Original Article

Assessment of oxygen saturation in retinal vessels of normal subjects and diabetic patients with and without retinopathy using Flow Oximetry System

Mohamed A. Ibrahim[1,2*], Rachel E. Annam[1*], Yasir J. Sepah[2], Long Luu[3], Millena G. Bittencourt[1], Hyun S. Jang[1], Paul Lemaillet[3], Beatriz Munoz[4], Donald D. Duncan[5], Sheila West[4], Quan Dong Nguyen[2], Jessica C. Ramella-Roman[3,6]

[1]Retinal Imaging Research and Reading Center (RIRRC), Wilmer Eye Institute, Johns Hopkins University, School of Medicine, Baltimore, MD, USA; [2]Ocular Imaging Research and Reading Center (OIRRC), Stanley M. Truhlsen Eye Institute, University of Nebraska Medical Center, Omaha, NE, USA; [3]Department of Biomedical Engineering, The Catholic University of America, Washington, DC, USA; [4]Dana Center for Preventive Ophthalmology, Johns Hopkins University, Baltimore, Maryland, USA; [5]Department of Electrical and Computer Engineering, Portland State University, Oregon, USA; [6]Department of Biomedical Engineering, and Herbert Wertheim College of Medicine, Florida International University, Miami, FL, USA

*These authors have contributed equally to the preparation of this manuscript and serve as co-first authors. Dr. Nguyen and Dr. Ramella-Ramon have collaborated equally in the conduct of this study.

Correspondence to: Jessica Ramella-Roman, Ph.D. Associate Professor, Department of Biomedical Engineering, and Herbert Wertheim College of Medicine, Florida International University, E6 2610, 10555 W Flagler St, Miami, FL 33174, USA. Email: jramella@fiu.edu.

Purpose: To assess oxygen saturation (StO_2) in retinal vessels of normal subjects and diabetic patients with and without retinopathy using the modified version of the Flow Oximetry System (FOS) and a novel assessment software.

Methods: The FOS and novel assessment software were used to determine StO_2 levels in arteries and veins located between 1 and 2 mm from the margin of the optic disc and in the macular area.

Results: Eighteen normal subjects, 15 diabetics without diabetic retinopathy (DM no DR), and 11 with non-proliferative diabetic retinopathy (NPDR) were included in final analysis. The mean [± standard deviation (SD)] StO_2 in retinal arteries was 96.9%±3.8% in normal subjects; 97.4%±3.7% in DM no DR; and 98.4%±2.0% in NPDR. The mean venous StO_2 was 57.5%±6.8% in normal subjects; 57.4%±7.5% in DM no DR; and 51.8%±6.8% in NPDR. The mean arterial and venous StO_2 across the three groups were not statistically different (P=0.498 and P=0.071, respectively). The arterio-venous differences between the three study groups, however, were found to be statistically significant (P=0.015). Pairwise comparisons have demonstrated significant differences when comparing the A-V difference in the NPDR group to either normal subjects (P=0.02) or diabetic patients without DR (P=0.04).

Conclusions: The arterio-venous difference was greater, and statistically significant, in patients with NPDR when compared to normal subjects and to patients with diabetes and no retinopathy. The mean venous StO_2 was lower, but not statistically significant, in NPDR compared with diabetics without retinopathy and with normal subjects.

Keywords: Diabetes; oximetry; oxygen; retina

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Introduction

Diabetes mellitus (DM) is a metabolic disease that causes considerable worldwide morbidity and mortality. In 2010, researchers estimated that 285 million people worldwide had diabetes, and that these numbers will probably increase by 54% by 2030 (1). In the United States, about 10.9 million persons were diagnosed with diabetes in 2010 (2). Among the many complications of DM, the micro-vascular morbidities can be quite severe and can affect multiple organ systems resulting in complications such as retinopathy, nephropathy, and neuropathy, among others. Diabetic retinopathy (DR) is ranked as the leading cause of blindness and visual disability in the middle-aged/working American population (3), and these complications can lead to a reduction in life expectancy and can have a considerable impact on quality- and disability-adjusted life years indices as well as the cost of health care for affected patients (4). Therefore, it is essential to understand the progression of the disease with an aim to possibly prevent or delay the development of its complications.

Though the pathogenesis of DR is not fully understood, DR is believed to be associated with changes in oxygen saturation (StO_2) in retinal vessels (5), and its development has been linked to changes in partial pressure of oxygen (pO_2) in the retina (6). In addition, hypoxia is one of the main factors involved in the pathogenesis of DR, and eventually neovascularization (7). We believe that showing the changes in retinal oxygenation and metabolism in patients with no or early retinopathy will give clinicians a better and earlier insight on diabetic progression. However, since early microvascular changes may not be easily detected on clinical examination compared to late changes, at which point they are usually irreversible, changes in the hemoglobin StO_2 in retinal arteries and veins may indicate very early microangiopathy, which could help clinicians detect vascular compromise in diabetic patients long before retinopathy is seen clinically. Recently several devices have appeared, both in laboratory and clinical settings, capable of monitoring either StO_2 (8-11) in the retinal arterioles and venules or flow velocity in the superficial retina capillary network (12,13).

