Retinal Vessel Oxygen Saturation in Healthy Individuals

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Purpose. We measured oxygen saturation in retinal vessels of healthy eyes to determine the effects of age, sex, and cardiovascular parameters, as well as the reliability of the measurements and topographic differences.

Methods. The Oxymap T1 retinal oximeter is based on a fundus camera. It simultaneously captures retinal images at two different wavelengths and estimates retinal vessel oxygen saturation. Mean saturation of main retinal arterioles and venules was measured in 120 healthy individuals aged 18–80 years (median 47 years). Of the 120 participants 44 (37%) were male (49 years) and 76 (63%) female (44 years).

Results. Oxygen saturation was 92.2 ± 3.7% (mean ± SD) in retinal arterioles and 55.6 ± 6.3% in venules. No significant difference in oxygen saturation was found between left and right eyes. The inferotemporal quadrant had lower oxygen saturation in arterioles and venules ($P < 0.0001$). Arteriolar oxygen saturation was stable with age. Venular oxygen saturation in males decreased by 1.9 ± 0.6% (mean ± SEM) per 10 years of age ($P = 0.003$) and by 0.7 ± 0.4% in females ($P = 0.068$). Arteriovenous (AV) difference increased by 1.5 ± 0.5% per 10 years in males ($P = 0.004$) and 1.0 ± 0.4% ($P = 0.007$) in females. For every 10 mm Hg increase in ocular perfusion pressure, oxygen saturation in arterioles increased by 0.9 ± 0.4% ($P = 0.024$) and in venules by 1.2 ± 0.7% ($P = 0.075$).

Conclusions. Retinal arteriolar oxygen saturation is stable in healthy individuals, while there is a significant decrease in venular oxygen saturation with age in males and a similar trend in females. AV difference increases significantly with age for both sexes. Our study provided normative data for spectrophotometric retinal oximetry in the Caucasian population. (*Invest Ophthalmol Vis Sci.* 2012;53:5433–5442) DOI: 10.1167/iovs.12-9912

The fundus of the eye provides a direct view of retinal arterioles and venules. Imaging them can provide information on oxygen saturation in central nervous system vessels and can relate to metabolic changes in eye diseases as well as oxygen saturation in central vessels in systemic diseases. Metabolic imaging is especially of interest in ophthalmology as many important eye diseases have metabolic foundations, such as diabetic retinopathy, or direct involvement of retinal blood vessels, such as retinal artery and vein occlusions.

After more than a decade of development, the research group has built a noninvasive dual-wavelength spectrophotometric retinal oximeter. This is an important milestone in the quest for metabolic imaging of the retina, a journey that began more than 60 years ago with the landmark work of Hickam and Frayser.[1,2] They were the first to measure the retinal oxygenation noninvasively in persons using photographic methods by applying special filters to obtain fundus images with two wavelengths of light. The work was continued by Laing et al. who also developed two-wavelength retinal oximetry to obtain oxygen saturation in retinal vessels in rabbits.[3] Later, very important contributions to retinal oximetry were made by Delori et al.[4,5] and Schweitzer et al.,[6] who developed different multiwavelength oximetry systems. With advancing technology, Beach et al.[7,8] presented a digital imaging system to measure oxygen saturation in retinal vessels by two-wavelength imaging capturing both images at the same time. Crittin et al. used similar optical approach in developing their two-wavelength oximetry system,[9] while Hammer et al. developed an oximetry instrument that also captured two fundus images simultaneously with two wavelengths of light, but with a different optical approach.[10] Other groups using diverse approaches also have contributed to the field of retinal oximetry, such as Denninghoff et al., who developed a multiwavelength oximetry system,[11,12] Ramella-Roman et al. who introduced a multiaperture camera system for retinal oxygen saturation measurement,[13] Kagemann et al. who used Fourier domain optical coherence tomography to assess spectral oximetry,[14] Li et al. who used an adaptive optics confocal scanning laser ophthalmoscope to measure oxygen saturation in small retinal vessels,[15] Khoobehi et al. who developed a hyperspectral system that has been used mainly for oxygen saturation measurements of optic nerve heads in monkeys,[16,17] Harvey et al. who developed a hyperspectral instrument for measuring retinal oxygen saturation in vivo and in a model eye,[18–20] and Humayun's group who developed different hyperspectral system for measuring oxygen saturation within retinal vessels.[21,22] For review see the report of Harris et al.[23]

