Regional Patterns of Retinal Oxygen Saturation and Microvascular Hemodynamic Parameters Preceding Retinopathy in Patients With Type II Diabetes

Julia Hafner,1 Laurin Ginner,2,3 Sonja Karst,1 Rainer Leitgeb,2,3 Michael Unterluggauer,2,3 Stefan Sacu,1 Christoph Mitsch,1 Christoph Scholda,1 Eleonore Pablik,4 and Ursula Schmidt-Erfurth1

1Department of Ophthalmology and Optometry, Medical University of Vienna, Vienna, Austria
2Center of Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria
3Christian Doppler Laboratory for Innovative Optical Imaging and its Translation to Medicine, Medical University of Vienna, Vienna, Austria
4CeMSIIS, Institute for Medical Statistics, Medical University of Vienna, Vienna, Austria

Correspondence: Sonja Karst, Department of Ophthalmology and Optometry, Medical University Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria; sonja.karst@meduniwien.ac.at.

Submitted: June 30, 2017
Accepted: September 30, 2017

PURPOSE. Alterations in retinal oxygen metabolism and retinal microcirculation are signs of impending diabetic retinopathy (DR). However, if specific retinal regions are primarily affected is so far unknown. The purpose of this study was to investigate if retinal oxygen saturation (SO2) and microvascular hemodynamic parameters follow a distinct regional pattern in patients with diabetes but no DR.

METHODS. Patients with type II diabetes without clinically apparent DR were imaged as follows: SO2 in peripapillary vessels was assessed with dual-wavelength oximetry. Optical coherence tomography angiography (OCTA) scans were acquired with a prototype system using a swept-source laser with an effective 400 kHz A-scan rate and 16° field of view. Regional flow indices termed “flux” were calculated for the peripapillary microvasculature. Parafoveal capillary density was evaluated with the commercially available AngioVue OCTA.

RESULTS. Twenty-nine eyes of 16 consecutive patients (59 ± 10 years, 6 females) were included in this study. SO2 differed significantly between quadrants (P < 0.001), with a decreasing pattern from the upper nasal through the lower nasal, the upper temporal and the lower temporal quadrant in arterioles and venules. In contrast, peripapillary flux followed an increasing trend from nasally to temporally. Peripapillary and parafoveal microvascular hemodynamic parameters demonstrated no significant regional variability as observed for retinal oxygenation.

CONCLUSIONS. Metabolic imaging identified regional differences in retinal SO2 without an associated topographic variance in microvascular hemodynamics in type II diabetes without DR. Future studies should focus on the mechanisms causing this heterogeneity in metabolic demand.

Keywords: diabetic retinopathy, retinal hemodynamics, retinal oximetry, biomarkers in disease

Microaneurysms and capillary closure are the earliest fundoscopically detectable lesions in diabetic retinopathy (DR). These changes are known to be nonuniformly distributed across the retina. The superior temporal quadrant is affected first. Morphologic lesions in this quadrant are twice as prevalent as in the inferior nasal one, where signs tend to appear latest in disease development.1,2 This distribution has also been reported for acellular capillaries and pronounced retinal dilatation of veins, mirroring hyperglycemic severity.3 Applying ultrawide field imaging techniques, it has recently been proposed that similar to the pattern found in the posterior pole, microaneurysms, venous beading, and hemorrhages are more pronounced in the temporal than nasal field of the retinal periphery.4

However, these morphologic lesions only appear secondary to early disturbances in the retinal microcirculation and retinal metabolism of patients with diabetes. The structure of the retinal microvasculature is unique: It feeds this highly metabolically active tissue whilst limiting the extent of the vascular beds to minimize optical interference to the photoreceptors5: The inner retina is perfused by three interconnected capillary plexi that include a superficial capillary plexus (SCP), which is found in the retinal nerve fiber layer (RNFL) adjacent to the optic nerve head (ONH) and migrates into the ganglion cell layer toward the macula, as well as an intermediate and a deep capillary plexus (DCP), which are located at the two borders of the inner nuclear layer (INL).6 The vascular layers in the center of the macula form a terminal capillary ring surrounding the foveal avascular zone (FAZ), which is totally dependent on blood supplied by diffusion from the choriocapillaris. This ring may have irregularities and ragged borders, ultimately leading to an enlargement of the FAZ associated with capillary dropout, a potential sign for impending diabetic macular ischemia.7 Objective quantification of such
early features of microvascular dysfunction is now feasible with functional imaging including optical coherence tomography angiography (OCTA). The benefits of OCTA for a high resolution visualization of subtle changes in the macular region of patients with diabetes have already been outlined in previous studies. There has though been little focus on functional evaluation of the peripapillary microcirculation.