The Flow Oximetry System (FOS) (*Figure 1*) is a novel non-invasive system developed by our group to measure retinal StO_2 and retinal blood flow. The FOS system is capable of calculating StO_2 in the retina by acquiring spectroscopic sensitive images of the retinal vessels, making it the first device to simultaneously measure retinal oxygenation and blood flow. The device used in this study is a modified dual-length version of earlier prototypes that has been reported elsewhere (14). The results obtained with the FOS were co-registered with optical coherence tomography (OCT) thickness map obtained with a commercial system (Spectralis HRA+OCT™, Heidelberg Engineering Inc., Vista, CA, USA). OCT is a non-invasive, non-contact imaging technique that produces high resolution, cross-sectional images of human tissue. Recent work has suggested that change in retinal thickness, and more importantly the volume, can be used to assess the rate of change of disease, or progression and regression. In patients with macular edema followed by the vascular damage, the earliest change detected was a collection of excessive fluid in the Muller cells. After the loss of Muller cells, the fluid accumulated in the outer plexiform layer of Henle. The photoreceptor layer may be damaged due to this increased intraretinal pressure. Thus, OCT thickness and volume may serve as potential useful indicators of early change the patients with diabetes.)

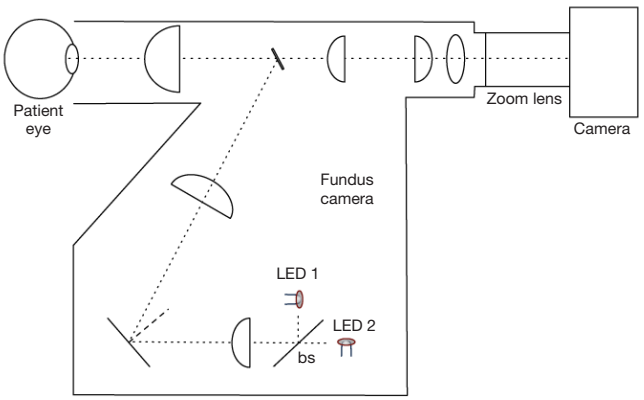

Figure 1 The layout of the FOS Device. All light sources were replaced with controllable LED systems. The imager consisted of a fundus camera combined with a zoom lens. FOS, Flow Oximetry System; LED, light emitting diode.

Methods

Flow Oximetry System (FOS)

The new FOS uses a simple two-wavelength algorithm to ascertain StO_2 levels in the retina. This method was developed by other groups (15-18) and was adapted to our instrumentation. The flow assessment is conducted with a red-blood-cells-tracking technique previously presented by

our group (19).

The combined flow and oximetry system consists of a modified Fundus Camera (Zeiss FF3, Jena, Germany), where both the original light sources (white light lamp for focusing and a flash light for image acquisition) were replaced with two light engines (Enfis, Swansea, UK, LED1 and LED 2 in *Figure 1*). The light engines were centered at 520 and 630 nm (15 nm FWHM). It is to note that Bosschaart *et al.* have shown 522 nm to be an isosbestic wavelength in human blood (20). A 30:70 beam-splitter was used to maximize the throughput of the green light source so to optimize image quality. This was necessary due to the low backscattered remission from the retina in that wavelength range. A color camera (24 bit, Prosilica Genie, Billerica, Massachusetts, 1,024 pixels × 1,024 pixels, chip size 3 mm × 3 mm) was combined with a zoom lens with focal length of 150-450 mm, f/5.6-f/3.2, (Computar, Commack, New York) and connected to the fundus system through its imaging port. A custom black epoxy attachment was constructed to align the camera to the system as well as obtain a solid connection between camera and fundus system. The camera was capable of 60 frames per second acquisition.

Image acquisition, was controlled through a custom made software, (Matlab®, MathWorks, Natick, Massachusetts). After the software was started, the camera entered focus mode where only the green light engine was active. In this mode, the engine was pulsated at 60 Hz and controlled by the camera; pulses were 3 μs in duration and camera gain was set to maximum. A short delay 0.5 μs between camera acquisition and pulse start was added programmatically to avoid any issues with the light source ramp up time. The short pulse setting was used to focus the fundus system and allow the operator to locate a region of interest within the patient retina. At the same time by using these settings, the subject compliance and comfort were maximized. When the operator considered all imaging parameters to be satisfactory, image acquisition was triggered through either a button in the general user interface or with a foot pedal connected to a data acquisition card (National Instruments, Austin, Texas) controlled by the program. The acquisition phase lasted a total of 4 seconds equal to 240 frames. During acquisition both the red and green light engine were active at 60 Hz. The pulse duration was increased to 6 μs so to have more energy deposition and clearer images. Even with this larger pulse the energy level of the combined light engines was well below the retina threshold of damage. Once the acquisition time was expired, the 240 frames were saved in an uncompressed Audio Video Interleave (AVI) format.

