

# Association Between Corneal Changes and Retinal Oximetry in Diabetes Mellitus

Lisa Ramm , Eberhard Spoerl, Naim Terai, Robert Herber , Lutz E Pillunat

Department of Ophthalmology, University Hospital Carl Gustav Carus, TU Dresden, Dresden, 01307, Germany

Correspondence: Lisa Ramm, Department of Ophthalmology, University Hospital Carl Gustav Carus, TU Dresden, Fetscherstr 74, Dresden, 01307, Germany, Tel +49 351 458 3381, Fax +49 351 458 4335, Email [lisa.ramm@uniklinikum-dresden.de](mailto:lisa.ramm@uniklinikum-dresden.de)

**Purpose:** Diabetes mellitus (DM) causes different corneal changes that are associated with the severity of diabetic retinopathy. To identify the pathophysiological reasons for this, corneal tomography and optical densitometry (COD) were combined with retinal oximetry.

**Methods:** Patients with DM and healthy subjects were included in this pilot study. Spatially resolved corneal thickness and COD were assessed using the Pentacam HR (Oculus). The pachymetry difference ( $PAC_{Diff}$ ) was calculated as an indicator of an increase in the peripheral corneal thickness. Oxygen saturation ( $SO_2$ ) of the retinal vessels was measured using the Retinal Vessel Analyzer (Imedos Systems UG). Subsequently, the associations between corneal and retinal parameters were analyzed.

**Results:** Data from 30 patients with DM were compared with those from 30 age-matched healthy subjects. In DM, arterial ( $P = 0.048$ ) and venous ( $P < 0.001$ )  $SO_2$  levels were increased, and arteriovenous  $SO_2$  difference was decreased ( $P < 0.001$ ). In patients,  $PAC_{Diff}$  was higher than that in healthy subjects ( $P < 0.05$ ), indicating a stronger increase in peripheral corneal thickness. The COD was reduced in DM ( $P = 0.004$ ). The  $PAC_{Diff}$  of concentric rings with a diameter of 4 mm ( $r = -0.404$ ;  $P = 0.033$ ) to 8 mm ( $r = -0.522$ ;  $P = 0.004$ ) was inversely correlated with the arteriovenous  $SO_2$  difference. Furthermore,  $PAC_{Diff}$  4 mm was negatively associated with arterial  $SO_2$  ( $r = -0.389$ ;  $P = 0.041$ ), and the COD of the peripheral corneal areas correlated positive with arterial  $SO_2$  (COD total 10–12 mm:  $r = 0.408$ ;  $P = 0.025$ ).

**Conclusion:** These associations might indicate a common pathogenesis of corneal and retinal changes in DM, which could be caused by reduced oxygen supply, mitochondrial dysfunction, oxidative stress, and cytokine effects.

**Plain Language Summary:** Retinal changes are particularly important for ophthalmologists in the management of diabetes mellitus. These are primarily consequences of diabetic vascular changes that can lead to a lack of oxygen. However, there is also evidence of significant changes in the cornea of patients with diabetes. In the present study, the associations between changes in corneal thickness profile, optical density of the cornea, and oxygen saturation of retinal vessels in diabetes mellitus were demonstrated for the first time. Therefore, this study could contribute to clarifying the possible causes of corneal changes in patients with diabetes.

**Keywords:** diabetes mellitus, retinal oximetry, corneal optical densitometry, pachymetry, retinal vessel analyzer, pentacam HR

## Introduction

Chronic hyperglycemia induces morphological and functional changes in various organ systems of the human body. In addition to acute metabolic complications, the course of the disease is determined by the chronic vascular changes.<sup>1</sup> In the eye, the consequences of retinal microangiopathy (diabetic retinopathy (dRP) and maculopathy (dMP)) are most significant.<sup>2</sup> In addition to the damage, particularly to small blood vessels, caused by the high blood sugar level, direct hyperglycemic effects on the retinal cells probably also play a role.<sup>2</sup> Furthermore, there is increasing evidence that DM affects corneal microstructure and function.<sup>3</sup> In patients with DM, the incidence of ocular surface diseases has increased.<sup>2,4</sup> Potential reasons include reduction in corneal innervation, changes in tear film composition, and epithelial cells with an accumulation of advanced glycation end-products (AGEs). Furthermore, disturbed corneal barrier function

and delayed wound healing are conceivable.<sup>3,4</sup> Changes in growth factor concentrations, cytokine levels, oxidative stress, changes in corneal proteoglycans and glycosaminoglycans, and inflammatory components may be associated.<sup>3-5</sup>

Degeneration of neuronal fibers with a reduction in corneal sensitivity has been reported, which correlates with the stage of dRP.<sup>6</sup> Furthermore, cellular changes, with a reduction in epithelial cell density, were present in DM.<sup>7</sup> In addition, keratocyte loss<sup>8</sup> and reduction in endothelial cells<sup>9</sup> are associated with disease duration and HbA<sub>1c</sub> values. Different studies have reported an increase in central corneal thickness (CCT) in DM.<sup>9-11</sup> The rise in CCT is associated with the severity of peripheral neuropathy,<sup>10</sup> disease duration,<sup>9</sup> and the stage of dRP.<sup>11</sup> Furthermore, changes in the peripheral corneal thickness profile are associated with the stage of dRP and presence of dMP.<sup>12</sup> However, hyperglycemia-induced changes in the corneal stroma are hardly known.<sup>3</sup> The stroma accounts for approximately 90% of the whole corneal volume<sup>2</sup> and mainly determines corneal biomechanical properties.<sup>3,4</sup> In this context, corneal biomechanics are also altered in DM<sup>5,13</sup> and these changes correlate with the stage of dRP and presence of dMP as well.<sup>13</sup> Furthermore, an increase in corneal autofluorescence<sup>14</sup> and a reduction in corneal optical density<sup>15</sup> were detectable, which also showed significant associations with the severity of dRP.<sup>14,15</sup>