Another early hallmark, regarded as a direct measure of metabolic dysfunction even before the presence of DR, is the alteration of oxygen saturation in retinal vessels. Retinal oxygenation has been proposed to be altered in patients with diabetes ranging from no DR to vision-threatening DR. Yet, as for retinal blood flow changes in diabetes, literature findings on oxygen saturation still seem inconsistent: There are studies demonstrating significantly increased arteriolar and venular oxygen saturation in patients with proliferative DR (PDR) compared with controls, without a trend of increasing oxygen saturation with increasing severity of the disease. Results of a different study suggest that only venular oxygen saturation increases with increasing severity of DR. Retinal oxygenation has been proposed to be altered in patients with diabetes ranging from no DR to vision-threatening DR. Yet, as for retinal blood flow changes in diabetes, literature findings on oxygen saturation still seem inconsistent: There are studies demonstrating significantly increased arteriolar and venular oxygen saturation in patients with proliferative DR (PDR) compared with controls, without a trend of increasing oxygen saturation with increasing severity of the disease.

There is evidence for a geographic variability in the distribution of fundoscopically manifest lesions in DR. However, current hypotheses on the pathogenesis of DR fail to account for this incidence, and so far it is still unknown if there is topographic variance in retinal metabolic and microvascular hemodynamic parameters, which may be precious biomarkers for DR development. Therefore, the purpose of this study was to investigate if retinal oxygen saturation (SO2) and macular as well as peripapillary hemodynamic parameters follow a distinct regional pattern in patients with diabetes without DR.

METHODS

All study investigations were conducted in accordance with the tenets of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Medical University of Vienna (EK1134/2015) and registered in the European Clinical Trials Database (EudraCT-201560002359634). All patients gave written informed consent to retinal imaging as part of the study protocol.

Study Cohort

We included consecutive adult patients with a history of type II diabetes for at least 6 months without fundoscopic signs of DR in this cross-sectional study. Exclusion criteria were other ocular diseases (e.g., glaucoma, retinal detachment, macular hole, age-related macular degeneration, retinal vascular occlusion, macular dystrophies), media opacities, structural damage to the center of the macula, active intraocular inflammation, and previous intraocular surgery. After Early Treatment Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) testing, a slit-lamp examination of the anterior segment, intraocular pressure measurement, and fundus biomicroscopy, ophthalmic imaging was performed as follows.

OCTA of the Peripapillary Region. Inner retinal peripapillary microvascular flow profiles were assessed with OCTA centered on the ONH using a prototype system with a swept source laser at a central wavelength of 1050 nm and bandwidth of 110 nm. The sweep rate of the light source is 200 kHz, which is virtually doubled to achieve 400 kHz A-scan rate applying spectral splitting in post processing. This enables OCTA imaging with a field of view of up to 16° with sufficient sampling in order to guarantee high resolution for the OCTA images. The x-scanner (fast axis) scans linearly, whereas the y-scanner (slow axis) scans in steps with a vertical sampling of 400 steps. For OCTA, four tomograms are recorded at each sampling point in y-direction, resulting in a total of 1600 tomograms per volume. The lateral number of sampling points is 800 for 200 kHz and 1600 for the split spectrum method at 400 kHz, respectively. Detailed specifications of the system have already been published. In post processing, the retinal layers were segmented automatically using a graph-based approach. The boundaries for the analysis were set from the inner limiting membrane (ILM) to the retinal pigment epithelium (RPE) in order to visualize the retinal vasculature only and to avoid interfering signal from the choroid. Further en face analysis was conducted within a circle of 800 pixels diameter (corresponding to approximately 5.2 mm diameter, adjusted to axial length of the eye) centered on the ONH, excluding the area of the ONH itself (mean area excluded: 6562 pixels corresponding to 1.11 mm²). After automatic segmentation of large vessels with subsequent exclusion of these, we calculated a microvascular flow index termed “flux,” which has been defined as “mean flow signal intensity” ranging from 0 to 1. Flux was analyzed for the total