The color images produced by the camera were processed into their basic Bayer components; Red, Green, and Blue images. The 240 frames stack of Green images was used for the flow assessment, while both Red and Green stacks were used for the oximetry measurements. Minor cross talk between the images was noticed. The stacks of images were also registered with an algorithm presented elsewhere (19). Frames with large movement artifacts were discarded, both techniques can utilize as little as 20 frames so only a stable segment (small to no-motion present) of the full stack was ultimately used. When measuring oxygen within the retinal capillaries, frames for the Red and Green stack were averaged producing an average R and G image. These two images were then processed using the aforementioned algorithm based on calculation of the vessel optical density, where reflectance values on the vessels where normalization by reflectance values of nearby background.

Images obtained with an OCT system were co-registered with the FOS maps. A simple algorithm was devised for this purpose. Three characteristics points were selected in both the FOS image and the OCT enface image (typically vessel bifurcations were used), once the coordinate of the location were known, the OCT enface and thickness map were padded through interpolation to the same size as the FOS image. Since the thickness maps were generated with approximately 20 OCT scans, this interpolation did not drastically reduce the OCT thickness resolution. An example of this process is shown in *Figure 2*.

In vitro calibration and validation of two-wavelength system was conducted utilizing an eye phantom (21) combined with a 100 μm diameter microfluidic channel (Translume, Ann Arbor, MI), an epoxy phantom background mimicking various layer of the retina (RPE, Choroid, and Sclera) and a syringe pump (Harvard Apparatus, Holliston, MA). The choice of vessel size for *in vitro* testing was determined by our system limitations; while the velocity assessment works best at low vessel size the opposite is true for StO_2 measurements (22).

While our previously reported (14) multi-wavelength system allowed for direct StO_2 extrapolation through minimization of the intensity values of light backscattered from capillaries to the curves of oxygenated and deoxygenated hemoglobin, the two wavelengths system uses a different principle based on monitoring of vessel optical density. Hence the validation approach was based on measuring the optical densities [OD =log (Ibackground/Ivessel)] for the two wavelength of interest (OD_{520} for 520 nm and OD_{630} for 630 nm) on vessels of known diameter

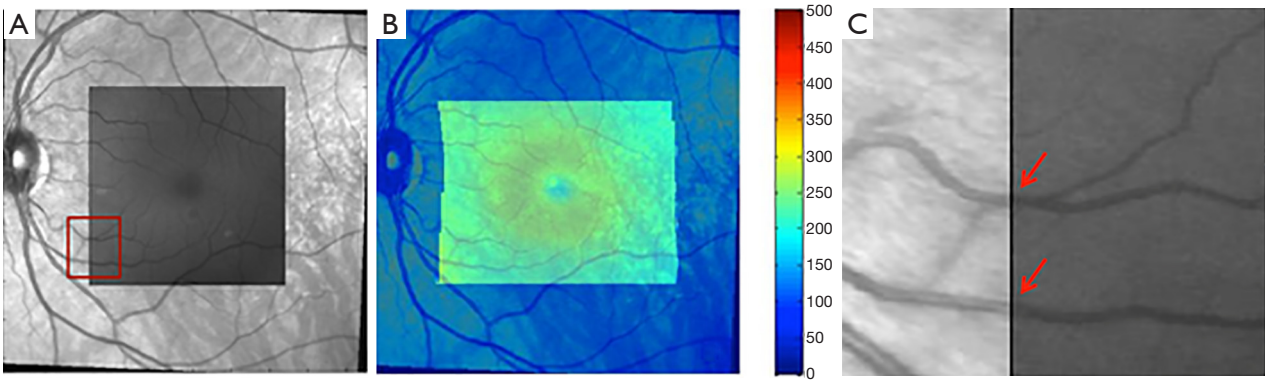

Figure 2 Co-registration of FOS images and OCT maps. Image A is the enface OCT image with the smaller FOS co-registered portion. Image B is the thickness map of the OCT. Image C shows is an enlargement of the portions shown in red in Image A. FOS, Flow Oximetry System; OCT, optical coherence tomography.

(100 µm) that were filled with known concentration of human blood from a local blood bank. StO_2 of the blood was modified through the addition of sodium hydrosulfite (Sigma, St. Louis, Missouri). Values between 50% and 100% StO_2 were created, as they were considered physiologically relevant. Before being imaged with the FOS, the same samples were measured with a bench-top oxygen sensor (Ocean Optics, Dundee, FL).