These associations lead to the assumption of a common pathogenesis for corneal and retinal changes in DM. Earlier studies using retinal oximetry revealed an increase in oxygen saturation (SO<sub>2</sub>) of the retinal arterioles and venules. This increase in SO<sub>2</sub> was associated with indicators of disease severity and the dRP.<sup>16</sup> In this context, the fundus of the eye offers an unique opportunity to directly visualize blood vessels and to measure tissue perfusion parameters. Furthermore, the arterial supply of the whole eye originates mainly from a common source: the ophthalmic artery. It is well known, that the stage of dRP is an indicator of the systemic microvascular stage and the risk of complications in DM. This may also be the case for hyperglycemia-induced corneal changes.<sup>3</sup>

To test the hypothesis of a common pathogenesis of corneal and retinal changes in DM, measurements of corneal parameters and retinal oximetry were combined in the current pilot study.

## Methods

This study adhered to the tenets of the Declaration of Helsinki and the local ethics committee of the University Hospital approved the study protocol. Adult patients with DM between October 2018 and April 2020 were included in this pilot study. In patients, the DM type, disease duration, antidiabetic therapy, and current HbA<sub>1c</sub> levels were assessed. The data were compared with those of age-matched healthy subjects. Only one eye randomly chosen from each participant was included in the study. Exclusion criteria were pre-existing corneal diseases, glaucoma, trauma, ocular surgery in the past (except for uncomplicated intravitreal injections), previous retinal vessel occlusion, and retinal diseases other than diabetic retinopathy or maculopathy. Data of participants with previous myocardial infarction, bypass operation, stroke, or peripheral artery occlusive disease were excluded because of their potential influence on retinal oximetry. All study participants underwent measurements using a Retinal Vessel Analyzer (RVA; Imedos Systems UG, Jena, Germany) and Pentacam (Pentacam HR, type 70,900, Oculus, Wetzlar, Germany).

The RVA consisted of a modified fundus camera (FF450; Carl Zeiss Meditec AG, Jena, Germany) equipped with a digital camera (KY-F75; JVC, Inc., Yokohama, Japan). Thirty-degree images of the fundus centered on the optic disc were taken. The SO<sub>2</sub> of retinal vessels was evaluated using a two-wavelength technique. By introducing a dual-bandpass transmission filter in the optical path of the camera, two images of different wavelengths (548 ± 10 nm and 610 ± 10 nm) were recorded simultaneously. For image acquisition the programs Visualis version 2.61 (Imedos Systems UG, Jena, Germany) and VesselMap version 3.10 (Imedos Systems UG, Jena, Germany) were used. The optical density of each retinal vessel was determined using the grayscale values of the images. SO<sub>2</sub> was proportional to the ratio of the optical densities of both captured images, and the saturation of each vessel was calculated accordingly.<sup>16</sup> Vessel segments within a peripapillary annulus (inner radius of 1 and outer radius of 1.5 optic disc diameters) were analyzed, and the mean values of all arterioles and venules were calculated.

Pentacam uses a rotating Scheimpflug camera to generate two-dimensional optical sections through the cornea and the structures lying behind, and the software reconstructs three-dimensional anterior and posterior surface tomography of the cornea. Spatially resolved corneal thickness was measured as the distance between the anterior and posterior border of the cornea.<sup>17</sup> In the current study, peripheral thickness change was calculated as the difference between the thinnest

corneal thickness (TCT) and the peripheral thickness value of concentric rings around the TCT. Rings with a diameter of 2–10 mm were used to calculate the  $PAC_{Diff}$ .<sup>12</sup>

Furthermore, Pentacam measured the corneal backscattered light and calculated the corneal optical density (COD) over a 12-mm-diameter area centered at the apex. The examined area was further divided into four annular zones (0–2 mm, 2–6 mm, 6–10 mm, and 10–12 mm in diameter), and for each zone, COD was given in three separate layers (anterior 120  $\mu$ m, central layer, and posterior 60  $\mu$ m).<sup>18</sup>

Data were analyzed using the SPSS software (Version 28, IBM Statistics, New York, USA). The Kolmogorov–Smirnov test was used to check for a normal distribution of the results. Because some parameters were not normally distributed, group comparisons were performed using the Mann–Whitney *U*-test. Associations among  $SO_2$  values, corneal thickness parameters, and COD values were examined using Spearman correlation analysis. Statistical significance was set at  $p \leq 0.05$ .

