Choroidal Oximetry With a Noninvasive Spectrophotometric Oximeter

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Purpose. The purpose of the study was to establish a new technology to measure hemoglobin oxygen saturation in human choroidal vasculature with a noninvasive spectrophotometric oximeter.

Methods. The fundus camera–based oximeter captures dual-wavelength oximetry images of the fundus and calculates optical density ratio (ODR), which is inversely related to hemoglobin oxygen saturation. Sixteen healthy and lightly pigmented individuals were imaged during normoxia and six during both normoxia and pure oxygen breathing (hyperoxia). ODR was measured for choroidal vessels, vortex veins, and retinal arterioles and venules.

Results. ODR was 0.10 ± 0.10 (mean ± SD) for choroidal vessels, 0.13 ± 0.12 for vortex veins, 0.22 ± 0.04 for retinal arterioles, and 0.50 ± 0.09 for retinal venules. Inhalation of pure oxygen lowered ODR levels in all vessel types; the decrease was 0.035 ± 0.028 in choroidal vessels ($P = 0.029$, paired t-test), 0.022 ± 0.017 in the retinal arterioles ($P = 0.022$, paired t-test), and 0.246 ± 0.067 in retinal venules ($P = 0.0003$, paired t-test).

Conclusions. The ODR can be measured noninvasively in the choroidal vessels of lightly pigmented individuals and is significantly lower in choroidal vessels than in retinal arterioles. This may suggest higher oxygen saturation but is also compatible with the reduced contrast of choroidal vessels at both wavelengths that is expected from scattering of light within the choroid. The decrease of ODR during hyperoxia was significant for all vessel types, which confirms that the oximeter is sensitive to changes in oxygen saturation in both choroidal and retinal vessels.

Keywords: oxygen, choroid, retina, optical density, hyperoxia

The human retina is dependent on two separate vascular systems, the retinal vessels and the choroid. Inner layers of the retina are served by the retinal vessels with oxygen and nutrition, while outer layers of the retina, including the highly metabolically active photoreceptors, are served mainly from the choroid. The choroid has extraordinarily high oxygen tension,[1] and the arteriovenous difference in oxygen content in the choroid is very low. Experiments on cats have demonstrated an arteriovenous difference in oxygen content of only 3%,[2] while it is around 35% in the retinal circulation.[3] Present knowledge of oxygenation in the choroid comes almost exclusively from invasive animal studies because lack of a safe and reliable noninvasive technology for oxygen studies in the choroid has prevented studies on humans until recently.

In 1961 Broadfoot et al.[4] reported a modified ophthalmoscope capable of noninvasively detecting changes in choroidal oxygen saturation in humans. This involved nonimaging measurement of the light reflected from the ocular fundus for illumination by light in four broad spectral bands during deep normal breathing and apnea. In 1975, Laing et al.[5] reported a nonimaging spectrophotometric choroidal oximeter that, following calibration, was able to continuously quantify choroidal oxygen saturation. It was composed of a fundus-monitoring unit, dual-wavelength (650 and 805 nm) light source and electrical system for synchronous processing of signals and calculation of the oxygen saturation. These choroidal oximetry studies establish the possibility of detecting differences in choroidal oxygenation in humans using noninvasive spectrophotometric methods. The technique used and described in this paper for spectrophotometric measurements of choroidal oxygen saturation is based on the same basic principles as Hickam et al.[6] and Beach et al.[7] used for calculating retinal oxygen saturation and was reported by Hardarson et al.[8] in 2006 and now recently by Geirsdottir et al.[9] Whereas previous measurements have determined oximetry averaged over an extended area of the choroid, we explore here the possibility of oximetric imaging of choroidal vessels.