After FOS image acquisition, the OD ratio ODR = OD_{630}/OD_{520} was calculated. Values of ODR measured with the FOS were linearly proportional to the ones measured with the spectrophotometer and could be related to the true values of StO_2. Each measurement was repeated three times.

The results of the velocity calibration have been presented elsewhere (19), here we simply present an example of our *in vitro* results where the syringe pump flow rate is increased to three different levels. As a consequence, the velocity within the capillary increases.

Subjects and characterization

Our study was designed as a prospective case-series study to assess retinal StO_2 in normal subjects, patients with DM without DR, and diabetic patients with non-proliferative diabetic retinopathy (NPDR). Our research adhered to the tenets of the Declaration of Helsinki (as revised in Edinburgh 2000) and was approved by the Johns Hopkins institutional review board. Patients were enrolled after giving their informed consent. Both type 1 and type 2 diabetics were included and duration of diabetes was noted. Subjects were required to have ocular media sufficiently clear to allow good quality ocular imaging. Exclusion criteria included subjects with corneal opacities, cataracts or dense vitreous hemorrhage, and patients with severe non-proliferative and proliferative DR. Current smokers and subjects with history of vitreo-retinal disease or surgery such as retinal detachment, epiretinal membrane, or vitreous hemorrhage were excluded. Other exclusion criteria included patients with other retinal or macular diseases other than that caused by diabetes and those with medical conditions that could interfere with the subject's ability to comply with study procedure such as inability to maintain steady head or eye positioning, as in patients with ataxia or nystagmus. Pupils were dilated prior to the image acquisition by standard dilation procedures using proparacaine hydrochloride 0.5%, tropicamide 1%, and phenylephrine hydrochloride 2.5%.

After dilation, a 4-second video was acquired. A total of 240 color frames (in 4 seconds) were acquired from each study eye. Analysis software was developed and used to calculate StO_2. Because the optical properties of the layers underlying the retinal blood vessels vary from one patient to another, a calibration step was required at the beginning of the image analysis for every patient. The software provided a manual mode for tracing the segments of vessels to be analyzed and the calculated StO_2 levels of the vessels were then overlaid on the gray scale image of the retina with false colors representation.

The FOS computerized assessment software was used to determine StO_2 levels in arteries and veins located in two main areas of the retina. The first area was the central 6 mm of the retina, excluding the foveal avascular zone (central 1 mm), and the second area was between 1 to 2 mm from the margin of the optic disc. While in the first area,

Table 1 Demographics of study subjects—study 1

Characteristics	Normal	Diabetic patients	
	(n=31)	No DR (n=25)	NPDR (n=21)
Mean age (SD), years	61.8 (11.4)	62.3 (13.8)	65.7 (13.3)
Female gender, n (%)	41.9 (55.6)	52 (46.7)	33 (36.4)
Mean duration of diabetes in years (SD)	NA	7.6 (9.0)	12.8 (9.5)

DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; SD, standard deviation; NA, not applicable.

Table 2 Demographics of study subjects—study 2

Characteristics	Normal	Diabetic patients	
	(n=18)	No DR (n=15)	NPDR (n=11)
Mean age (SD), years	56.2 (12.1)	63.5 (12.6)	66.9 (11.6)
Female gender, n (%)	10 (55.6)	7 (46.7)	4 (36.4)
Ethnicity, n (%)			
Caucasian	11 (61.1)	7 (46.7)	6 (54.5)
African American	4 (22.2)	3 (20.0)	3 (27.3)
Asian	2 (11.1)	2 (13.3)	0 (0)
Other/unknown	1 (5.6)	3 (20.0)	2 (18.2)
Diabetes duration, n (%)			
≤5 years	NA	9 (60.0)	3 (27.3)
>5 years	NA	6 (40.0)	8 (72.7)
Mean duration of diabetes in years (SD)	NA	5.2 (3.2)	12.8 (11.6)

DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; SD, standard deviation; NA, not applicable.

flow, oxygenation, and retinal thickness could be measured, the vessels surrounding the optics discs were too large for our flowmetry technique and retinal thickness was not measured, hence only oxygenation was considered. The image pixel size, which is calculated during the calibration portion of the study, was used to measure distances across the fundus image. The images were further sub-divided into four quadrants: superior, inferior, temporal, and nasal.

For the first study a total of 77 subjects (77 eyes) were included in the final analyses (31 normal subjects, 25 DM no DR patients, and 21 NPDR patients). *Table 1* shows the demographics and baseline characteristics of the study participants in different study groups. Measurements of the StO_2 and flow velocity were conducted. The measurements were made through tracing all vasculature in the central 6 mm of the retina, excluding the foveal avascular zone (central 1 mm). Arteries and veins were identified and traced. The average StO_2, velocities, and retina thickness, along single vessel and across multiple vessels and two main zones (1 and 2 mm diameter from the macula) was calculated.