## Results

Thirty eyes of patients with DM type 2 were included in this pilot study. Mean age was  $69.6 \pm 11.5$  years (range 47–94 years) and mean intraocular pressure amounted to  $15.2 \pm 4.3$  mmHg. The mean disease duration was  $21.1 \pm 13.2$  %, and the mean  $HbA_{1C}$  value was  $6.9 \pm 1.4$  %. Nine of the investigated eyes showed a dRP (one mild non-proliferative, four moderate non-proliferative, three non-proliferative, and one proliferative), eight patients showed a dMP with edema, and two patients had been treated by pan-retinal laser coagulation in the past. Furthermore, 13 patients had diabetic neuropathy, five had diabetic nephropathy, and seven had diabetic foot syndrome. Fourteen patients received oral antidiabetic therapy, nine were treated with insulin and seven patients received combination therapy.

$SO_2$  of retinal arterioles ( $100.7 \pm 6.5$  % vs  $97.4 \pm 4.9$  %,  $P = 0.048$ ) and venules ( $69.8 \pm 9.5$  % vs  $59 \pm 6.7$  %,  $P < 0.001$ ) was higher in DM patients than in healthy subjects. Furthermore, the arteriovenous  $SO_2$  difference was significantly reduced in the DM ( $30.9 \pm 7.5$  % vs  $38.5 \pm 5.5$  %,  $P < 0.001$ , Table 1).

The analysis of spatially resolved pachymetry readings showed higher differences between peripheral corneal thickness values and TCT in patients with DM than in the controls ( $P < 0.05$ , Table 2). This observation indicates a greater increase in the peripheral corneal thickness in patients with DM. In agreement, absolute pachymetry values of the concentric rings with a diameter of 8 mm ( $699.6 \pm 37.9$   $\mu$ m vs  $672.9 \pm 42.6$   $\mu$ m,  $P = 0.031$ ) and 10 mm ( $788.8 \pm 47.6$   $\mu$ m vs  $738.5 \pm 58.1$   $\mu$ m,  $P = 0.003$ ) were higher in patients than in healthy subjects (Table 2). Furthermore, in DM, COD was lower in most of the analyzed corneal areas and layers (total COD:  $21.8 \pm 6.6$  GSU vs  $27.3 \pm 5.3$  GSU,  $P = 0.004$ , Table 2).

**Table 1** Baseline Data and Oxygen Saturation Values of Retinal Vessels in Diabetes Mellitus Patients and Healthy Subjects

	DM Patients	Healthy Subjects	p value
Number	30	30	
Age (years)	$69.6 \pm 11.5$	$67.5 \pm 10.3$	0.539
IOP (mmhg)	$15.2 \pm 4.3$	$15.7 \pm 3$	0.412
Systolic BP (mmhg)	$154.5 \pm 20.5$	$130.7 \pm 13.3$	< 0.001
Diastolic BP (mmhg)	$86.7 \pm 11.4$	$77.3 \pm 10.2$	0.001
Mean arterial BP (mmhg)	$120.6 \pm 14.5$	$104 \pm 10.5$	< 0.001
Arterial $SO_2$ (%)	$100.7 \pm 6.5$	$97.4 \pm 4.9$	0.048
Venous $SO_2$ (%)	$69.8 \pm 9.5$	$59 \pm 6.7$	< 0.001
Arteriovenous $SO_2$ difference (%)	$30.9 \pm 7.5$	$38.5 \pm 5.5$	< 0.001

**Abbreviations:** DM, diabetes mellitus; IOP, intraocular pressure; BP, blood pressure;  $SO_2$ , oxygen saturation.

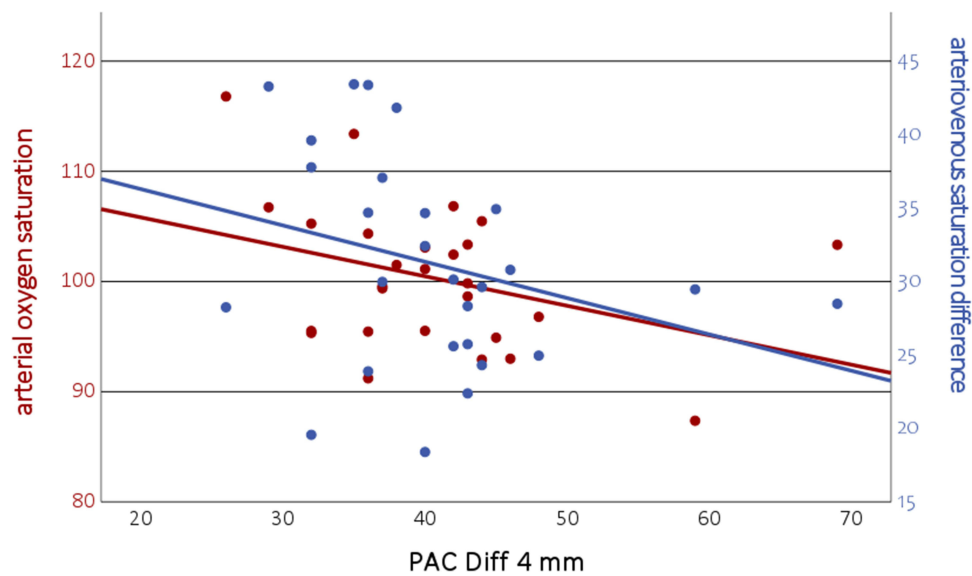
**Table 2** Results of Pentacam Measurements in Diabetes Mellitus Patients and Healthy Subjects