Choroidal oxygen saturation measurements are important from at least two points of view. First, they may allow study of pathophysiology of diseases in which choroidal ischemia and abnormalities in choroidal blood flow may play a role, such as age-related macular degeneration,[10–12] diabetic retinopathy,[13,14] and central serous choroioretinopathy.[15] Second, they may allow direct measurements of oxygen saturation in central vasculature. This is important in cardiovascular shock and severe injury, in which peripheral oxygenation as measured, for

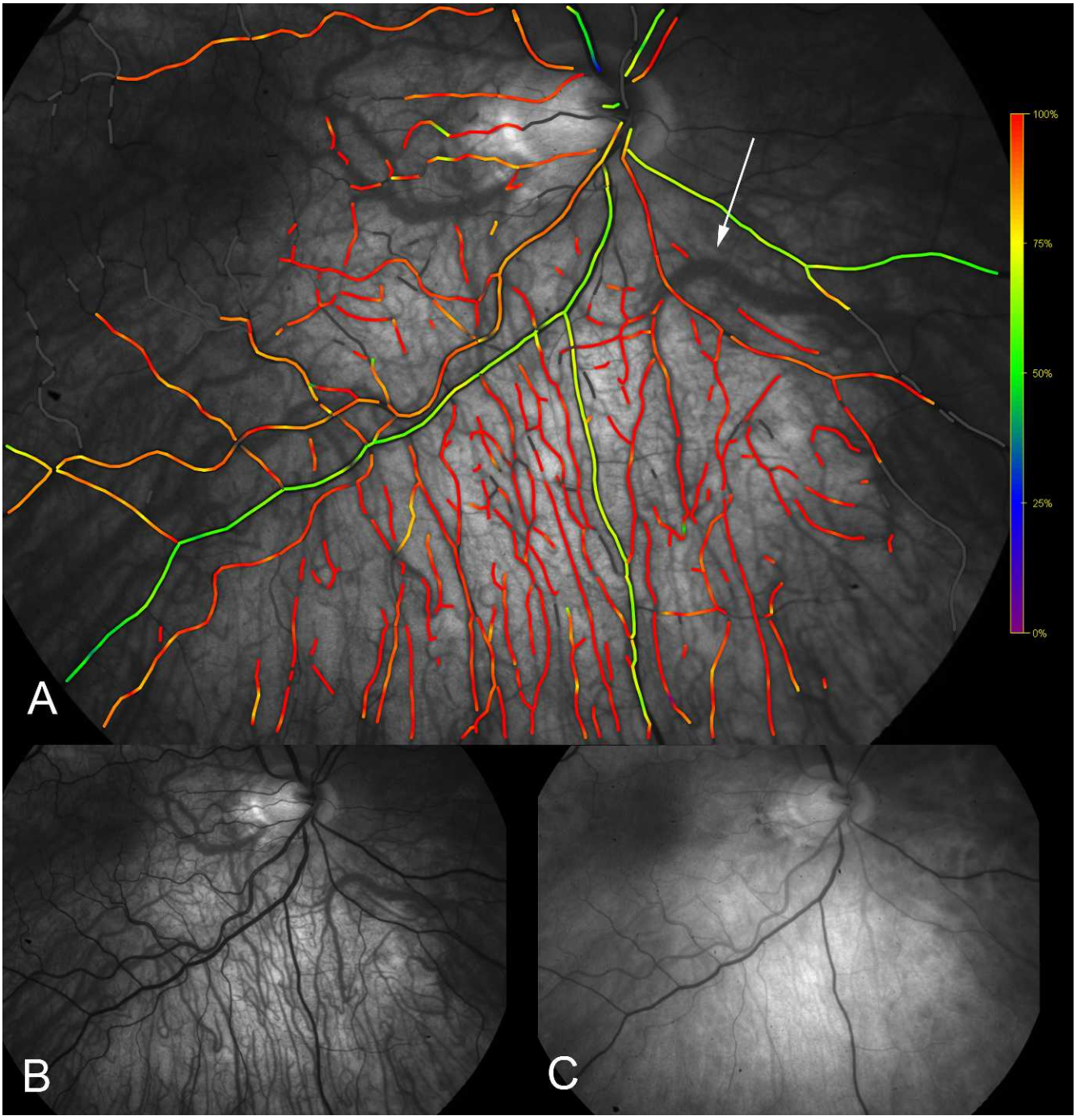

FIGURE 1. Choroidal oximetry image. (**A**) The vascular bed of the choroid is visible and measureable in lightly pigmented individuals with a spectrophotometric oximeter. The oxygen saturation in the vessels is color coded. *Red* represents the highest oxygen saturation and *purple* is the lowest (see *scale* on *right side*). *Arrow* is pointing at a visible choroidal vortex vein. (**B**) Oximetry fundus image taken with oxygen-insensitive wavelength (570 nm). (**C**) The same area of the fundus is captured simultaneously at an oxygen-sensitive wavelength (600 nm).

example, with finger pulse oximeter may be unreliable since the body favors vital organ perfusion including the eyes. Very little is known about the human choroidal oxygen saturation and the effect of disease, and therefore, with further technical development and additional studies, benefits of choroidal oximetry may emerge.