For the second study a total of 44 subjects (44 eyes) were included in the final analyses (18 normal subjects, 15 DM no DR patients, and 11 NPDR patients). *Table 2* shows the demographics and baseline characteristics of the study participants in different study groups. Age of participants ranged from 30 to 83 years, with the mean [± standard deviation (SD)] of 61 (±12.5) years. The mean duration of diabetes in patients without retinopathy was 5.2 (±3.2) years and was 12.8 (±11.6) years in patients with retinopathy. 61.1% of our patients were Caucasian, 22.2% were African Americans, 11.1% Asians, and 5.6% belonged to other races. In this phase of the study arteries and veins from either the superior and/or inferior quadrants were selected. Vessels within the temporal and nasal quadrants were excluded because of their small size. Measurements were taken from one major artery and one major vein in each quadrant. Only one eye per patient and only vessels clearly identified by an experienced ophthalmologist as arteries and veins were included in the final analysis. Eyes in which at least one artery and one vein in either quadrant could not be measured were excluded from the analysis. Vessel tracing was done manually and calculation of the StO_2 was automatically done using the computerized software. Patients in whom the duration of diabetes was unknown were eliminated from the final analyses.

Data was presented as mean and standard deviation (SD). Comparisons of means were done using variance analysis (ANOVA). Pairwise comparisons of the three groups of the study (normal *vs.* DM no DR, normal *vs.* NPDR, and DM no DR *vs.* NPDR) were performed using Bonferroni post-hoc analysis. All analyses were done with STATA statistical software, version 11 (STATA Corp, Texas).

Results

In vitro results

Various values of StO_2 of human blood were used during this phase of testing and the results obtained from the FOS system were compared to the one obtained with the oxygen sensor. ODR at the chosen wavelengths decreases linearly with increased StO_2; ODR data was fitted with a first order polynomial to minimize some of the experimental error. The results of this approach are shown in the insert of *Figure 3*. The corrected values of ODR were ultimately

used to calculate StO_2. Results are shown in *Figure 3*.

Figure 4 shows typical trace history map for increasing pump flow rate. The trace histories correspond to values of flow rate between 0.01 and 0.05 mL/h.

In vivo results

Figures 5 and 6 show results obtained in the first region under investigation (macular area). The region in the insert of *Figure 5* were averaged and separated into two data sets. Our analysis of flow and StO_2 values did not reveal any statistical significance when comparing study groups. *Figure 7* shows the average retinal thickness in the macular region.

When imaging areas in the proximity of the optic disc (*Figure 8*), some statistical differences arose. The mean StO_2 (±SD) in retinal arteries was 96.9%±3.8% (median; 98.9%), in normal subjects, 97.2%±3.6% (median; 98.9%) in patients with DM without DR, and 98.4%±2.0% (median; 99.1%) in patients with NPDR (*Table 3*). The mean venous StO_2 was 57.5%±6.8% (median; 57.7%) in normal subjects, 57.4%±7.3% (median; 58.1%) in patients with DM without DR, and 51.8%±6.8% (median; 51.4%) in patients with NPDR (*Table 3*). Among all patients, the minimum measured arterial StO_2 was 90.1% and the maximum was 100%, while the minimum measured venous StO_2 was 43.8% and the maximum was 66.8%.

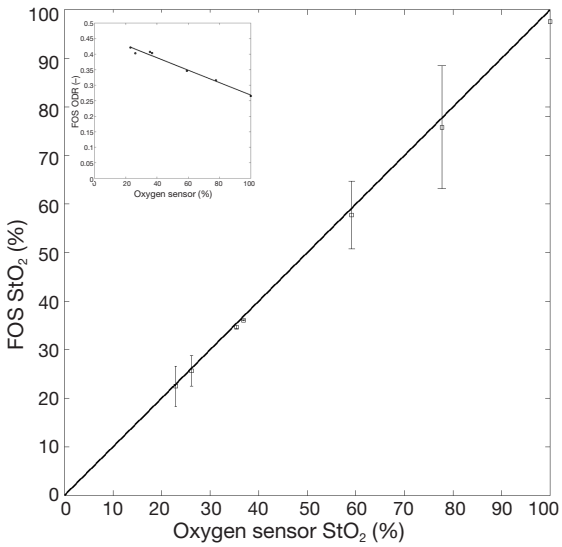

Figure 3 Capillary filled with human hemoglobin, different oxygen saturation values were obtained and measured with a bench-top oximeter. FOS, Flow Oximetry System; StO_2, oxygen saturation.