Number	DM Patients	Healthy Subjects	p value	
	30	30		
CCT ( $\mu\text{m}$ )	553 $\pm$ 100.6	557.2 $\pm$ 36.2	0.785	
<b>Spatially resolved pachymetry (in <math>\mu\text{m}</math>)</b>				
PAC TCT	551 $\pm$ 31.9	552.1 $\pm$ 33.7	0.994	
PAC 2 mm	561.4 $\pm$ 34.4	559.2 $\pm$ 33.7	0.846	
PAC 4 mm	588.1 $\pm$ 28.4	583.7 $\pm$ 34.6	0.695	
PAC 6 mm	634.4 $\pm$ 29.8	621.6 $\pm$ 36.7	0.215	
PAC 8 mm	699.6 $\pm$ 37.9	672.9 $\pm$ 42.6	0.031	
PAC 10 mm	788.8 $\pm$ 47.6	738.5 $\pm$ 58.1	0.003	
PAC <sub>Diff</sub> 2 mm	11.8 $\pm$ 7.3	8.5 $\pm$ 2.1	0.001	
PAC <sub>Diff</sub> 4 mm	40.5 $\pm$ 8.8	33 $\pm$ 5.2	< 0.001	
PAC <sub>Diff</sub> 6 mm	86.9 $\pm$ 16.4	70.9 $\pm$ 10.3	< 0.001	
PAC <sub>Diff</sub> 8 mm	152 $\pm$ 27.2	122.3 $\pm$ 18	< 0.001	
PAC <sub>Diff</sub> 10 mm	239.2 $\pm$ 42	189.7 $\pm$ 39.4	< 0.001	
<b>Corneal optical density (in GSU)</b>				
Anterior layer (total)	29.8 $\pm$ 9.5	36.5 $\pm$ 7.7	0.018	
Central layer (total)	19.9 $\pm$ 6	24.8 $\pm$ 5.6	0.008	
Posterior layer (total)	15.6 $\pm$ 4.7	20.5 $\pm$ 4.1	< 0.001	
All layers	0–2 mm	16.8 $\pm$ 5.6	19 $\pm$ 1.9	0.076
	2–6 mm	16.4 $\pm$ 4.9	19.3 $\pm$ 2.8	0.04
	6–10 mm	25.5 $\pm$ 8.9	34.1 $\pm$ 9.3	0.003
	10–12 mm	31.4 $\pm$ 10.4	40.9 $\pm$ 12.2	0.003
	total	21.8 $\pm$ 6.6	27.3 $\pm$ 5.3	0.004

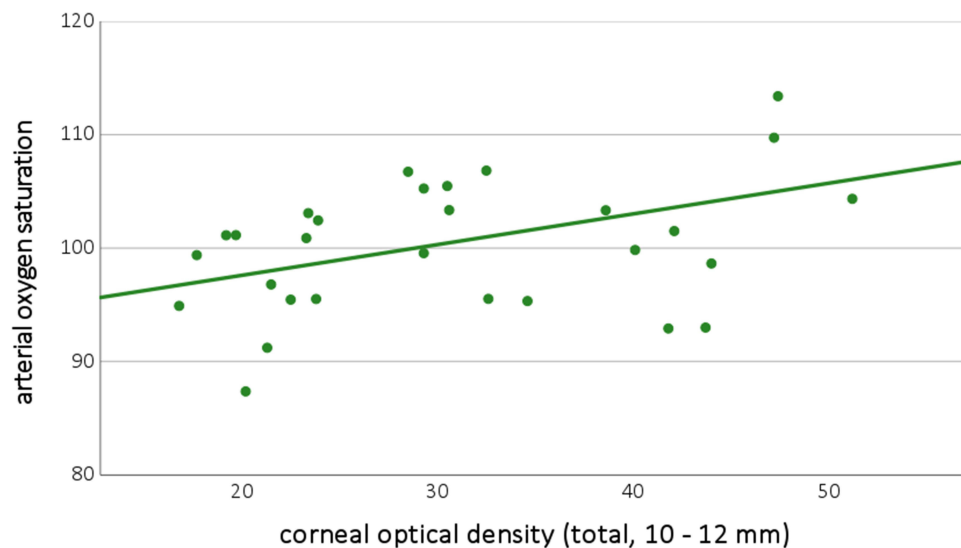
**Abbreviations:** DM, diabetes mellitus; CCT, central corneal thickness; TCT, thinnest corneal thickness; PAC, mean pachymetry values of concentric rings around TCT, circle diameter indicated in mm; PAC<sub>Diff</sub>, difference of pachymetry values between TCT and the concerning concentric circle.

In patients with DM, correlation analysis between  $\text{SO}_2$  of the retinal vessels and corneal thickness measurements revealed an inverse association between arteriovenous saturation difference and the thickness difference between the TCT and peripheral concentric rings with a diameter of 4–8 mm (PAC<sub>Diff</sub> 4 mm:  $r = -0.404$ ,  $P = 0.033$ ; Figure 1; PAC<sub>Diff</sub> 5 mm:  $r = -0.441$ ,  $P = 0.019$ ; PAC<sub>Diff</sub> 6 mm:  $r = -0.474$ ,  $P = 0.011$ ; PAC<sub>Diff</sub> 7 mm:  $r = -0.452$ ,  $P = 0.016$ ; PAC<sub>Diff</sub> 8 mm:  $r = -0.522$ ,  $P = 0.004$ ). Furthermore, arterial  $\text{SO}_2$  inversely correlated with the difference between the TCT and peripheral ring with a diameter of 4 mm ( $r = -0.389$ ,  $P = 0.041$ , Figure 1).