FIGURE 1. (A) Segmentation boundaries from ILM (red line) to RPE (orange line). (B) Peripapillary OCTA with 16° field of view. (C) Field of analysis for retinal quadrants after segmentation of major vessels.
peripapillary ring area. In addition, flux was assessed for the superior temporal, inferior temporal, superior nasal and inferior nasal section of the ring (Figs. 1A–C).

**OCTA of the Macular Region.** Macular microvascular variables were analyzed using the AngioVue OCTA system (RTVue-XR Avanti; Optovue, Fremont, CA, USA), which applies a split-spectrum amplitude decorrelation angiography (SSADA) software algorithm (version 2016.1.0.2). The device acquires 70,000 A-scans per second to compose OCTA volumes consisting of $304 \times 304$ A-scans. All investigators were instructed to repeat imaging until scans with a signal strength index of at least 72 were obtained. All analyses were performed in the $3 \times 3 \text{mm}$ OCTA volumes by the same masked investigator (JH). Images with segmentation errors or artifacts (e.g., double vessel pattern or dark areas from motion, blur and floaters that obscure vessel signal) were excluded. The OCTA images of the superficial and deep capillary network were generated separately using the automated software algorithm of the machine: The superficial network extends from 3 µm below the ILM to 15 µm below the inner plexiform layer (IPL). The deep capillary network extends from 15 to 70 µm below the IPL. Vessel density was defined as the proportion of vessel area with blood flow over the total area measured. It was evaluated in the superficial and deep capillary network of the parafoveal area with a diameter of 3 mm, excluding the central millimeter of the fovea (Fig. 2). The following variables of interest were evaluated: total parafoveal, as well as superior, temporal, inferior, and nasal parafoveal vessel density in percent. Additionally, the size of the FAZ ($\text{mm}^2$) was evaluated in the superficial and deep plexus by automated delineation using the nonflow area tool (Figs. 3A, 3B).

**Retinal Oximetry.** Fundus camera-based, dual-wavelength retinal oximetry (Oxymap ehf., Reykjavik, Iceland) was performed to measure SO2 in major peripapillary arteries and veins, which were defined by a diameter of $\geq 93 \mu \text{m}$ and a segment length of $\geq 465 \mu \text{m}$. We adhered to these thresholds as SO2 results become unreliable in thin and short vessel segments.24 All study visits were scheduled in the mid-afternoon to exclude the possibility of circadian fluctuations in SO2. The precise oximetry technique has been described previously.25 In brief, the oximeter records images reflected from the retina at an oxygen-sensitive (600 nm), as well as an oxygen-insensitive (570 nm) wavelength. An inverse linear relation between the optical density ratio measured at the two wavelengths and SO2 is assumed.25 Studies have shown that this retinal oximetry technique is remarkably stable with high

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**Figure 2.** Parafoveal vessel density analysis of the central area of 3 mm diameter excluding the central 1 mm; parameters of interest are highlighted in red.

**Figure 3.** (A) Superficial FAZ area in $3 \times 3 \text{mm}$ volume scan. (B) Deep FAZ area in $3 \times 3 \text{mm}$ volume scan.
reproducibility and repeatability of measurements in healthy subjects as well as in diseased retinas.12,26–29

The same masked investigator (JH) performed all analyses with the built-in software (version 2.4.0). The best quality image with the ONH in its center was chosen from every patient for further analysis according to a standard protocol given by the manufacturer: Two circles of 1.5 and 3 times the optic disc diameter were created, and all vessel segments (≥93 µm diameter and ≥465 µm length) between these two circles were selected (Fig. 4). The mean SO2 and mean diameter of the selected arteries and veins were then automatically analyzed. Different blood flow in vessels was taken into account because the software adjusts for artifactual SO2 changes as a result of vessel width by weighing the means with the fourth power of the vessel diameter.30 The location of each vessel segment was registered according to which retinal quadrant they belonged to (superior temporal, inferior temporal, superior nasal, and inferior nasal quadrant section of the ring). The saturation in venules (SO2v) was subtracted from the saturation in arterioles (SO2a) to obtain the oxygen extraction (arterio-venous oxygen saturation difference).