Modern spectrophotometric retinal oximetry takes advantage of the great development of digital cameras and computer technology that has taken place in the last few decades. Spectrophotometric retinal vessel oximetry values have been shown to be reliable and relatively stable in healthy individuals.[6,24] Oximetry has demonstrated changes in retinal vessel oxygenation in several eye disorders, including retinal vein occlusions,[25,26] diabetic retinopathy,[27–29] and glaucoma,[30,31] and it also has displayed an effect of pharmacologic and surgical interventions for glaucoma.[32–35] This technology has demonstrated as well the changes in retinal oxygen metabolism

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with changes in illumination[36] and by flicker light stimulation.[37] Traustason et al. have shown that systemic hypoxia in Eisenmenger syndrome can be measured reliably with retinal oximetry. [38]

All of these studies were performed with either an older version of the Oxymap oximeter or another type of oximeter. In our study, the latest Oxymap retinal oximeter is described and oximetry was performed on healthy individuals to provide basic normative data for a Caucasian population. The reliability of the measurements was tested by comparing the retinal vessel oxygen saturation between the right and left eyes. The effects of age, sex, and cardiovascular parameters on retinal vessel oxygenation were described as well as the topographic differences within the eye.

Methods

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

Study Population

A total of 148 allegedly healthy persons of Caucasian origin were recruited for the study. Exclusion criteria were any history or signs of retinal or optic nerve diseases, any eye disease that could affect the quality of images, eye trauma, any known or suspected adverse effects to pupil dilation, diabetes mellitus, severe cardiovascular or respiratory diseases, pregnancy, and breastfeeding. In all, 28 subjects had to be excluded from the analysis; two due to missing images, five with low quality images, 11 with drusen, six with glaucoma, two with diabetes mellitus, one with cataract, and one with pigment abnormality. Therefore, 120 persons were included in the study.

All subjects went through a standard study protocol. After consenting to the study, the subjects answered a questionnaire on medical history, medications, and smoking. Measurements were made of blood pressure and heart rate (Omron M6 Comfort [HEM-7000-E]; Omron Healthcare Europe, Hoofddorp, The Netherlands), finger pulse oximetry (Ohmeda Biox 3700; Ohmeda, Boulder, CO), best corrected visual acuity (Snellen chart), and intraocular pressure (iCare TAO1 Tonometer; Tiolat Oy, Helsinki, Finland). Ocular perfusion pressure was calculated as 2/3 mean brachial artery pressure – intraocular pressure, where mean arterial pressure was 2/3 diastolic pressure + 1/3 systolic pressure. Pupils were dilated with 1% tropicamide (Mydriacyl; S.A. Alcon-Couvreur N.V., Puurs, Belgium), which in some cases was supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Lake Forest, IL). After examination of the dilated fundus, a regular 50° color fundus image was taken (Zeiss FF 450plus fundus camera; Carl Zeiss Meditec AG, Jena, Germany) before the participants headed for oximetry.

In all, 210 eyes from 120 persons aged 18 to 80 years were analyzed (Table 1). Both eyes of a participant were used only for the comparison between right and left eyes ($n = 105$). For all other analyses, only one eye was chosen for each subject by randomization. If a person had only one eligible eye, that eye was used for the analyses. Median age of the participants was 47 years (males 49 years; females 44 years). Four females (20, 24, 72, 76 years) had to be excluded from the multivariate analyses due to missing information on blood pressure, intraocular pressure, or smoking status.