In healthy subjects, no significant association between corneal thickness and arterial or venous  $\text{SO}_2$  was detected (for example arterial  $\text{SO}_2$  and PAC<sub>Diff</sub> 2 mm:  $r = 0.168$ ,  $P = 0.376$ , PAC<sub>Diff</sub> 4 mm:  $r = 0.152$ ,  $P = 0.424$ , PAC<sub>Diff</sub> 6 mm:  $r = 0.084$ ,  $P = 0.661$ ; venous  $\text{SO}_2$  and PAC<sub>Diff</sub> 2 mm:  $r = -0.171$ ,  $P = 0.366$ , PAC<sub>Diff</sub> 4 mm:  $r = -0.155$ ,  $P = 0.414$ , PAC<sub>Diff</sub>



**Figure 1** Association between oxygen saturation of retinal arterioles (in %), retinal arteriovenous saturation difference (in %), and difference between central thinnest corneal thickness and peripheral thickness of the cornea measured at a concentric ring with a diameter of 4 mm (PAC Diff 4 mm, in  $\mu\text{m}$ ) in diabetes mellitus.



**Figure 2** Correlation between retinal arterial oxygen saturation (in %) and peripheral corneal optical density (of all layers at 10 to 12 mm diameter, in  $\mu\text{m}$ ) in diabetes mellitus patients.

6 mm:  $r = -0.138$ ,  $P = 0.469$ ). However, the arteriovenous saturation difference weakly correlated with  $\text{PAC}_{\text{Diff}} 2 \text{ mm}$  ( $r = 0.374$ ,  $P = 0.041$ ),  $\text{PAC}_{\text{Diff}} 4 \text{ mm}$  ( $r = 0.408$ ,  $P = 0.025$ ) and  $\text{PAC}_{\text{Diff}} 6 \text{ mm}$  ( $r = 0.368$ ,  $p = 0.045$ ).

Furthermore, in DM, the COD of the peripheral corneal zones was associated with arterial  $\text{SO}_2$ : COD in the 10–12 mm area of the anterior layer ( $r = 0.422$ ,  $P = 0.02$ ), central layer ( $r = 0.4$ ,  $P = 0.028$ ), and total COD of the 10–12 mm zone ( $r = 0.408$ ,  $P = 0.025$ , [Figure 2](#)) correlated with arterial  $\text{SO}_2$ . However, no association between COD values and venous  $\text{SO}_2$  (total COD of the 10–12 mm zone:  $r = 0.031$ ,  $P = 0.869$ ) or arteriovenous saturation differences (total COD of the 10–12 mm zone:  $r = 0.339$ ,  $P = 0.067$ ) was detected in DM. Similarly, in healthy subjects, no significant correlations were detected between COD and oxygen saturation values (total COD of the 10–

12 mm zone and arterial  $\text{SO}_2$ :  $r = 0.223$ ,  $P = 0.236$ ; venous  $\text{SO}_2$ :  $r = 0.094$ ,  $P = 0.62$ ; arteriovenous  $\text{SO}_2$  difference:  $r = -0.014$ ,  $P = 0.943$ ).

## Discussion

### Corneal Thickness Profile

In the past, not only a hyperglycemia-induced increase in CCT<sup>9,10</sup> but also changes in the corneal thickness profile<sup>12</sup> were detected in DM. The observation of a stronger peripheral thickness increase in the cornea in the DM<sup>12</sup> group was confirmed in the current study. Potential reasons for the diabetic CCT increase include alterations in corneal ground substances with the accumulation of advanced glycation end products, glycosylation of proteoglycans and glycosaminoglycans, increased crosslinking of collagen fibrils,<sup>5</sup> and thickening of basal membranes.<sup>19</sup> In addition, these changes may induce an increase in the peripheral corneal thickness. Furthermore, changes in corneal hydration may be a causative factor. In addition to central endothelial changes,<sup>9,10</sup> alterations in the peripheral corneal endothelium have been reported,<sup>20</sup> which may result in corneal swelling. Moreover, a healthy cornea is free of blood vessels and limbal capillaries supply only a small peripheral proportion.<sup>2</sup> Therefore, systemic hyperglycemia may influence the corneal periphery. Furthermore, the distribution pattern of proteoglycans within the cornea may also be causative.<sup>21</sup> The properties of proteoglycans depend on their glycosaminoglycan (GAG) side chains and their proportions vary within the cornea.<sup>2,21,22</sup> Different water-binding properties of GAGs influence corneal hydration<sup>22,23</sup> and the spatial orientation of collagen fibrils. Therefore, GAGs act as spacers between collagen fibrils and affect their orientation,<sup>21</sup> which may influence the corneal thickness profile.

### Corneal Optical Density

COD was used to obtain further information on the microstructural changes in the corneal tissue. To quantify the clarity of the cornea, the backscattering of incident light during tissue passage can be measured.<sup>24</sup> Light scattering is a complex process that depends on different factors, such as the refractive indices of collagen and interfibrillar matrix, structure of collagen fibrils, and hydration of the cornea.<sup>23</sup> As previously mentioned, hydration and collagen structure are mainly determined by proteoglycans.<sup>23</sup> Therefore, changes in the proteoglycan distribution can cause deviations in COD. In the current study, COD was reduced in DM.

