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Regional variation in human retinal vessel oxygen saturation

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ABSTRACT

The purpose of this study was to investigate regional differences in oxygen saturation of blood in first degree retinal vessels using a novel non-flash hyperspectral retinal camera (Photon etc Inc). Nine healthy individuals (mean age 24.4 ± 3.6 yrs, 5 males) were imaged at 548, 569, 586, 600, 605 and 610 nm wavelengths. Optical density values were extracted with the aid of Image-J software for blood oxygen saturation (SO_2) determination. Arteriolar and venular SO_2 were measured at three locations (ranging 1–3 optic nerve head radii) from the disc margin along the vessels in the superior and inferior temporal quadrants. Retinal SO_2 was significantly higher in the superior temporal arteriole and venule as compared to the inferior temporal vessels ($p = 0.033$ and $p = 0.032$ for arterioles and venules, respectively). SO_2 was not significantly different between the three measurement sites for any of the given vessels imaged ($p > 0.05$). In conclusion, greater SO_2 values were found in the superior temporal first degree retinal arterioles and venules in young healthy individuals than in the equivalent inferior vessels. However, there were no detectable differences in retinal SO_2 along each of the major vessels, a finding that is consistent with the concept of these vessels not contributing primarily to gas exchange. Moreover, the SO_2 was consistently higher in the arterioles than in the equivalent venules ($p < 0.0001$).

1. Introduction

The intricacy of the retinal vascular complex and the high-oxygen demand of the retina, combined with an absence of perfusion redundancy, makes the retina an especially vulnerable target for ischemic diseases. The reliable determination of retinal blood oxygenation is anticipated to be a valuable outcome measure in any posterior segment pathology with a potential ischemic component such as diabetic retinopathy, age-related macular degeneration, vascular occlusions and even possibly glaucoma. Non-invasive hyperspectral imaging of the retina and evaluation of the retinal blood oxygenation are promising new tools for investigating the pathophysiology of retinal disease.

Previous research has investigated changes in retinal oxygen saturation (SO_2) in healthy individuals as well as in patients with systemic and ocular pathologies. Retinal arteriolar oxygen saturation has been found to be stable in healthy individuals, whilst a significant decrease in venular oxygen saturation with increasing age in males has been noted (Geirsdottir et al., 2012). Venous oxygen saturation measured using the dual-wavelength oximetry technique has also been shown to be significantly higher in patients with diabetic retinopathy than in healthy controls (Hammer et al., 2009). A more recent study has reported significantly higher oxygen saturation values both in arterioles and venules of diabetic patients compared to controls (Hardarson and Stefansson, 2012). Moreover, arteriolar SO_2 values have been found to be significantly lower in patients with normal tension glaucoma compared to controls (Michelson and Scibor, 2006). A majority of these findings have been evaluated around the optic nerve or a pre-defined discrete area such as the major inferior temporal retinal vessels. As such, it is yet unclear whether variation in techniques and the chosen retinal location of interest can bias the outcome of retinal SO_2 assessment.

Given that oxygen diffusion occurs at the level of the exchange vessels (i.e. the smaller vessels downstream of the arterioles), the amount of arterial oxygenation might be expected to fall along the vessel tree. The aim of this study was to evaluate optical density and SO_2 in the major retinal vessels at three discrete points along the venules and arterioles and to compare these values between the superior temporal and inferior temporal quadrants. The study utilized a novel prototype retinal hyperspectral camera.

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2. Materials and methods

2.1. Participants

Nine healthy volunteers (mean age = 24.4 ± 3.6, age range 21–29 years, 5 males) were recruited for this study. All participants were non-smoking, free of any media opacities, ocular and systemic pathologies and were not taking any medication. Volunteers' best-corrected visual acuity was 0.00, or better, using the logarithm of the minimum angle of resolution (LogMAR) ETDRS chart number 1. Eyes were dilated using a single drop of 1% tropicamide (Mydriacyl, Alcon Canada Inc, Mississauga, Canada) prior to a thorough fundoscopic examination and after intraocular pressure measurements using Goldmann tonometry. This study was approved by the Research Ethics Board of the University Health Network, Toronto, Canada. Written informed consent was obtained from all the volunteers and the study protocol adhered to the tenets of the Declaration of Helsinki.

2.2. Imaging procedure

A prototype hyperspectral camera (Photon etc, Montreal, Canada) was used for imaging. The system is based on a custom-built high definition (1392 × 1040 pixel) mydriatic fundus camera incorporating a tunable laser source (TLS) as the light source. The TLS is able to transmit wavelengths within a spectral range of 420–1000 nm (visible to near-infrared) with a bandwidth of 2 nm allowing rapid wavelength selection from the stable and supercontinuum light source (Leukos-SM-30-OEM, Leukos Innovative Optical Systems, Limoges, France). The TLS is electronically tunable and the system incorporates an automatic spectral calibration system to achieve precise and accurate (<1 nm) wavelength selection. Application of the TLS along with a white light source eliminates the use of a conventional Xenon flash lamp and thus all images are acquired at safe and comfortable light levels. The field of view for each image is 37.4° diagonally, 29° horizontally and 22° vertically and the focus range of the camera lens covers +10 to −10 diopters.