While the arterial StO_2 tended to be highest in patients with NPDR when compared to the other groups, the venous StO_2 tended to be lowest in this same group of patients (*Table 3*). Nevertheless, no statistically significant difference was found when the mean arterial and mean venous StO_2 were compared across the three groups using the one-way ANOVA test (*Table 4*). Comparison of the mean arterio-venous (A-V) difference across the three groups, however, was found to be statistically different (P=0.015) (*Figure 9*). Pairwise comparisons of the mean A-V difference among the three groups, adjusted for multiple comparisons, using Bonferroni's post-hoc analysis (*Tables 3,4*) demonstrated statistically significant differences between the normal subjects and the NPDR group (P=0.02) and between diabetic patients without DR and patients with NPDR (P=0.042). However, no statistically significant difference was found when we compared the A-V difference for normal subjects and diabetic patients without DR (*2*).

Though we did not compare the mean StO_2 across the different quadrants, there were no major differences in the measured StO_2 values between the superior or inferior quadrants.

The retinal vascular system is an environment where a

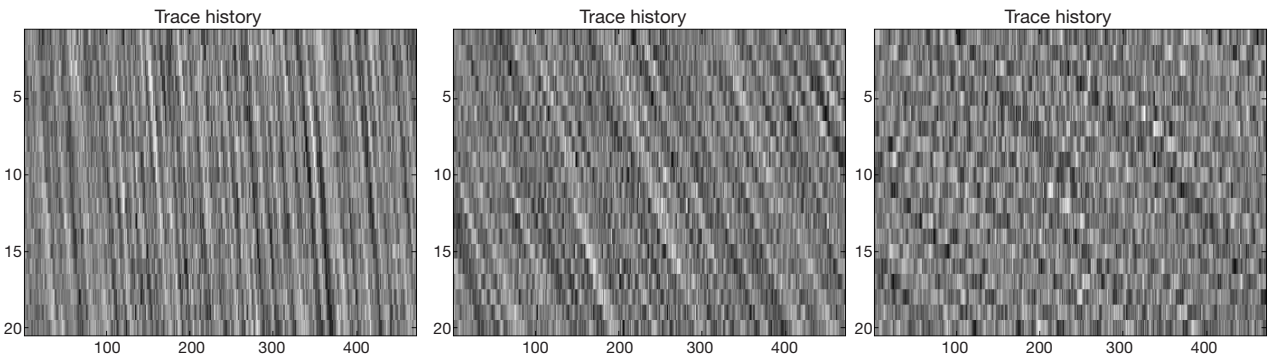

Figure 4 Trace history of 100 μm vessel at different flow rates (0.01, 0.03, 0.05 mL/h).

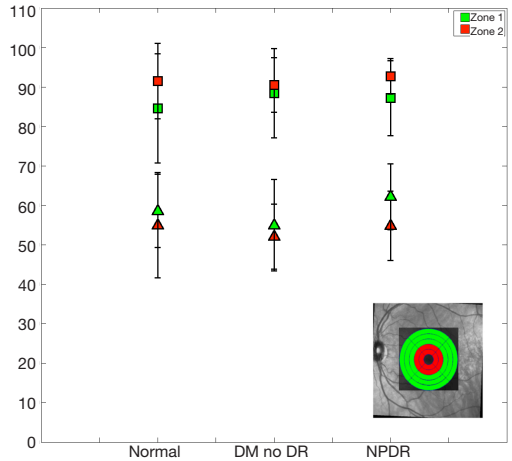

Figure 5 Oxygen saturation averaged across vessels in the region of interest of the insert. Values are in percent oxygen saturation. DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy.

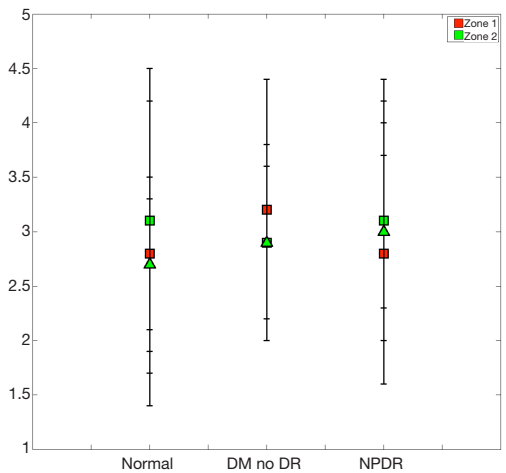

Figure 6 Average velocities in mm/sec for venules and arterioles in the regions shown in the insert of *Figure 5*. DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy.

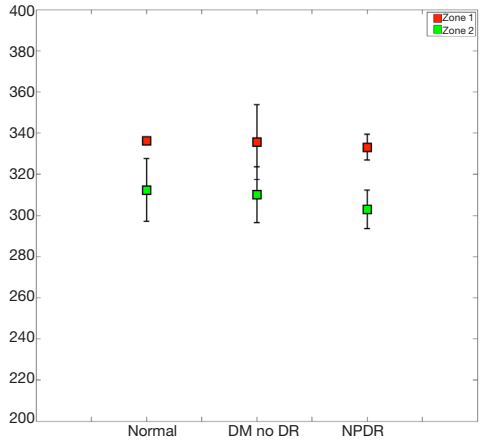

Figure 7 Average retinal thickness of the regions shown in the insert of *Figure 5*. Values are in μm. DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy.