The stroma accounts for approximately 90% of total corneal volume.<sup>2</sup> Using *in vivo* confocal microscopy, Hillenaar et al showed that keratocyte nuclei are the main source of stromal backscattering of light.<sup>25</sup> Additionally, a reduction in corneal keratocytes has been reported in DM, which is associated with the disease duration and HbA<sub>1c</sub> level.<sup>8</sup> Therefore, the loss of corneal keratocytes introduced by chronic hyperglycemia may be the cause of COD reduction. Keratocytes play an essential role in the balance between proteoglycans and the degradation of metabolic products.<sup>26</sup> In cases of reduced keratocyte function, the accumulation of metabolites might introduce corneal thickening and changes in the thickness profile.

### Retinal Oximetry

In DM, arterial and venous  $\text{SO}_2$  were increased and arteriovenous differences were reduced. In agreement with this, earlier studies showed an increase in  $\text{SO}_2$  of retinal vessels, which was correlated with the severity of dRP,<sup>16</sup> and vascular changes of the retina were considered indicators of systemic diabetic complications. On the one hand, a reduced oxygen delivery to the tissue may be reasonable. This could be caused by blood shunting, capillary non-perfusion, increased oxygen affinity of hemoglobin, thickening of capillary basement membranes, shortened arteriovenous passage time, and dysfunction of the vascular endothelium.<sup>16</sup> On the other hand, mitochondrial dysfunction or tissue degeneration may reduce the metabolic oxygen demand.<sup>16</sup>

### Association Between Corneal and Retinal Changes

Following the hypothesis of reduced oxygen release into the tissue, the retinal arteriovenous  $\text{SO}_2$  difference was reduced in DM. With increasing HbA<sub>1c</sub> levels, the oxygen affinity of hemoglobin rises<sup>27</sup> and the oxygen supply declines. This

stronger oxygen affinity of hemoglobin exists not only in the retina but also in all tissues of the body. As mentioned above, the peripheral portion of the cornea is supplied by the limbal capillaries.<sup>2</sup> In addition, oxygen delivery may be suboptimal in DM. The reduced availability of oxygen might cause a reduction in the number of corneal cells as a result of apoptosis. The consequence could be keratocyte loss, leading to a reduced COD in patients with DM. In addition, few or inactive cells cannot maintain the optimal corneal condition, and changes in proteoglycan and water distribution occur. This result may be due to the changes in the corneal thickness profile. In agreement with this, in the current study, a reduction in the arteriovenous  $SO_2$  difference (indicating reduced oxygen extraction) was associated with a stronger peripheral thickness increase in DM. Furthermore, retinal arterial  $SO_2$  was positively correlated with the COD of the peripheral corneal areas in DM. A possible explanation could be that, unlike the central cornea, the peripheral tissue is mainly supplied by limbal capillaries.<sup>2</sup> As mentioned above, loss of corneal keratocytes may be the cause of COD reduction in DM.<sup>8,25</sup> Therefore, assuming a systemically similar perfusion situation, one might speculate that a sufficient supply of high arterial  $SO_2$  may prevent keratocyte loss in peripheral corneal regions. However, further studies are needed to clarify whether the intake of oxygen in the corneal tissue may be diminished in diabetes or whether other causes lead to keratocyte loss.

However, there is another possible explanation for mitochondrial dysfunction. In systemic DM pathogenesis, the formation of reactive oxygen species (ROS) by dysfunctional mitochondria appears to be the primary event.<sup>1,28</sup> Therefore, ROS can induce proinflammatory cytokines,<sup>29</sup> and chronic inflammation is a part of the diabetic complications.<sup>1</sup> Furthermore, AGE formation during hyperglycemia is accompanied by ROS release.<sup>1</sup> Thus, an increase in oxidative stress and ROS accumulation may be important for the development of dRP.<sup>30</sup>

In addition, corneal mitochondria are affected by DM. Earlier studies have shown impaired function and altered morphology of the mitochondria of endothelial cells.<sup>31</sup> The increase in corneal autofluorescence correlated with the severity of dRP.<sup>14</sup> It may show changes in the metabolism of mitochondrial flavoproteins, which are influenced by the glucose level, oxygen supply, and respiratory state of corneal mitochondria.<sup>14</sup>

As mentioned above, ROS not only have direct damaging effects on cells and molecules, but are also able to induce proinflammatory cytokines.<sup>29</sup> Accordingly, in DM, a significant association between  $SO_2$  in retinal vessels and the concentration of proangiogenic and proinflammatory cytokines in aqueous fluid has been found.<sup>32</sup> Cytokine<sup>33</sup> and AGE-induced ROS production<sup>34</sup> can induce apoptosis in corneal cells. Therefore, it is conceivable that mitochondrial dysfunction, oxidative stress, and an increase in proinflammatory cytokines lead to malfunction and death of corneal keratocytes.<sup>8</sup> This, in turn, could lead to changes in COD and corneal thickness profile. These and similar changes probably occur not only in the cornea but also in other organs. Therefore, retinal oximetry may be an indicator of the systemic oxygen supply. This assumption is in line with previous hypotheses of systemic mitochondrial dysfunction in patients with chronic hyperglycemia<sup>1</sup> and the common pathogenesis of corneal and retinal alterations in DM.