One eye was randomly selected for imaging. Similar to any conventional camera, the participants were seated with their chin in the chinrest and forehead against the head support. Each subject was instructed to maintain fixation on an external red light source in front of the non-study eye. Multi spectral retinal images were taken using specific wavelengths between 500 and 650 nm (i.e. 548, 569, 586, 600, 605 and 610 nm) at an exposure time of 80 ms for each wavelength resulting in an average acquisition time of 3 s. Participants were instructed to maintain steady fixation during the complete image acquisition paradigm.

2.3. Image registration

The PhySpec software (Photon etc., Montreal, QC, Canada) was used to normalize the cubes (i.e. a 3-D graphical representation of *x*, *y* co-ordinates as a function of wavelength) and ultimately correct for wavelength-dependent optical scaling and fine involuntary eye movements. The reference image for scaling was chosen at 570 nm because the vessels are most distinct at this wavelength. A portion of the image containing the optic nerve head (ONH) was selected and all images in the cube were aligned with this selected region of the reference image. Additionally, a Gaussian filter was used to improve the details of each cube.

2.4. Optical density, oxygen saturation and vessel diameter calculations

The system is capable of automatic intensity spectra determination at manually specified locations by providing the reflected light intensity values (for both arterioles and venules) that are extracted at each selected location for the spectrum of selected wavelengths. Retinal vessel oxygen saturation was measured in one major arteriole and venule at three locations on the vessels (i.e. at 1 optic nerve head radius, at 2 radii and at 3 radii distance) in the inferior and superior temporal quadrants of the study eye. Image-J software (http://rsb.info.nih.gov/ij/) line tool was used for precise *x*, *y* co-ordinate determination of the retinal point of interest. A line of length three times of the vessel width was drawn perpendicular to the vessel and the optical density values were extracted using an Image-J macro. Due to the lack of an intrinsic algorithm to measure the optical density along the wavelength band, the concept of the optical density ratio by Beach et al. (1999) of two monochromatic wavelengths (in this case 586 and 605 nm as found to be more repeatable than other oxygen sensitive wavelengths) for the determination of the retinal vessel oxygen saturation was used. The *a* and *k* constant values were calculated based on previously published work on automated retinal oximetry (Hardarson et al., 2006). To measure the vessel diameter, all images were set at 570 nm as both arterioles and venules are most distinct at this wavelength and standardized scaling (300% magnification) was adopted. Image-J software was used to draw a perpendicular demarcation line between the outer edges of the vessel walls (Fig. 1).

2.5. Statistical analysis

Statistical Package for the Social Sciences (SPSS) v16 was used for statistical analyses. Data was tested for normality of distribution using Shaphiro-Wilk test and histograms ($p > 0.254$ for all). Repeated measure analysis of variance (re-ANOVA) was used to compare SO_2 values amongst three regions of the vessels (arterioles and venules separately). Independent sample *t*-test was used to compare vessel

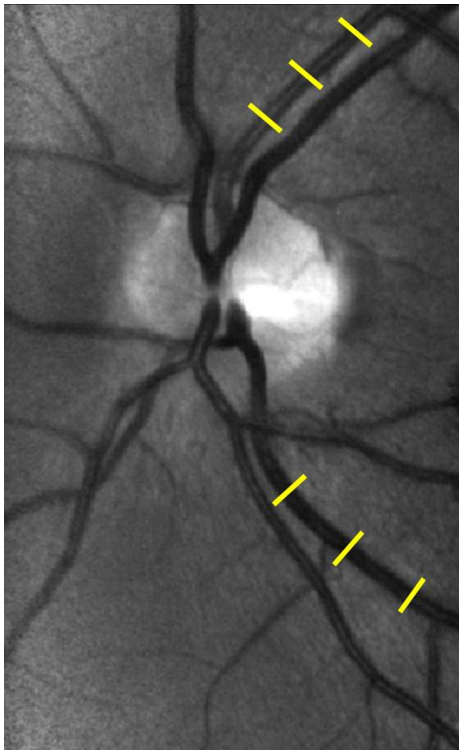

Fig. 1. An example of the analysis procedure undertaken using Image-J software. The yellow lines (3× length of vessel diameter perpendicular to the longitudinal orientation of the vessel) indicate three regions of interest selected for vessels within the superior and inferior temporal quadrants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)