Retinal Oximetry

Oximeter. The dual wavelength noninvasive retinal oximeter Oxymap T1 (Oxymap ehf., Reykjavik, Iceland) is composed of two digital cameras (Insight IN1800, 1600 × 1200 square pixels; Diagnostic Instruments Inc., Sterling Heights, MI), a custom-made optical adapter, an image splitter, and two narrow band-pass filters (Fig. 1). It is coupled to a fundus camera base (Topcon TRC-50DX; Topcon Corporation, Tokyo, Japan) and simultaneously yields two fundus images of the same area of the retina with two different wavelengths of light, 570 and 600 nm (Fig. 2). Two narrow 5 nm bandpass filters (full width at half maximum transmittance) were used to acquire the images at the two wavelengths. A broader 80 nm bandpass filter also was placed in the light path of the fundus camera (585 nm center wavelength) to limit unnecessary light exposure to the subjects' eyes, thereby only allowing light between 545 and 625 nm to exit the camera lens.

Table 1. Total Number of Healthy Caucasian Participants in Each Age Group in the Study, Subdivided by Sex

Age (y)	Males	Females	Totals
18 to 29	4	20	24
30 to 39	12	12	24
40 to 49	7	13	20
50 to 59	5	15	20
60 to 69	10	13	23
70 to 80	6	3	9
Totals	44	76	120

Figure 1. The dual wavelength retinal oximeter Oxymap T1 (Oxymap ehf.) is composed of two digital cameras (Insight IN1800, 1600 × 1200 square pixels; Diagnostic Instruments Inc.), a custom-made optical adapter, and an image splitter. It is coupled to a fundus camera base (Topcon TRC-50DX; Topcon Corporation).

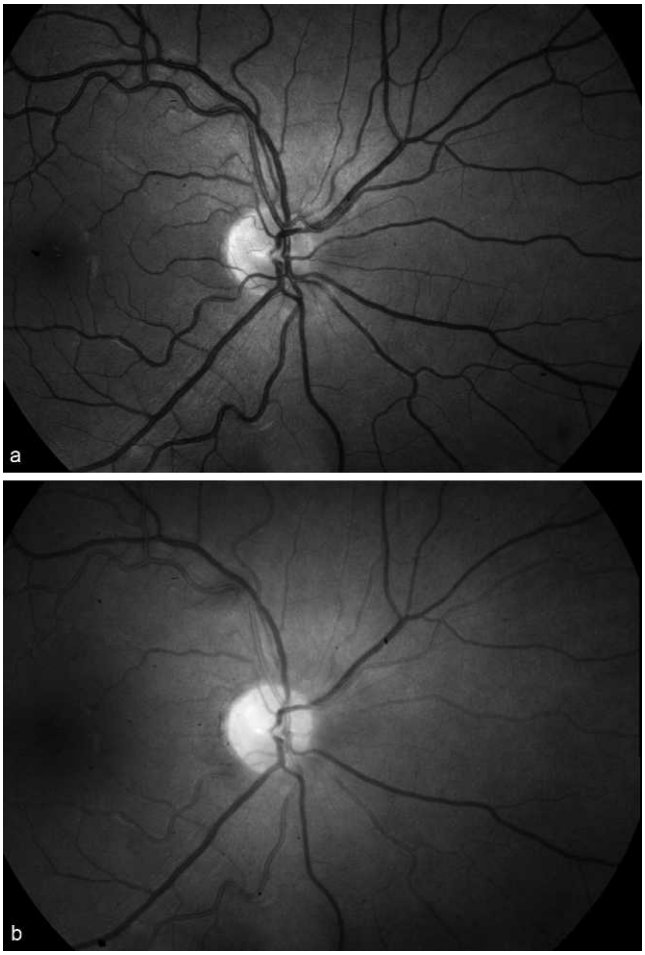

Figure 2. The two monochrome images, which the Oxymap T1 oximeter acquires at the two different wavelengths of light. (**a**) 570 nm is the reference isosbestic wavelength that is insensitive to oxygen. (**b**) 600 nm is the oxygen sensitive wavelength, and demonstrates how the light absorbance of the vessels changes with different oxygen saturation of arterioles and venules.