The goal of the study was to establish a new technology to measure hemoglobin oxygen saturation with a spectrophotometric oximeter in the choroidal vasculature in healthy human volunteers.

METHODS

Oxymap T1, a Spectrophotometric Retinal Oximeter

The fundus camera–based oximeter, Oxymap T1 (Oxymap ehf., Reykjavik, Iceland) captures dual-wavelength oximetry images of the retina. The fundus camera is a Topcon TRC-50DX (Topcon Corporation, Tokyo, Japan). The oximeter is attached on top of the fundus camera and is comprised of a custom-

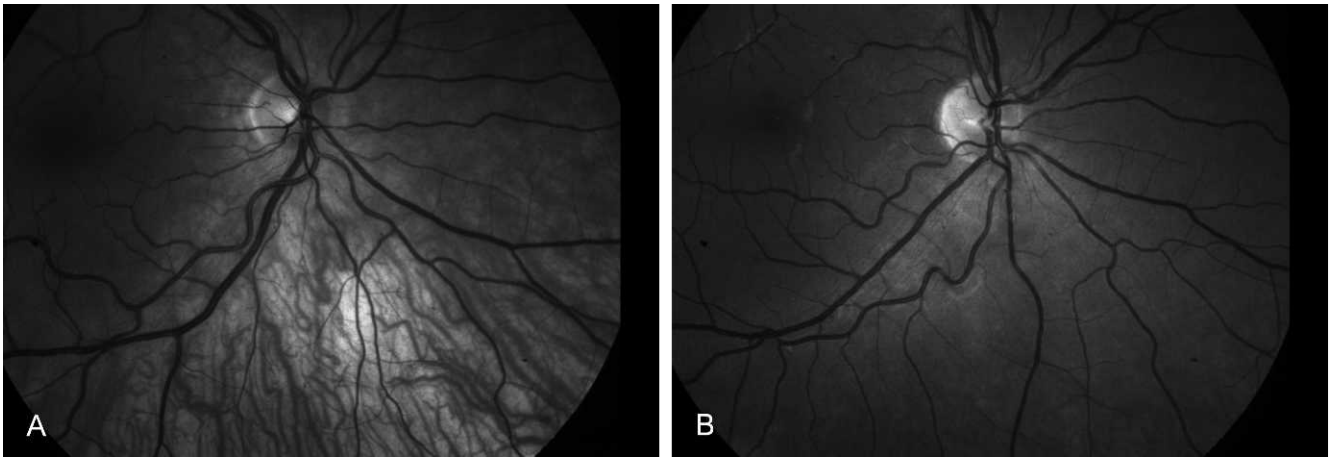

FIGURE 2. Lightly pigmented versus pigmented. Comparison between (**A**) a lightly pigmented individual with visible choroidal vasculature and (**B**) a pigmented individual, choroidal vasculature invisible. The 16 subjects were selected for the study from 148 participants in a normative study solely because their choroidal vasculature was visible and measureable with the retinal oximeter, Oxymap T1.

made optical adapter, image splitter, and two highly sensitive digital cameras (Insight IN1800, 1600 × 1200 square pixels; Diagnostic Instruments Inc., Sterling Heights, MI). The fundus image, captured by the fundus camera (Topcon TRC-50DX; Topcon Corporation), is split into two, and one image is sent to each camera. One camera captures the image at 570 nm (insensitive to oxygen saturation, Fig. 1B), while the other captures the same area of the fundus at 600 nm (sensitive to oxygen saturation, Fig. 1C).

