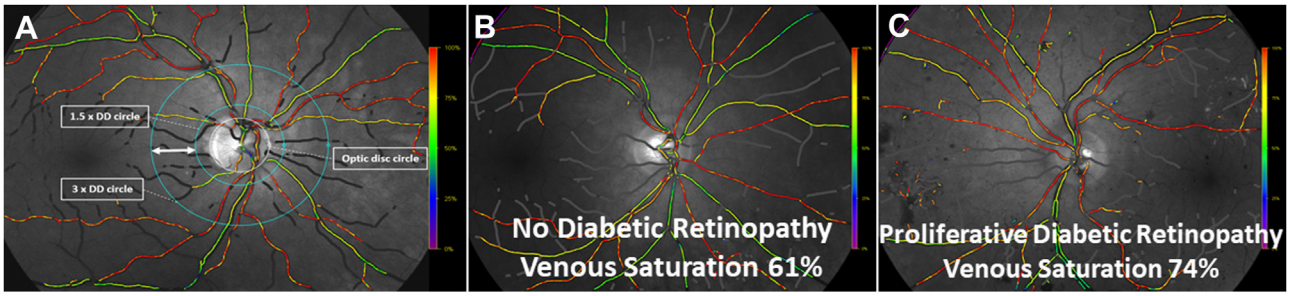

Figure 2. Retinal oximetry image maps. **A,** Annotated oximetry images with the white circle indicating the optic disc borders. The small and large blue circles are centered to the optic disc and their diameter is 1.5 and 3 times the diameter of the optic disc circle (white), respectively. Oximetry measurements were obtained from the vessels included within the measurement area as indicated by the white arrows. **B,** Retinal oximetry map of an eye with no diabetic retinopathy and a venous saturation of 61%. **C,** Retinal oximetry map of an eye with proliferative diabetic retinopathy with a venous saturation of 74%. DD = disc diameter.

spectrophotometric retinal oximetry calculates the difference in absorbance of oxygenated and nonoxygenated blood by using the reflection of light at 2 different wavelengths, one at 600 nm where the oxyhemoglobin is sensitive and one at 570 nm where it is not. Oxygen saturation in arterioles and venules was calculated by the extraction of the relative reflectance from the larger vessels and the surrounding fundus tissue at these 2 different wavelengths. The captured images were evaluated by a trained grader (C.M.P.) and were analyzed using the Oxymap Analyzer V2.1 software, which automatically detects all pixels of the retinal vessels and classifies them as arterioles or venules. The optic disc was manually selected and demarcated with a circle and then the software automatically created 2 concentric larger circles, centered on the optic disc using a diameter of 1.5 and 3 times that of the optic disc circle (Fig 2). Oximetry measurements were obtained from the vessels within the area between these 2 larger circles. Venous oxygen saturation (VO_2) and the arterial-venous saturation (A-VO_2) difference were determined. All overlapping, branching, or intersecting vessels within the measurement area were excluded.

Statistical Analysis

Nonparametric analyses (Wilcoxon rank-sum tests) were used to compare distributions of continuous variables between groups of eyes with versus without FA PPL. The chi-square test was used to compare frequencies of categorical variables as appropriate. Logistic multivariate regression models were used to adjust for the possible confounding parameters of diabetes type and duration, DR grade, and HbA1c levels. These multivariate models used repeated measures to account for correlations between eyes from individual participants in the study. An alpha value of ≤ 0.05 was considered significant. All analyses were performed using SAS software, version 9.4 (SAS Inc).

Results

A total of 42 eyes from 21 participants were evaluated in the study. The participant demographic and ocular characteristics are detailed in Table 1. The distribution of ETDRS DR severity based on UWF color images of the cohort was 12.5% no DR, 39.6% mild nonproliferative DR (NPDR), 18.8% moderate NPDR, 12.6% severe NPDR, and 12.6% proliferative DR (PDR). Fluorescein angiography PPL were present in 59.5% of the eyes.

On UWF-FA images, there was a significantly greater mean NPA ($P = 0.0012$, Wilcoxon rank-sum test) and NPI ($P = 0.0018$) associated with worsening DR severity (Fig 3). These relationships remained significant after adjusting for age, type and duration of diabetes, and HbA1c ($P = 0.0010$ for NP, $P = 0.0020$ for NPI, logistic multivariate regression). In multivariate analysis, the presence of FA PPL was significantly associated with larger total NPA (Table 2).