## Limitations

This study has some limitations. It was an observational cross-sectional pilot study that showed differences and connections, but could not provide proof of causality. It is therefore just speculation about possible connections. For this reason, a longitudinal evaluation may be beneficial for identifying pathogenetic associations. Moreover, the necessary group matching reduced the number of participants, and all the patients were Caucasian. Since this was an exploratory study with limited number of cases, the p-values were not adjusted.<sup>35</sup> Consequently, the results should be cautiously interpreted. Furthermore, the study could gain from confocal microscopy. In addition, direct oximetry of limbal corneal capillaries would be desirable and could potentially be performed in a future study. However, the strength of the current study lies in the combination of the different measurement procedures in patients with DM.

## Conclusion

In conclusion, in this pilot study, earlier results of changes in  $SO_2$  of retinal vessels, reduction in COD, and alterations in corneal thickness profile in DM were confirmed. The significant association between retinal  $SO_2$  values and corneal parameters may indicate the common pathogenesis of hyperglycemia-induced corneal and retinal changes. The possible mechanisms include reduced oxygen supply, mitochondrial dysfunction, oxidative stress, and cytokine effects in DM.

## Data Sharing Statement

The dataset analyzed in this study is available from the corresponding author upon reasonable request.

## Statement of Ethics

This study adhered to the tenets of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the University Hospital Carl Gustav Carus, Dresden, TU Dresden, Germany (EK 424102016 and EK 91032013). Before enrolment, all participants were informed of the study purpose, methods, and data analysis, and informed consent was obtained from all participants.

## Disclosure

The authors declare no conflicts of interest in this work.

## References

1. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137–188. doi:10.1152/physrev.00045.2011
2. Bowling B. *Kanski's Clinical Ophthalmology: A Systematic Approach*. 8th ed. Elsevier; 2017.
3. Del Buey MA, Casas P, Caramello C, et al. An update on corneal biomechanics and architecture in diabetes. *J Ophthalmol*. 2019;2019:7645352. doi:10.1155/2019/7645352
4. Shih KC, Lam KSL, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. *Nutr Diabetes*. 2017;7(3):e251. doi:10.1038/nutd.2017.4
5. Scheler A, Spoerl E, Boehm AG. Effect of diabetes mellitus on corneal biomechanics and measurement of intraocular pressure. *Acta Ophthalmol*. 2012;90(6):e447–451. doi:10.1111/j.1755-3768.2012.02437.x
6. Česká Burdová M, Kulich M, Dotřelová D, Mahelková G. Effect of diabetes mellitus type 1 diagnosis on the corneal cell densities and nerve fibers. *Physiol Res*. 2018;67(6):963–974. doi:10.33549/physiolres.933899
7. Qu JH, Li L, Tian L, Zhang XY, Thomas R, Sun XG. Epithelial changes with corneal punctate epitheliopathy in type 2 diabetes mellitus and their correlation with time to healing. *BMC Ophthalmol*. 2018;18(1):1. doi:10.1186/s12886-017-0645-6
8. Kalteniece A, Ferdousi M, Azmi S, Marshall A, Soran H, Malik RA. Keratocyte density is reduced and related to corneal nerve damage in diabetic neuropathy. *Invest Ophthalmol Vis Sci*. 2018;59(8):3584–3590. doi:10.1167/iovs.18-23889
9. Calvo-Maroto AM, Cerviño A, Perez-Cambrodi RJ, García-Lázaro S, Sanchis-Gimeno JA. Quantitative corneal anatomy: evaluation of the effect of diabetes duration on the endothelial cell density and corneal thickness. *Ophthalmic Physiol Opt J Br Coll Ophthalmic Opt Optom*. 2015;35(3):293–298. doi:10.1111/opo.12191
10. Kumar N, Pop-Busui R, Musch DC, et al. Central corneal thickness increase due to stromal thickening with diabetic peripheral neuropathy severity. *Cornea*. 2018;37(9):1138–1142. doi:10.1097/ICO.0000000000001668
11. Jha A, Verma A, Alagorie AR. Association of severity of diabetic retinopathy with corneal endothelial and thickness changes in patients with diabetes mellitus. *Eye Lond Engl*. 2021. doi:10.1038/s41433-021-01606-x
12. Ramm L, Spoerl E, Pillunat LE, Terai N. Is the corneal thickness profile altered in diabetes mellitus? *Curr Eye Res*. 2020;45(10):1228–1234. doi:10.1080/02713683.2020.1741009
13. Ramm L, Herber R, Spoerl E, Pillunat LE, Terai N. Factors influencing corneal biomechanics in diabetes mellitus. *Cornea*. 2020;39(5):552–557. doi:10.1097/ICO.0000000000002275
14. Stolwijk TR, van Best JA, Oosterhuis JA, Swart W. Corneal autofluorescence: an indicator of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 1992;33(1):92–97.
15. Ramm L, Spoerl E, Pillunat LE, Terai N. Corneal densitometry in diabetes mellitus. *Cornea*. 2020;39(8):968–974. doi:10.1097/ICO.0000000000002310
16. Hammer M, Vilser W, Riemer T, et al. Diabetic patients with retinopathy show increased retinal venous oxygen saturation. *Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Klin Exp Ophthalmol*. 2009;247(8):1025–1030. doi:10.1007/s00417-009-1078-6
17. Oliveira CM, Ribeiro C, Franco S. Corneal imaging with slit-scanning and Scheimpflug imaging techniques. *Clin Exp Optom*. 2011;94(1):33–42. doi:10.1111/j.1444-0938.2010.00509.x
18. Cankaya AB, Tekin K, Kiziltoprak H, Karahan S, Yilmazbas P. Assessment of corneal backward light scattering in the healthy cornea and factors affecting corneal transparency. *Jpn J Ophthalmol*. 2018;62(3):335–341. doi:10.1007/s10384-018-0584-7
19. Hashemi H, Asgari S, Mehravaran S, Emamian MH, Fotouhi A. Five-year changes of anterior corneal indices in diabetics versus non-diabetics: the shahroud eye cohort study. *Curr Eye Res*. 2019;44(1):30–33. doi:10.1080/02713683.2018.1521977
20. McNamara NA, Brand RJ, Polse KA, Bourne WM. Corneal function during normal and high serum glucose levels in diabetes. *Invest Ophthalmol Vis Sci*. 1998;39(1):3–17.
21. Ho LTY, Harris AM, Tanioka H, et al. A comparison of glycosaminoglycan distributions, keratan sulphate sulphation patterns and collagen fibril architecture from central to peripheral regions of the bovine cornea. *Matrix Biol J Int Soc Matrix Biol*. 2014;38:59–68. doi:10.1016/j.matbio.2014.06.004
22. Castoro JA, Bettelheim AA, Bettelheim FA. Water gradients across bovine cornea. *Invest Ophthalmol Vis Sci*. 1988;29(6):963–968.
23. Meek KM, Leonard DW, Connon CJ, Dennis S, Khan S. Transparency, swelling and scarring in the corneal stroma. *Eye Lond Engl*. 2003;17(8):927–936. doi:10.1038/sj.eye.6700574
24. Özyol P, Özyol E. Assessment of corneal backward light scattering in diabetic patients. *Eye Contact Lens*. 2018;44(Suppl 1):S92–S96. doi:10.1097/ICL.0000000000000331