The Oxymap Analyzer (Oxymap Analyzer software 2.2.1, version 3847; Oxymap ehf.) analyzes the images and measures brightness at the measured vessels (I) and to the side of the vessels (I_0). The brightness at the vessel is reduced by light absorbance by the blood in the vessel, while the brightness to the side of the vessel is not. The light absorbance of the blood vessel can be described with the optical density (OD):

$$OD = \log\left(\frac{I_0}{I}\right). \quad (1)$$

The OD at 570 nm is not sensitive to oxygen saturation, whereas the OD at 600 nm decreases with oxygen saturation. The optical density ratio (ODR),

$$ODR = \frac{OD_{600}}{OD_{570}} \quad (2)$$

is therefore sensitive to oxygen saturation, while being relatively insensitive to other effects, such as vessel diameter. The ODR has an inverse and approximately linear relationship to oxygen saturation ($SatO_2$).[16,17]

$$SatO_2 = a + b \times ODR. \quad (3)$$

For further explanation on Oxymap T1 (Oxymap ehf.) and calibration see Geirsdottir et al.[9]

Study Protocol

The study was approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority. All volunteers signed an informed consent before participation in the study. The study adhered to the tenets of the Declaration of Helsinki.

Sixteen subjects were selected from 148 healthy participants from a normative study on retinal oxygen saturation.[9] All subjects were Caucasian, 4 men and 12 women, age of 40 ± 14 years (mean ± SD). The 16 subjects were selected for the study from the 148 participants solely because the choroidal vasculature was visible and measureable with the retinal oximeter (Fig. 1A). Figure 2 shows a lightly pigmented fundus in which choroidal vessels are visible, and for comparison a more densely pigmented fundus, in which these vessels are invisible. Supplementary Figure S1 shows fundus images (570 and 600 nm) from the entire group of 16 eyes. All subjects went through the same standard study and imaging protocol. Five images were acquired from each subject with four different angles of gaze: (1) macula centered, (2) optic disc centered, (3) optic disc down (superior fundus), (4) optic disc up (inferior fundus), and (5) optic disc centered (see Palsson et al.[18]). All images were acquired with the same settings on the fundus camera (Topcon TRC-50DX; Topcon Corporation): 50° field of view, small aperture setting on, and flash intensity 50 W. For further explanation of study and imaging protocol, see Geirsdottir et al.[9]

Analysis

ODRs of choroidal and retinal vessels were obtained using the Oxymap analyzing software (Oxymap Analyzer software 2.2.1, version 3847; Oxymap ehf.). For the normoxia part of the study ($n = 16$) image 4 in the imaging protocol was used for analysis (optic disc up, inferior fundus, Fig. 1A), because the choroidal vessels were most visible in the inferior fundus. The right eye was analyzed in all cases except for two because of bad image quality. ODR was measured for six segments of choroidal vessels (arterioles and venules), one segment of a choroidal vortex vein, one segment of a retinal arteriole, and one segment of a retinal venule. The six choroidal vessel segments were averaged for each subject to get a good average of the area where the choroidal vessels were most visible and also so that the measurement would cover similar area as the retinal arteriole and venule measured for each subject. All measured vessel segments (of all categories) were greater than 50 pixels in length and had a diameter greater than 8.0 pixels (approximately 74 μm[19]). Measurements were not continued beyond branching points. Otherwise, no upper limits were used for length of vessels or diameter. Because most visible choroidal vessels looked alike and it could not be determined whether they are arterioles or venules, they were simply called choroidal vessels. The vortex veins in the choroid were