**Statistical Analysis**

A statistician from the Department for Medical Statistics (Medical University of Vienna) performed all statistics using SAS (version 9.4) and R (version 3.3.3). All variables are presented as mean ± standard deviation (SD) for continuous and as percentages for categorical variables. Differences between measurements in the four quadrants of the retina were analyzed using a mixed linear regression model. To adjust for multiple measurements in one patient, the individual patient was included as a random factor in this model. The level of significance for the comparison between quadrants was set to 0.0083, due to Bonferroni correction for the multiple testing. If significant differences between the quadrants were found, pairwise testing between the quadrants followed. This pairwise testing was done with $t$-tests under $P$ value adjustment with Tukey’s honest significant difference (HSD). For all these secondary analyses, the level of significance was set to 0.05. Unpaired $t$-tests were used to evaluate differences between our sample and the corresponding normative values in a healthy population published by Jani et al.31 We did not receive access to the original data of Jani et al.,31 and therefore could not calculate the exact standard error of the mean for the repeated measurements in that sample. Hence, we estimated the standard error of the mean for the $t$-test as SD/$\sqrt{\text{number of patients}}$. This is a very conservative approach to avoid false positive findings, but might result in a lack of power. Boxplot graphs were used for graphic visualization of differences between variables.

**RESULTS**

We included 29 eyes (14 right, 15 left eyes) of 16 patients (6 females) for the analyses of this study. Baseline demographic
TABLE 1. Baseline Patient Characteristics

Baseline Demographic and Clinical Characteristics of Patients With Type II Diabetes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, n (%)</td>
<td>6 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>59 ± 10</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration, mean ± SD, y</td>
<td>13 ± 14</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin A1c, mean ± SD, %</td>
<td>7.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>OAD + insulin†, n (%)</td>
<td>9 (56.3)</td>
<td></td>
</tr>
<tr>
<td>Systolic/diastolic BP ≥ 140/90†, n (%)</td>
<td>12 (75)</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>10 (62.5)</td>
<td></td>
</tr>
</tbody>
</table>

BP, blood pressure.
† Oral antidiabetic medication intensified with subcutaneous insulin therapy.
‡ Based on the medical records provided by the primary care physician or specialist for internal medicine.

and clinical characteristics of the patients are summarized in Table 1.

Regional Patterns of SO2

All study patients were of central European descent with a similar fundus pigmentation, providing the precondition for a comparison of SO2 results. The mean vessel diameter was 132.12 ± 10.79 μm and 158.81 ± 15.23 μm for arterioles and venules, respectively, resulting in a mean arterio-venous ratio of 0.84 ± 0.1. The mean SO2a was 96.88% ± 6.07% and the mean SO2v was 62.43% ± 9.51%. It followed a trend being higher in the inferior temporal, followed by the inferior nasal, superior nasal, and superior temporal region (Table 2), but without statistically significant differences between these quadrants. Table 2 illustrates that the highest arterial and venous SO2 were found in the superior nasal quadrant, followed by the inferior nasal, superior temporal, and inferior temporal quadrant. Significant differences were demonstrated between the four quadrants for SO2a as well as SO2v (P < 0.001). The arterial oxygen saturation in the inferior temporal sector was statistically significantly lower than in the superior nasal and the inferior nasal sector; additionally, the superior nasal quadrant showed statistically significantly higher arterial oxygen saturation than the superior temporal quadrant. Venous oxygen saturation was statistically significantly lower in the inferior temporal than in the superior nasal, superior temporal, and inferior temporal sector. Regional differences in SO2a and SO2v as well as arterio-venous differences are presented in Figure 5.