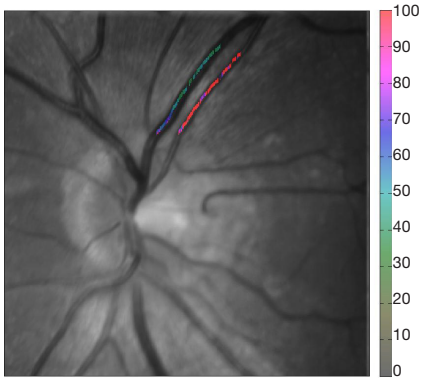

Figure 8 Typical images of results (artery ~100% and vein ~55%).

constant balance of oxygen pressure and StO_2 is required for normal functioning of the eye (23). Therefore, changes in StO_2 may indicate onset of hypoxia and microvascular retinal changes that may aid in clinical diagnosis of early diabetic eye changes. In our study, we found that, compared to individual changes in StO_2 of retinal veins and arteries, calculation of the A-V differences might be a better predictor of tissue oxygenation. Such finding can be explained by Fick's principle, which states that the consumption of oxygen in a tissue is proportional to the rate of blood flow through that tissue times the difference in StO_2 between the arterial and venous circulations in the same tissue (24). It is important to note that studies exploring the regional oxygen differences in the retina found that vessels further away from the macula demonstrated lower arterial and venous StO_2 compared to vessels closer to the macula, independent of vessel diameter (23). Skov Jensen *et al.* have also suggested that peripheral and macular retinal blood vessels show differences in their ability to autoregulate their metabolic needs; this was true in healthy individuals as well as in patients with peripheral or macular DR (25). Therefore, depending on the location of measurement of StO_2 in the retina, the results can differ

Table 3 Arterial and venous StO_2

StO_2	Normal (N=18)	Diabetic patients	
		No DR (N=15)	NPDR (N=11)
Mean artery StO_2 (SD)/median	96.9 (3.8)/98.9	97.2 (3.6)/98.9	98.4 (2.0)/99.1
Mean vein StO_2 (SD)/median	57.5 (6.8)/57.5	57.4 (7.3)/58.1	51.8 (6.8)/51.4
Artery-vein StO_2 (SD)/median	39.4 (7.0)/38.5	39.8 (6.2)/39.1	46.6 (6.7)/45.0

DR, diabetic retinopathy, NPDR, non-proliferative diabetic retinopathy; StO_2, oxygen saturation.

Table 4 ANOVA and Bonferroni post-hoc analysis

StO_2	P values			P values
	Normal *vs.* DM no DR	DM no DR *vs.* NPDR	NPDR *vs.* normal	
On-way ANOVA				
Artery StO_2				0.498
Vein StO_2				0.071
Artery-vein StO_2				0.015
Bonferroni post-hoc analysis				
Artery StO_2	1.00	1.00	0.75	
Vein StO_2	1.00	0.137	0.107	
Artery-vein StO_2	1.00	0.042	0.02	

Comparison of means was done using ANOVA, student *t*-test, and Bonferroni tests. StO_2, oxygen saturation; DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; ANOVA, analysis of variance.

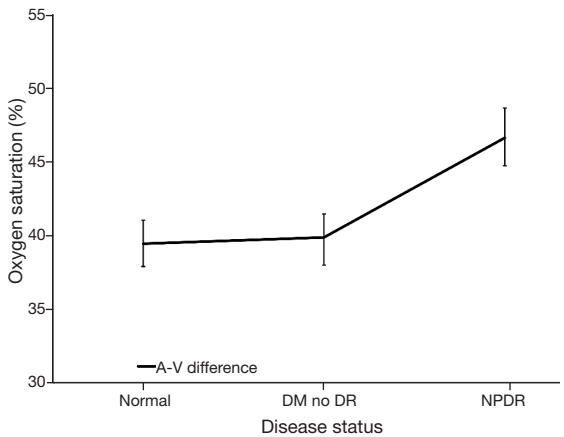

Figure 9 The arterial venous (A-V) difference distribution across normal subjects and diabetic patients with and without retinopathy. DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy.

widely. To avoid such location-dependent variation of StO_2, the measurements in our study were taken from vessels in standard location in the peri-papillary region.

Our results show that diabetics with NPDR were more likely to have a higher arterial but lower venous StO_2 with statistically significant higher A-V difference when compared to normal subjects and to diabetic patients without retinopathy. This is contrary to the results reported by Hammer *et al*. 2009 where patients with mild NPDR had lower A-V difference when compared to normal subjects (9). Hardarson and Stefánsson 2012 reported a higher venous StO_2 in patients with DR. However in their study, they have stratified their sample to patients with background retinopathy, patients with macular edema, and patient with pre-PDR/PDR. Therefore, it is unlikely that our results can be directly compared as it is unknown where patients with mild NPDR would fit in such classification (5).