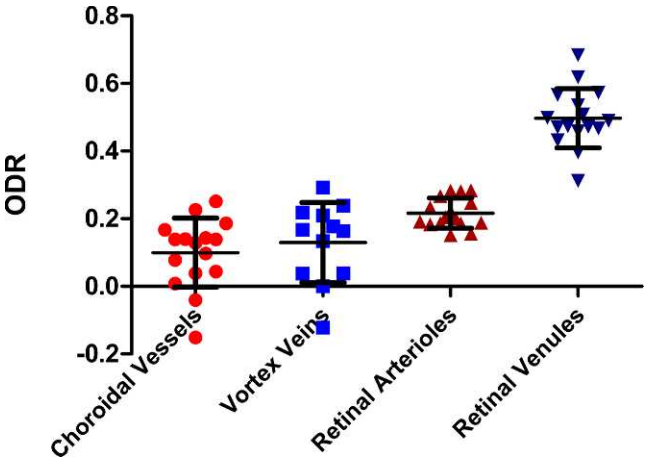

FIGURE 3. ODR during normoxia. Mean ODR, which is inversely related to hemoglobin oxygen saturation, is shown for choroidal vessels, vortex veins, retinal arterioles, and retinal venules under normal oxygen breathing condition (normoxia). The *bars* show one standard deviation. The difference between choroidal vessels and retinal arterioles is statistically significant ($P = 0.0012$, paired *t*-test, $n = 16$). There is no statistically significant difference between choroidal vessels and vortex veins ($P = 0.175$, paired *t*-test, $n = 12$).

recognized by their vortex pattern and wide diameter as indicated with an arrow in Figure 1A. Only 12 of 16 subjects had visible and measurable vortex veins in the inferonasal quadrant of the fundus.

Six of the 16 subjects (two men and four women, age of 42 ± 19 years [mean ± SD]) also inhaled 100% oxygen for 10 minutes (6 L/min, mask covering mouth and nose). Retinal oximetry images were obtained before and immediately after inhalation. Images were analyzed the same way as before except for the choroidal vortex veins, which were not visible in the hyperoxia part of the study because images were acquired with different angle of gaze, optic disk was centered (image 2 in the imaging protocol).

To test the repeatability of the choroidal measurement, the left eye was analyzed using the same criteria as was used for the right eye. Three subjects were excluded, two because of bad image quality and one because the left eye did not have six visible and measureable choroidal segments.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software Inc., La Jolla, CA). A paired *t*-test was applied to compare ODR values for choroidal vessels and retinal arterioles and to compare normoxia and hyperoxia, all categories of measured vessels. For repeatability test a paired *t*-test and one-way ANOVA was applied.

RESULTS

Normoxia

The mean ODR was 0.10 ± 0.10 (mean ± SD) for choroidal vessels, 0.13 ± 0.12 for choroidal vortex veins, 0.22 ± 0.04 for retinal arterioles, and 0.50 ± 0.09 for retinal venules (Fig. 3). According to a paired *t*-test the difference between the choroidal vessels and retinal arterioles was statistically significant ($P = 0.0012$, $n = 16$), but the difference between choroidal vessels and vortex veins was not statistically significant ($P = 0.175$, $n = 12$).

Hyperoxia

Inhalation of 100% oxygen ($n = 6$) lowered ODR levels in choroidal vessels, retinal arterioles, and retinal venules. The decrease in ODR between normoxia and hyperoxia was statistically significant for all vessel types (Fig. 4). The decrease was 0.035 ± 0.028 for choroidal vessels ($P = 0.028$, paired *t*-test), 0.022 ± 0.017 for retinal arterioles ($P = 0.022$, paired *t*-test), and 0.246 ± 0.067 for retinal venules ($P = 0.0003$, paired *t*-test). In addition, the difference between choroidal vessels and retinal arterioles remained statistically significant during hyperoxia ($P = 0.021$, paired *t*-test).

Repeatability

The difference between the right and the left eye was not significant ($P = 0.14$, paired *t*-test). Standard deviation between measurements of the right and left eye in the same individual was 0.07 (Fig. 5).