In this cohort, retinal oximetry measures were significantly associated with the presence of FA PPL. There was significantly higher mean VO_2 in eyes with FA PPL compared with eyes without FA PPL ($P = 0.0116$) (Table 2). Similarly, the presence of FA PPL was significantly associated with decreased A-VO_2 compared with the absence of FA PPL ($P = 0.0017$). In multivariate

Table 1. Study Participant Demographic and Ocular Characteristics

Participants (N = 21)	**Total N (%) or Mean ± SD**
Female	14 (34.8)
Age (yrs)	40.6 ± 10.4
Diabetes type 1	32 (76.2)
Duration of diabetes (yrs)	21.4 ± 10.0
HbA1c (%)	7.7 ± 1.0
Eyes (N = 42)	**Total N (%)**
Diabetic retinopathy severity by ultra-widefield color images (masked to ETDRS 7-fields)	
No DR (level 10)	4 (9.5)
Mild NPDR (level 35)	19 (45.2)
Moderate NPDR (levels 43, 47)	9 (21.4)
Severe and very severe NPDR (level 53)	4 (9.5)
PDR (level 65, 71, 75)	6 (14.2)
FA PPL present	25 (59.5)

DR = diabetic retinopathy; FA PPL = predominantly peripheral lesions on ultrawidefield fluorescein angiography; HbA1c = hemoglobin A1c; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SD = standard deviation.

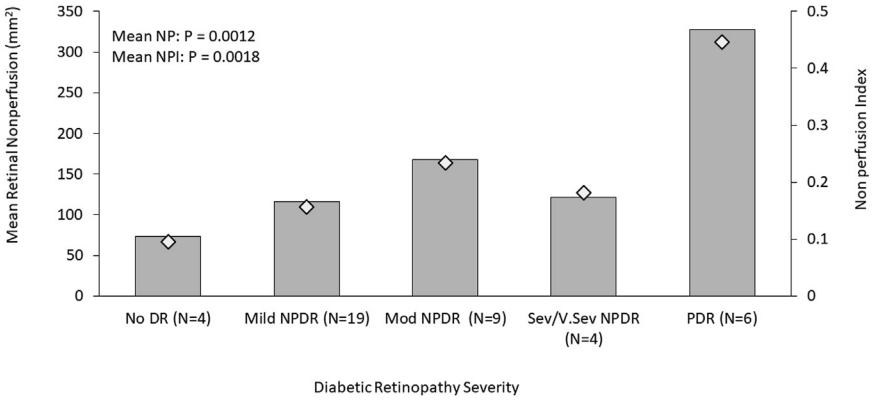

Figure 3. Relationship of diabetic retinopathy severity with retinal nonperfused area and nonperfusion index. Bars represent the average total retinal nonperfused area on UWF-FA in each DR severity group. The mean overall NPIs for each DR severity group are shown as diamond symbols. DR = diabetic retinopathy; FA = fluorescein angiography; Mod Sev = moderately severe; NPDR = nonproliferative diabetic retinopathy; NPI = nonperfusion index; PDR = proliferative diabetic retinopathy; Sev/V.Sev = severe or very severe; UFW = ultra-widefield.

analysis, the presence of FA PPL remained significantly associated with higher VO_2 and lower A-VO_2 (Table 2). No statistical significance was observed between retinal oximetry measurements and NP.

In this cohort, we did not observe a direct association between retinal oximetry measurements and DR severity (Table 3), despite the strong associations with established markers of DR progression on UWF-FA. There was higher VO_2 and lower A-VO_2 in eyes with FA PPL, potentially indicating larger areas of retinal ischemia, but these findings were not statistically significant.

Discussion

Results from this study demonstrate that the presence of FA PPL, apart from their established association with retinal NP, is highly associated with increased VO_2 and decreased A-VO_2 difference, both of which are retinal oximetry measures indicative of conditions such as A-V shunting, reduced oxygen consumption, reduced retinal cell viability, increased NPA, and possibly retinal ischemia. These associations with retinal oximetry measures are independent of DR severity and remain significant after adjusting for age, diabetes type and duration, and HbA1c.

The results of this study align with findings from a larger prospective cohort, emphasizing the relationship between retinal NP, FA PPL, and increasing DR severity. These results underscore the importance of UWF retinal imaging for disease severity stratification in DR.[5,14,22] Our prior work and results from the multicenter, nationwide DRCR Retina Network Protocol AA confirmed that a high NPI at baseline and the presence of FA PPL were associated with a greater risk of DR worsening over a 4-year follow-up period.[7,19] Furthermore, studies on widefield swept-source OCT angiography have shown that lower vessel density and increased NPA are associated with increasing DR severity in eyes with DR.[23,24] Capillary loss, peripheral vessel narrowing, and greater NPA have also been linked with PPL and worsening of DR severity.[25] In the present cohort, bivariate analysis showed that greater NPA and NPI were associated with presence of FA PPL. We conducted an exploratory analysis of disease progression in 17 patients who had follow-up visits for $\geq$4 years (mean follow-up duration: 4.4 $\pm$ 2.1 years). Progression by $\geq$1 step in the clinical ETDRS severity scale was observed in 6 eyes (14.3%) from 4 patients, including 1 eye progressing from severe NPDR to PDR, 2 eyes from moderate NPDR to PDR, 1 eye from mild NPDR to PDR, and 1 eye from mild NPDR to moderate NPDR. All eyes that showed