25. Hillenaar T, Cals RHH, Eilers PHC, Wubbels RJ, van Cleynenbreugel H, Remeijer L. Normative database for corneal backscatter analysis by in vivo confocal microscopy. *Invest Ophthalmol Vis Sci.* 2011;52(10):7274–7281. doi:10.1167/iovs.11-7747
26. Yam GHF, Riau AK, Funderburgh ML, Mehta JS, Jhanji V. Keratocyte biology. *Exp Eye Res.* 2020;196:108062. doi:10.1016/j.exer.2020.108062
27. Schweitzer D, Lasch A, van der Vorst S, et al. Change of retinal oxygen saturation in healthy subjects and in early stages of diabetic retinopathy during breathing of 100% oxygen. *Klin Monatsbl Augenheilkd.* 2007;224(5):402–410. doi:10.1055/s-2007-963156
28. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature.* 2000;404(6779):787–790. doi:10.1038/35008121
29. Cejka C, Cejkova J. Oxidative stress to the cornea, changes in corneal optical properties, and advances in treatment of corneal oxidative injuries. *Oxid Med Cell Longev.* 2015;2015:591530. doi:10.1155/2015/591530
30. Barot M, Gokulgandhi MR, Mitra AK. Mitochondrial dysfunction in retinal diseases. *Curr Eye Res.* 2011;36(12):1069–1077. doi:10.3109/02713683.2011.607536
31. Aldrich BT, Schlötzer-Schrehardt U, Skeie JM, et al. Mitochondrial and morphologic alterations in native human corneal endothelial cells associated with diabetes mellitus. *Invest Ophthalmol Vis Sci.* 2017;58(4):2130–2138. doi:10.1167/iovs.16-21094
32. Tayyari F, Khuu LA, Sivak JM, et al. Retinal blood oxygen saturation and aqueous humour biomarkers in early diabetic retinopathy. *Acta Ophthalmol.* 2019;97(5):e673–e679. doi:10.1111/aos.14016
33. Sagoo P, Chan G, Larkin DFP, George AJT. Inflammatory cytokines induce apoptosis of corneal endothelium through nitric oxide. *Invest Ophthalmol Vis Sci.* 2004;45(11):3964–3973. doi:10.1167/iovs.04-0439
34. Shi L, Yu X, Yang H, Wu X. Advanced glycation end products induce human corneal epithelial cells apoptosis through generation of reactive oxygen species and activation of JNK and p38 MAPK pathways. *PLoS One.* 2013;8(6):e66781. doi:10.1371/journal.pone.0066781
35. Cipriani V, Quartilho A, Bunce C, Freemantle N, Doré CJ. Ophthalmic statistics note 7: multiple hypothesis testing—to adjust or not to adjust. *Br J Ophthalmol.* 2015;99(9). doi:10.1136/bjophthalmol-2015-306784

## Clinical Ophthalmology

Dovepress

### Publish your work in this journal

Clinical Ophthalmology is an international, peer-reviewed journal covering all subspecialties within ophthalmology. Key topics include: Optometry; Visual science; Pharmacology and drug therapy in eye diseases; Basic Sciences; Primary and Secondary eye care; Patient Safety and Quality of Care Improvements. This journal is indexed on PubMed Central and CAS, and is the official journal of The Society of Clinical Ophthalmology (SCO). The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/clinical-ophthalmology-journal>