DISCUSSION

ODR, which correlates inversely to hemoglobin oxygen saturation, can be measured in the choroidal vasculature in lightly pigmented individuals with a spectrophotometric oximeter. Even though calculation of oxygen saturation from choroidal ODR has not been attempted, a low ODR is consistent with high oxygen saturation and high saturation in the choroid is in agreement with earlier studies on oxygenation in animals.[20,21] Shahidi et al.[20] and Shakoor et al.[21] used noninvasive phosphorescence imaging system to measure pO_2 in rat eyes and measured higher pO_2 in the choroid than in retinal arterioles under normal and increased oxygen breathing conditions; this agrees with our findings in the human eye.

While measuring the choroidal vessel ODR is new in this field, measurements on the retinal arterioles and venules is not new and our measurements on the retinal vessels are also in good agreement with previous studies made using an automated image analysis technique based on dual-wavelength oximetry similar to our technique.[7,22]

Alm and Bill[2] found that the arteriovenous difference in oxygen content in the cat choroid is only 3%. This agrees with our experience that choroidal arterioles and venules are difficult to distinguish with spectrophotometric oximetry and that choroidal vortex veins only have slightly higher ODR (indication of lower oxygen saturation) than the other choroidal vessels (Fig. 3).

The Oxymap T1 oximeter (Oxymap ehf., Reykjavik, Iceland) has been shown to be sensitive to changes in oxygen saturation in retinal vessels and to give repeatable and reliable results when measuring hemoglobin oxygen saturation and vessel diameter.[8,9,18,19] This is the first time it has been applied to choroidal vessels. By using the standard Oxymap T1 retinal calibration for the choroidal ODR the calculated hemoglobin oxygen saturation is 107 ± 12% (mean ± SD, $n = 16$) for the choroidal vessels, 106 ± 13% for the vortex veins (mean ± SD, $n = 12$), 94 ± 5% for retinal arterioles (mean ± SD, $n = 16$), and 59 ± 9% (mean ± SD, $n = 16$) for retinal venules. This calibration is obviously not appropriate for the vessels within the choroid, but the oxygen saturation for the retinal vessels compares well to our previous results on retinal oxygen saturation.[8,9] Subjects for this study were selected because their choroidal vasculature was visible (due to light pigmentation) and measureable. That does not seem to affect the results on oxygen saturation for the retinal arterioles and venules. Inhalation of 100% oxygen (hyperoxia, $n = 6$) lowered the ODR levels for all measured vessel types, both choroidal and retinal, which corresponds to an increase in oxygen