Specialized software (Oxymap Analyzer software 2.2.1, version 3847; Oxymap ehf.) automatically selects measurement points on the oximetry images, and measures brightness on the measured vessels (I) and to the side of the vessels (I_0) at each wavelength. The light absorbance by the blood in the vessels influences the brightness on the vessel but not the brightness to the side of the vessel. The light absorbance can be described with the optical density (OD):

$$OD_\lambda = \log(I_0/I). \quad (1)$$

Optical density is sensitive to oxygen saturation at 600 nm, but not at the reference isosbestic wavelength, 570 nm. Figure 2 shows the two monochrome images, which the oximeter acquires at the two different wavelengths. At the oxygen insensitive wavelength 570 nm (Fig. 2a) the arterioles and venules are similarly dark whereas at 600 nm the light absorbance decreases with increased oxygen saturation and arterioles appear much brighter than venules (Fig. 2b). The optical density ratio (ODR) can be calculated from optical density at the two wavelengths:

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

ODR, therefore, is sensitive to oxygen saturation, and has an inverse and approximately linear relationship to oxygen saturation ($SatO_2$):[7,23]

$$SatO_2 = a*ODR + b, \quad (3)$$

where a and b are constants. The calibration of a and b for Oxymap T1 was achieved by matching the optical density ratios from healthy individuals, measured with Oxymap T1, with saturation measurements performed in a study with a calibrated device by Schweitzer et al.[6] They found that mean oxygen saturation of retinal arterioles and venules was 92.2% and 57.9%, respectively. That resulted in the calibration constants $a = -1.1755$ and $b = 1.1917$ (see page S1 in Supplementary Material, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9912/-/DCSupplemental).

When the oximeter is calibrated correctly, the Oxymap Analyzer software automatically processes the two monochrome images and displays the results as a pseudocolor fundus image as shown in Figure 3.

Imaging. The participant was seated comfortably in front of the fundus camera. All images were taken by the same experienced research scientist in a dark room, with the only light sources being the fundus camera and computer screen. The fundus was illuminated with the lowest setting of the aiming light that allowed for a good view of the fundus and appropriate alignment of the fundus camera. The flash intensity was set to 50 Ws and the small aperture setting was turned on. If needed, the small pupil setting was used (registered by the user if used). Images of the right eye were taken first and then of the left eye. Generally, 5 images were taken (some for other purposes than presented here), but occasionally further images were added to acquire an image of sufficient quality for every area. An image with the optic disc in the center normally was the second and fifth in the imaging protocol. The time between images (flashes) of the same eye averaged approximately 30 seconds.

Analysis. For each subject, the first good quality image with the optic disc in the center was used for analysis. Figure 3 shows a pseudocolor fundus image of the right eye of one participant generated automatically by the Oxymap T1 oximeter. The colors indicate oxygen saturation in the retinal vessels according to the scale to the right in the figure. Arterioles generally are orange to red, indicating oxygen saturation approximately 90 to 100%. Venules can vary from blue to yellow, but normally are green, indicating oxygen saturation approximately 50 to 60%. In each eye, oxygen saturation was measured in all major retinal arterioles and venules above 8 pixels in vessel width. Eight pixels in vessel width equal approximately 74 micrometers.[39] Vessel segments were selected by the user in a standardized manner, but vessel width, length, and location within the image had to be selected according to the protocol specified in Table 2 (see also Fig. 4).

After detailed vessel segment selection, the Oxymap Analyzer software automatically measured the oxygen saturation within each selected vessel. Means and SDs of oxygen saturation measurements of the fundus were determined. According to Beach et al.[7] and Hammer et al.,[10] there is an artifactual decrease in measured saturation values with increased vessel diameter in retinal arterioles and venules. Therefore, a correction for vessel size was performed. The correction factor was determined by comparing saturation and vessel diameter just before and just after bifurcation of vessels 8 pixels or larger in diameter on both sides of the bifurcation. The saturation can be assumed to be the same on both sides of the bifurcation (at least for arterioles), but the vessel diameter is different. Although we analyzed vessels of the same size (8 pixels or larger) during the calibration of the oximeter and during the analysis of the current data, we still have a large range of vessel diameters in arterioles and especially in venules, from 8 up to 25 pixels. Within this range there is an effect of vessel diameter that must be accounted for. Our testing for correction factor with this version of the oximeter software resulted in the same correction factor for arterioles and venules. As a result, all measurements were corrected by adding 1.16% to the saturation value for each pixel above the mean diameter for arterioles and venules. Similarly, 1.16% was subtracted for each pixel below mean diameter (see page S1 in Supplementary Material, http://www.iovs.org/lookup/suppl/ doi:10.1167/iovs.12-9912/-/DCSupplemental).