Table 2. Nonperfusion Extent on Ultra-widefield Images and Retinal Oximetry Data

	FA PPL Absent (N = 17 Eyes) (Mean ± SD)	FA PPL Present (N = 25 Eyes) (Mean ± SD)	*P* Value	Adjusted *P* Value*
NPA (mm^2)	112.8 ± 78.7	181.7 ± 111.0	0.0108	0.0446
NPI	0.16 ± 0.13	0.24 ± 0.15	0.0190	0.1115
VO_2 Sat. (%)	67.5 ± 4.7	71.3 ± 4.1	0.0116	0.0256
A-VO_2 Sat. (%)	30.1 ± 4.1	25.6 ± 3.9	0.0017	0.0079

A-VO_2 Sat = arterial/venous oxygen saturation difference; FA PPL = predominantly peripheral lesions on ultrawidefield fluorescein angiography; NPA = nonperfusion area; NPI = nonperfusion index; SD = standard deviation; VO_2 Sat = venous oxygen saturation.
*Adjusted *P* value for age, diabetes type, diabetic retinopathy severity, diabetes duration, and hemoglobin A1c.

Table 3. Relationship of Retinal Oximetry Measurements with DR Severity Distribution and Presence of FA PPL

	No DR (N = 4) (Mean ± SD)		Mild NPDR (N = 19) (Mean ± SD)		Moderate NPDR (N = 9) (Mean ± SD)		Severe NPDR (N = 4) (Mean ± SD)		PDR (N = 6) (Mean ± SD)		*P* Value
	(−) FA PPL (N = 5)	*(+) FA PPL (N = 1)*	*(−) FA PPL (N = 9)*	*(+) FA PPL (N = 10)*	*(−) FA PPL (N = 2)*	*(+) FA PPL (N = 7)*	*(−) FA PPL (N = 1)*	*(+) FA PPL (N = 3)*	*(−) FA PPL (N = 2)*	*(+) FA PPL (N = 4)*	
VO_2 (%)	68.6 ± 5.7	70.4 ± 0	67.7 ± 4.4	71.5 ± 4.1	64.5 ± 7.7	71.5 ± 4.6	68.1 ± 0	72.1 ± 6.0	67.5 ± 1.5	70.4 ± 3.9	0.97
A-VO_2 (%)	28.4 ± 4.3	30.0 ± 0	29.3 ± 3.5	24.3 ± 3.6	31.7 ± 6.2	27.1 ± 3.4	38.6 ± 0	25.0 ± 4.1	29.3 ± 0.3	25.4 ± 5.6	0.77

A-VO_2 = arterial/venous oxygen saturation difference; DR = diabetic retinopathy; (−) FA PPL = without the presence of predominantly peripheral lesions on ultra-widefield fluorescein angiography; (+) FA PPL = with the presence of predominantly peripheral lesions on ultra-widefield fluorescein angiography; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SD = standard deviation; VO_2 = venous oxygen saturation.

progression had FA PPL at baseline, with a progression rate of 37.5%, compared with 0% in eyes without FA PPL ($P = 0.0167$). These findings highlight the association between baseline FA PPL and an increased risk of DR worsening. These data reinforce the importance of identifying noninvasive methods to quantify retinal NP in eyes at risk for DR development and worsening.

Protocol AA from the DRCR Retinal Network, which was a prospective multicenter longitudinal trial, has established both FA PPL and retinal NP to be significant predictive markers for DR disease progression that are independent of DR severity.[7,19] Previous larger studies on retinal oximetry highlighted the heterogeneity of eyes within each DR severity level, suggesting the need to identify progression markers that are independent of DR severity. In this study, the presence of FA PPL was found to be significantly associated with retinal oximetry measurements and provides preliminary evidence to potentially support the value of retinal oximetry as a noninvasive measure of retinal ischemia and as a marker for an increased risk of DR worsening.

The retinal capillary network is distinct from the other vascular networks due to its autoregulation and response to varying metabolic demands.[26] Retinal oximetry utilizes blue wavelengths to quantify retinal metabolic activity by calculating the relative hemoglobin oxygen saturation based on the different light absorption.[12] Studies on retinal oximetry and widefield scanning laser ophthalmoscopy have shown that hyporeflective areas on red-free scanning laser ophthalmoscopy images are associated with peripheral retinal NP and ischemia in eyes with DR.[27,28] In this study, there were no significant associations between retinal NP or NPI and retinal oximetry measurements. This is possibly due to the limited sample size within each severity level and the heterogeneous distribution of NP among the eyes even within the same DR severity level. However, we have shown the significant association between the presence of FA PPL with the retinal oximetry measures of retinal ischemia. Specifically, the presence of FA PPL in the retina is strongly associated with increased VO_2 and decreased A-VO_2 difference, independent of DR severity, age, diabetes' type and duration, and HbA1c.