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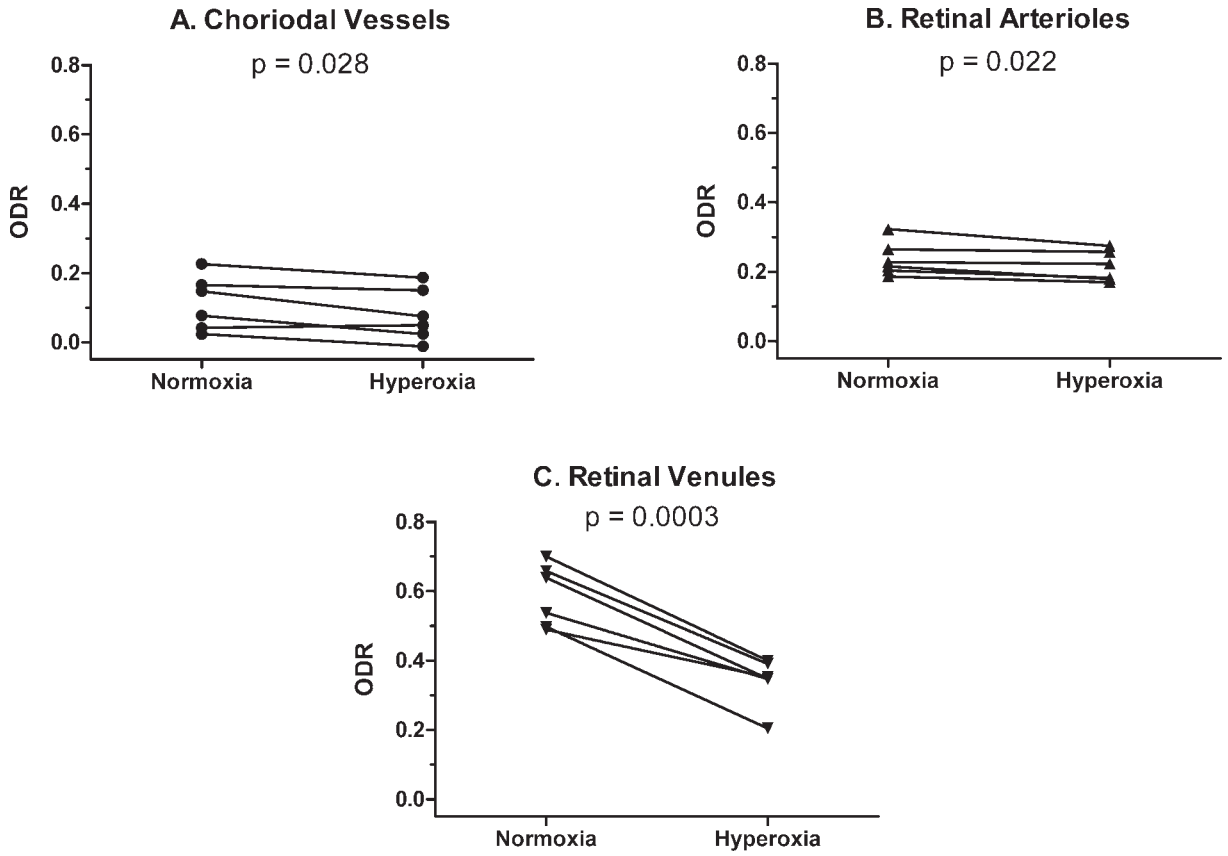

FIGURE 4. ODR during normoxia and hyperoxia. ODR is inversely related to hemoglobin oxygen saturation. It is shown for (**A**) choroidal vessels, (**B**) retinal arterioles, and (**C**) retinal venules under normal oxygen breathing condition (normoxia) and with subjects breathing 100% oxygen (hyperoxia). According to paired *t*-test there is a statistically significant difference for choroidal vessels ($P = 0.028$), retinal arterioles ($P = 0.022$), and retinal venules ($P = 0.0003$) between normoxia and hyperoxia.

saturation. By using the standard retinal calibration, the increase in hemoglobin percentage was found to be 4% for choroidal vessels, 2% for retinal arterioles, and 26% for the retinal venules. This demonstrates that with hyperoxia the oximeter is sensitive to changes in oxygen saturation for both the choroidal and retinal vessels. (Vortex veins were not visible on images taken for the hyperoxia part of the study.)

We measured only individuals with the most visible choroidal vessels, which included only 16 individuals from a group of 148 healthy individuals. Of these, only six were available for the hyperoxia experiment. The small sample sizes may make the parametric statistical tests used vulnerable to deviations of the population from normal distribution. We therefore recalculated all *P* values using the Wilcoxon signed rank test. This did not change the conclusions of the study, although the difference between normoxia and hyperoxia in choroidal vessels became borderline statistically significant ($P = 0.063$), and the same was true for the comparison of retinal arterioles and choroidal vessels during hyperoxia ($P = 0.059$).

The repeatability of retinal oxygen measurements with the oximeter has been determined previously.[18] The standard deviation of repeated measurements was 1.0% for arterioles and 1.4% for venules. Repeatability was not tested in the same way in this study but can nonetheless be estimated. We calculated the standard deviation between measurements of the left and right eye in the same individual. This is displayed in ODR values in the results. If the ODR values are transformed into saturation (with standard retinal calibration), the standard deviation between measurements of the left and right eye in the same individual was 7% (Fig. 5). Although the study was not designed to estimate repeatability, these values indicate that the variability is greater for the choroidal measurements than it is for retinal measurements.