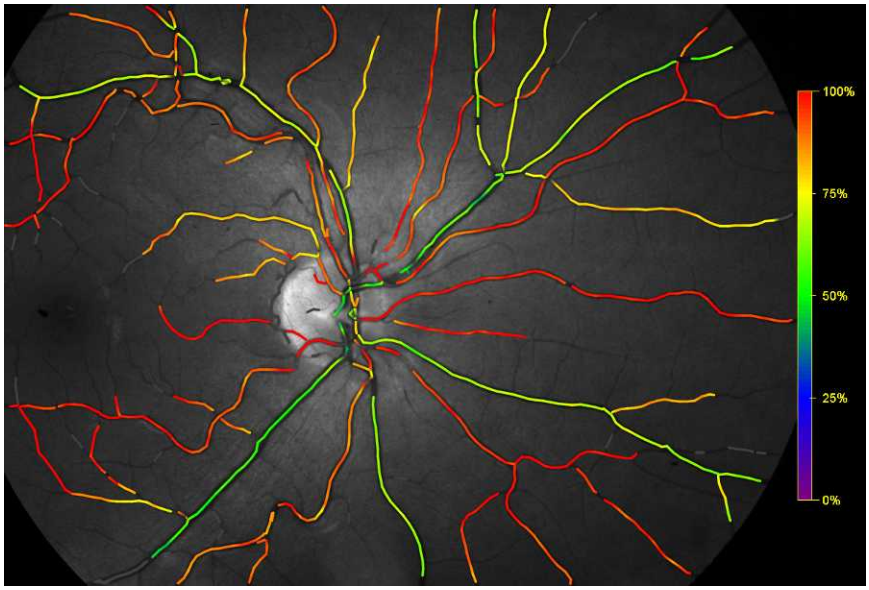

Figure 3. Pseudocolor fundus map generated automatically by the Oxymap T1 oximeter. *Colors* indicate oxygen saturation in retinal vessels (*scale to the right of the image*). Arterioles generally are *orange to red*, indicating oxygen saturation approximately 90 to 100%. Venules can vary from *blue to yellow* but normally are *green*, indicating oxygen saturation approximately 50 to 60%.

Statistical Analysis

Statistical analyses were performed using the R software package, version 2.14.1 (The R Foundation for Statistical Computing, available in the public domain at www.r-project.org) and Prism, version 5 (GraphPad Software Inc., La Jolla, California). For all analyses, $P < 0.05$ was considered statistically significant.

Multivariate analysis for arterioles, venules, and arteriovenous (AV) difference included age, sex, current smoking status, finger pulse oxygen saturation (measured), and ocular perfusion pressure. We felt that ocular perfusion pressure was the most relevant variable in these analyses and, since it is derived from systolic, diastolic, and intraocular pressure measurements, it is not possible to add those three variables individually into the multiple linear regression model alongside ocular perfusion pressure. Moreover, the models for arterioles and venules also took into account the interaction between age and sex:

$$SatO_2 = x_0 + x_1 \times age + x_2 \times sex + x_3 \times \text{smoker} + x_4 \times pulseox + x_5 \times perfusion + x_6 \times age \times sex, \quad (4)$$

where the x_i are the coefficients in Table 4, *smoking* equals 1 for current smokers but otherwise 0, *sex* equals 0 for males and 1 for females, *pulseox* is the finger pulse oximeter reading in percentage, and *perfusion* is the ocular perfusion pressure in mm Hg. Regarding the coding of the *sex* variables (male 0, female 1), the coefficient for *sex* (x_2) and the interaction of *age* and *sex* (x_6), where relevant, denotes the difference between males and females.