Furthermore, past retinal oximetry results in eyes with vision-threatening DR have demonstrated retinal regional oxygen saturation differences in larger vessels with higher VO_2 and lower A-VO_2 values in venules draining the macula.[29] In patients with type 2 diabetes without clinically apparent DR, retinal oximetry measurements detected regional differences within the peripapillary area without any noticeable changes in the microvascular hemodynamics, implying that detectable regional changes in retinal metabolic activity may be linked with disease heterogeneity and risk of worsening.[30] In eyes with NPDR and type 1 diabetes, age and lower A-VO_2 were associated with the presence of DR independently of other known factors for developing DR.[31] Moreover, longitudinal data of repeated oximetry results over a 3-year follow-up period have shown an increase in VO_2 and a decrease in A-VO_2 over time in eyes with DR, even before

any clinically observable changes. These findings suggest that oxygen saturation of the large vessels may be a sensitive marker to detect disease worsening.[32] In this cohort, the presence of FA PPL and their association with the underlying hypoxia markers of VO_2 and A-VO_2, independent of DR severity level and diabetes status, suggest that metabolic changes in the peripheral retina may precede the macular alterations and potentially indicate a higher long-term risk of disease progression.

In contrast to previous reports, we did not find a significant association between increasing DR severity and increases in VO_2. Prior studies have shown that hemoglobin oxygen saturation in retinal arterioles and venules increases with worsening DR severity.[32–34] However, in this cohort, we did not observe a direct association between retinal oximetry measurements and DR severity, despite strong associations with established markers of DR progression on UWF-FA. These findings suggest that retinal oximetry markers can reflect changes in retinal oxygenation specifically related to DR progression that may not be captured on the traditional DR severity scale, highlighting retinal oximetry as a potential noninvasive marker for risk of worsening independent of DR severity.

Limitations of this study include the exploratory nature of the analysis with limited patient numbers and without power calculation. Moreover, we focused on retinal oximetry measurements in large vessels within the peripapillary area, as the current retinal oximetry devices and software are limited to evaluating this region of the retina. Information of oxygenation throughout the retina, specifically in smaller vessels and capillaries, might be accomplished using UWF blue light imaging and could provide further insights regarding the pathophysiology of retinal tissue hypoxia and NP. Prior research has demonstrated that retinal oximetry measurements may vary with race and retinal pigmentation.[35] In this cohort, 95.3% of the patients were White, highlighting the need for future studies to investigate retinal ischemia markers for DR in more racially diverse populations. Advancements in retinal oximetry imaging technology combined with studies involving larger and more diverse sample sizes may allow for a more detailed evaluation of oxygenation and metabolic activity of the entire retina as we strive to predict eyes at increased risk of DR progression.

Past studies have established the increased risk of DR progression with the presence of FA PPL. Our data show that FA PPL presence is associated with retinal oximetry measures consistent with the presence of venous shunting or reduced retinal oxygen consumption, possibly indicative of greater areas of retinal ischemia. These findings underscore the potential value of retinal oximetry as a noninvasive measure of retinal ischemia and as a marker for an increased risk of DR worsening.

Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. This single-site, cross-sectional observational study was performed at the Beetham Eye Institute of the Joslin Diabetes Center in Boston, a tertiary referral center for diabetes care. The study protocol and design were consistent with the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board of the Joslin Diabetes Center. Informed consent was obtained from all patients before study participation, and the conduct of the study complied with the Health Insurance Portability and Accountability Act.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Sampani, Ashraf, Sun, Aiello, Silva

Data collection: Sampani, Ashraf, Pitoc, Rhee, Tolson, Cavallerano, Sun, Aiello, Silva

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Overall responsibility: Ashraf, Sun, Aiello, Silva, Sampani

Abbreviations and Acronyms:
A-VO_2 = arterial-venous saturation; **DR** = diabetic retinopathy; **FA** = fluorescein angiography; **HbA1c** = hemoglobin A1c; **NP** = nonperfusion; **NPA** = nonperfusion area; **NPDR** = nonproliferative diabetic retinopathy; **NPI** = nonperfusion index; **PDR** = proliferative diabetic retinopathy; **PPL** = predominantly peripheral lesions; **UWF** = ultra-widefield; **VO_2** = venous oxygen saturation.

Keywords:
Diabetic retinopathy, Retinal ischemia, Retinal nonperfusion, Retinal oximetry, Ultra-widefield angiography.

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