Regional Patterns of Peripapillary Microvascular Hemodynamics

The mean peripapillary flux in this cohort of type II diabetic patients was 0.117 ± 0.030. The highest flux values were found in the superior temporal, followed by the inferior temporal and superior nasal quadrant, with the lowest flux in the inferior nasal region (Table 2). This regional pattern differed from that observed for SO2 (following a trend of higher flux temporally than nasally) but contrary to SO2 results, we found no statistically significant difference in flux between the four retinal quadrants.

Regional Patterns of Macular Microvascular Hemodynamics

The segmentation algorithm of the Angiovue software allows a differentiated analysis between a “superficial FAZ” and a “deep FAZ.” The means were 0.310 ± 0.137 mm² and 0.424 ± 0.226 mm² for the superficial and deep FAZ, respectively, in this patient cohort. The parafoveal vascular density in the measurement area was higher in the DCP (61.28% ± 5.37%) than in the SCP (52.57% ± 4.31%) (P < 0.001).

Parasfoveal vascular density tended to be higher superiorly (52.47 ± 5.03 in the SCP, 62.06 ± 5.08 in the DCP) than inferiorly (51.84 ± 5.11 in the SCP, 61.51 ± 4.28 in the DCP), and higher nasally (53.18 ± 4.40 in the SCP, 60.93 ± 4.35 in the DCP) than temporally (52.80 ± 4.35 in the SCP, 60.69 ± 3.85 in the DCP) for the SCP and DCP, but without statistically significant difference between these parafoveal sectors.

DISCUSSION

We present the first study to investigate the presence of a distinct regional pattern in SO2, parafoveal vessel density, and peripapillary flux in patients with type II diabetes without DR. The superior nasal quadrant displayed the highest arteriolar and venular SO2 levels, which then followed a decreasing pattern over the inferior nasal, superior temporal, and inferior temporal quadrant. As explained by the manufacturer, the retinal oximeter is only capable of giving SO2 values relative to the normative values defined for a healthy Icelandic population. Our results therefore sometimes exceeded 100%. Similar findings have been reported previously in patients with diabetes and may be explained by an alteration of the optical densities in these patients as well as the different retinal pigmentation compared to Icelandic individuals. We compared the mean SO2 levels of our patient cohort (96.88% ± 6.07% and 62.43% ± 9.51% for arteriolar and venular SO2, respectively) with the corresponding values (90.4% ± 4.5% and 55.3% ± 7.1% for arteriolar and venular SO2, respectively) in a retinal oximetry normative database of healthy individuals from diverse ethnic backgrounds.

Table 2. Peripapillary SO2 and Flux for Quadrants

<table>
<thead>
<tr>
<th>Retinal Quadrant</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial Oxygen Saturation, %</td>
<td>96.12 ± 5.30</td>
</tr>
<tr>
<td>SN</td>
<td>99.52 ± 7.12</td>
</tr>
<tr>
<td>IT</td>
<td>93.70 ± 5.72</td>
</tr>
<tr>
<td>IN</td>
<td>98.22 ± 4.62</td>
</tr>
<tr>
<td>Venous Oxygen Saturation, %</td>
<td>63.63 ± 6.70</td>
</tr>
<tr>
<td>ST</td>
<td>65.68 ± 8.17</td>
</tr>
<tr>
<td>SN</td>
<td>56.26 ± 11.45</td>
</tr>
<tr>
<td>IN</td>
<td>64.00 ± 7.12</td>
</tr>
<tr>
<td>Oxygen Extraction Rate, %</td>
<td>32.86 ± 7.89</td>
</tr>
<tr>
<td>ST</td>
<td>34.14 ± 9.48</td>
</tr>
<tr>
<td>SN</td>
<td>37.06 ± 13.06</td>
</tr>
<tr>
<td>IN</td>
<td>34.35 ± 5.69</td>
</tr>
<tr>
<td>Peripapillary Flux</td>
<td>0.1174 ± 0.0325</td>
</tr>
<tr>
<td>ST</td>
<td>0.1159 ± 0.0346</td>
</tr>
<tr>
<td>SN</td>
<td>0.1163 ± 0.0338</td>
</tr>
<tr>
<td>IN</td>
<td>0.1143 ± 0.0333</td>
</tr>
</tbody>
</table>

ST, superior temporal; SN, superior nasal; IT, inferior temporal; IN, inferior nasal.
that a maldistribution of oxygen even occurs in patients with diabetes without DR.