The optical properties of the intravascular tissue in the choroid may play a role in these measurements. The ODR was lower in the choroidal vessels than in the retinal arterioles,

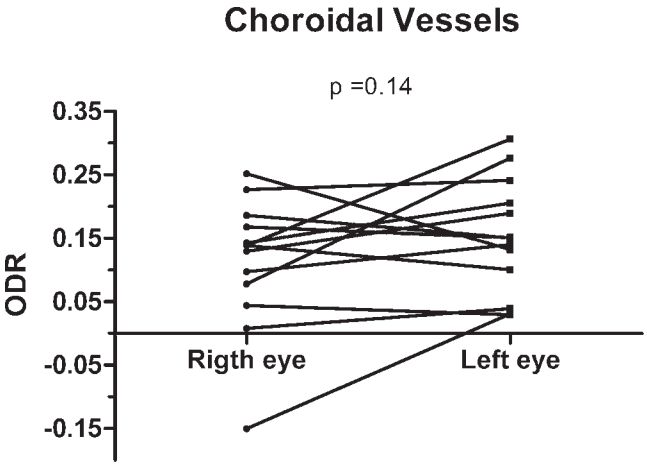

FIGURE 5. Repeatability: right versus left eye. For repeatability testing the left eye was measured using the same criteria as was used for the right eye. According to a paired *t*-test the difference was not significant ($P = 0.14$). Standard deviation between measurements of the right and left eye in the same individual was 0.07 ODR values.

potentially indicating higher oxygen saturation in the choroid; however, it is also observed that the OD of choroidal vessels is reduced at both 570 and 600 nm, which can affect the ODR and oxygen saturation conversion. This is compatible with scattering of light within the choroid reducing the contrast of choroidal vessels as follows. The OD of retinal vessels is measured against a bright background dominated by scattering from interstitial tissue. Choroidal blood vessels are embedded within this interstitial tissue and components of this tissue lying between blood vessels and the retinal pigment epithelium backscatter incident illumination, which reduces contrast of choroidal vessels; that is, it reduces OD. The magnitude of the scattering from interstitial tissue is approximately equal at both 570 and 600 nm but causes a proportionately greater reduction in the OD for the lower OD measurements at 600 nm. In consequence, there is a reduction in the ODR for choroidal vessels. If the reduction in ODR due to scattering is neglected, this would imply higher oxygenation levels and suggests nonrealistic oxygen saturation in excess of 100% in choroidal vessels. It is probable that the different illumination of the vessel by the surrounding choroidal tissue also has an effect, though this is expected to be less significant.

Different optical properties result from the fact that retinal vessels and choroidal vessels lie in different tissues at different depths. The result is that the standard calibration, which has been used for retinal vessels to transform ODR into oxygen saturation, is not appropriate for choroidal vessels. However, the lowering of ODR in the choroidal vessels with hyperoxia demonstrates that the oximeter is sensitive to changes in oxygen saturation in choroidal vessels as well as in retinal vessels.

It is furthermore observed that for some of the imaged retinas, the OD of choroidal vessels imaged at 600 nm is negative; that is, vessels appear brighter than the surrounding choroidal tissue, and this leads to the negative ODRs shown in Figure 3. Study of these images suggests that this brightness is associated with diffuse structure in the scattering interstitial tissue that correlates with the vessel structure and that there is insufficient contrast to detect an OD due to the vessel. This may be because the vessels are located more deeply within the choroidal tissue than the vessels for which positive ODs can be measured. In these cases the ODR for these vessels is effectively zero.

The physical optics leading to the observed ODR of choroidal vessels is inherently different from that underpinning oximetry of retinal vessels. The determination of choroidal vessel oxygenation will require some modification and refinement to the physical optics model established for retinal vessel oximetry, and this is the subject of ongoing investigation. It is clear, however, from these results that it is nevertheless possible to detect changes in choroidal vessel oxygenation associated with changes in inspired oxygen.

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