The increase in A-V difference in the NPDR group compared to the other groups could be due to an increase in oxygen consumption by the retina, a likely compensatory mechanism for the onset of hypoxia in some areas of the retina. Physiologically, the decrease in oxygen supply to a tissue usually sets off a cascade of adaptive mechanisms designed to maintain cellular activity at the lowest acceptable level to ensure survival of the affected tissue. Continued or worsening hypoxia leads to a failure of this compensatory mechanism, leading to cellular dysfunction and possibly irreversible cell damage (26). In mildly hypoxic but still viable retina as could be the case in mild NPDR, there may be increased extraction of oxygen the retina, which leads to a lower venous StO_2 and a higher A-V difference as evidenced in our study. As hypoxia worsens with further progression of the retinopathy, the number of viable areas in the retina will eventually decrease; at that stage, we may begin to see a decrease in the ability of the tissue to use oxygen, which may explain the higher venous StO_2 and lower A-V difference observed in the other studies. Calculating the A-V difference in the same vessels at the same location and monitoring

such A-V gradient over time may give a more accurate representation of disease progression.

Tiedeman *et al*. have found that the decrease in venous StO_2 in diabetic patients to be highly correlated with the glycemic state of the patient, as well as in patients who had a longer duration of diabetes (24), which is consistent with what we have observed in our study (*Table 1*). The authors have postulated that venous StO_2 in the retina depends on an auto-regulatory response in retinal tissues driven by oxygen demand and patients who were not able to auto-regulate adequately to an increasing oxygen demand, tended to extract more oxygen from the blood and subsequently had lower venous StO_2 (24). Patients with longer duration of diabetes are more prone to have uncontrolled hyperglycemia; therefore, it is plausible that the longer the duration of diabetes the higher the probability of retinopathy and hence, the increased probability of lower venous StO_2.

Increased oxygen delivery to the retinal tissue can also be explained in the absence of retinal hypoxia. Diabetic patients usually have higher metabolic demand, which is further complicated by the increase in glycosylated hemoglobin (HbA1c), which has higher affinity to oxygen. However, the insulin deficiency/resistance in those patients leads to increased levels of 2,3-bisphosphoglycerate (2,3-BPG) in the red blood cells (27). 2,3-BPG shifts the oxygen dissociation curve to the right, increasing oxygen delivery to the retinal tissues. The net result of increased affinity of HbA1c to oxygen and the increase in 2,3-BPG is complex and it is possible that early in the disease, the increased oxygen delivery to retinal tissues, as observed in our study, is derived by the dominance of the increased levels of 2,3-BPG.

Finally, the increase in A-V difference in the NPDR group may not indicate increased oxygen delivery to tissues. According to Fick's principle, oxygen consumption is the result of retinal blood flow and A-V difference. Therefore, the increase in oxygen extraction as indicated by the increased A-V difference in NPDR group can be negated by decreased retinal blood flow.

Our initial attempt to measure the StO_2 in the macular region has not showed any statistically significant differences or particular trends in all study groups either in StO_2 of blood velocity. This was true, whether the measured vessel is an artery, a vein, or representing an A-V differential. We believe this was the result of several factors. First, it was very difficult, even for experienced ophthalmologist, to differentiate 3rd and 4th order arteries from veins giving the very small caliber of the measured vessels. Second, in many instances, there were not enough vessels that could be confidently measured in the central 6 mm zone, especially in patients with NPDR, which resulted in exclusion of large number of data points with subsequent significant reduction of our sample size and hence, our statistical power. Finally, with very small vessels, the SDs of the averages along any measured vessel, whether it is a potential artery or vein, but especially with proposed arteries were too high.

Conclusions

We have described a normative range of arterial and venous StO_2 as obtained using the FOS and have shown that while arterial StO_2 tended to increase mildly in patients who have NPDR, venous StO_2 tended to decrease in this same group of patients when compared to the other groups, with significant increase in A-V difference. Our findings suggest increased oxygen extraction in eyes with early DR, which can be due to increased oxygen consumption by retinal tissue or secondary to a compensatory mechanism in response to reduction of blood flow in areas of retinal hypoxia. It is possible to explain the increase in oxygen extraction by right shift of the oxygen dissociation curve secondary to increased 2, 3-DPG levels or possibly a combination of all previous mechanisms. Our study also suggests that A-V difference could be a more accurate predictor of tissue oxygenation compared to measuring either retinal arterial or venous StO_2 alone.

Additional studies with a larger sample size, blood flow measurements, and inclusion of patients with more advanced/proliferative DR are indicated to confirm our findings and to further elucidate the utility of the FOS in clinical settings.

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