Even though our results depict regional differences in SO2, no definite pattern was found for peripapillary flux or parafoveal vessel density. Our special focus was on the peripapillary microvascular region as, at least to our knowledge, this area has not been examined in any former series of patients with type II diabetes. Flux was analyzed using an innovative OCTA technique in a 16° field of view around the ONH. Despite a trend of higher flux temporally than nasally to the ONH, we could not reveal statistically significant differences between the four quadrants. In the parafoveal region, we found a higher microvascular density in the deep than in the superficial capillary network, as recently described for healthy people. Additionally, we identified a trend toward a higher capillary density in the superior compared with the inferior sectors, but this difference was not statistically significant in either the SCP or DCP. The FAZ size of our patient cohort fits into the upper range of FAZ sizes reported to date. An enlargement of the FAZ has previously been described in patients with diabetes even without DR. However, the considerable intersubject variability in healthy people and the large overlap in size between healthy individuals and those with diabetes need to be emphasized. Furthermore, it has recently been shown that the vascular anatomy of the fovea cannot be reliably delineated with the OCTA systems currently available. Thus, FAZ size has been denoted as a poor diagnostic variable for DR, and was therefore not taken into account for further analyses and conclusions in this study.

Whilst parafoveal vessel density and peripapillary flux only provide information about the anatomic state of the retinal vasculature, retinal SO2 is a direct, noninvasive measure of retinal metabolic function. Coordination of metabolic demand is achieved by the close interaction between retinal neurons, glial cells, and blood vessels in the retinal “neurovascular units.” Hence, retinal SO2 should be sensitive to metabolic dysfunction or apoptosis of retinal neuronal cells. In theory, if early neurodegeneration occurs in a specific regional pattern across the retina, this would result in regionally different SO2 levels, but may not directly manifest in an altered pattern of microvascular density and flux, as suggested by our results.

To validate or refute of this hypothesis, further intensive research is required to (1) substantiate the pathologic nature of the topographic variance of SO2 in patients with diabetes, (2) define the exact cellular metabolism underlying these regional differences, (3) exclude the possibility that the absence of a distinct pattern in microvascular density and flux was due to technical limitations of the applied OCTA systems, and (4) investigate the regional pattern of these variables in the retinal periphery using ultrawide field imaging techniques, as our results are limited to the posterior pole of the retina.

The major limitation of our study is its small sample size, but it is the first approach to study the regional pattern of SO2 and microvascular density and flux in patients with diabetes without DR with three innovative, sophisticated imaging techniques including a complex prototype OCTA system. Given that the variables investigated may serve as precious biomarkers for the development and progression of DR in the future, knowledge about their topographic variability would facilitate early detection of disease activity. We cannot provide evidence that all these patients will definitely develop DR, but this explorative, cross-sectional study is an essential step toward selecting the best potential biomarkers that require validation in more cumbersome, longitudinal study designs.

In conclusion, the findings of this study suggest that retinal metabolic changes show a distinct regional distribution without an associated regional variance of microvascular hemodynamics in type II diabetes without DR. Future identification of the mechanisms contributing to this heterogeneity in retinal metabolic demand will help to improve our understanding of the pathogenesis of the disease.

**Acknowledgments**

Julia Hafner is a recipient of the DOC Fellowship of the Austrian Academy of Sciences.

Disclosure: **J. Hafner**, None; **L. Ginner**, None; **S. Karst**, None; **R. Leitgeb**, None; **M. Unterluggauer**, None; **S. Sacu**, None; **C. Mitsch**, None; **C. Scholda**, None; **E. Pablik**, None; **U. Schmidt-Erfurth**, None

**References**