Results

The oxygen saturation in the 120 eyes was 92.2 ± 3.7% (mean ± SD; 95% confidence interval [CI] 91.5–92.9%) in retinal arterioles and 55.6 ± 6.3% (95% CI 54.4–56.7%) in venules. These mean oxygen saturation values have been adjusted for vessel width as described in the Methods section. The AV difference was 36.7 ± 5.4% (95% CI 35.7–37.6%). Figure 5 shows that the retinal arteriolar oxygen saturation values were normally distributed (D'Agostino & Pearson omnibus normality

Table 2. Criteria for Choosing Retinal Vessel Segments for Measurement of Oxygen Saturation with the Oxymap T1 Retinal Oximeter

Retinal Vessel Selection	Criteria
Retinal vessel width	8 pixels or wider
Retinal vessel length	50 to 200 pixels (preferably as close to 200 pixels as possible)
Start vessel selection	As close to the optic disc but always exclude at least 15 pixels next to the optic disc (or a bright area surrounding it)
End vessel selection	After length of 200 pixels but never closer than 30 pixels to the edge of the image
If vessel branching occurs less than 50 pixels from the selection start (as close to the optic disc as explained above) and if the smaller of the daughter vessels is:	
(a) 6 pixels or less in diameter	(a) Ignore the vessel branching and analyze the segment from the optic disc
(b) Wider than 6 pixels	(b) Start the analysis from the vessel branching and measure segments further away from the optic disc
Exclude vessel segments specifically	If the background points are affected by extremes in brightness (undetected nearby vessels, laser scars, hemorrhages and so forth)

Vessel width in pixels (1 pixel ≈ 9.3 μm[39]), vessel length in pixels (count), and location of chosen vessel segments within the pseudocolor fundus image were selected in a standard manner.

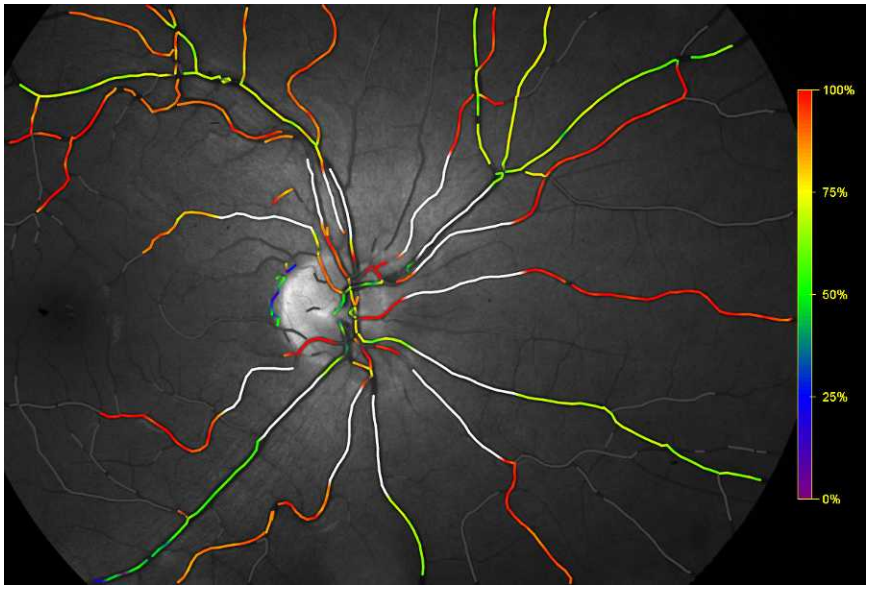

Figure 4. Pseudocolor fundus map showing vessels segments selected for analysis (*white vessel segments in image*). The oximeter was set to detect all vessels 8.0 pixels or more in diameter, while smaller vessels appear *gray* in figure. Vessel segments 50 to 200 pixels in length intended for analysis were selected manually (*white overlay*) and analyzed by the user in a standardized manner according to the protocol in Table 2. All other vessel segments (*in color or gray*) were not analyzed.

test $K^2 = 5.2$; $P = 0.07$). The distribution of venous saturation was skewed to the left and did not pass a normality test ($K^2 = 9.5$; $P = 0.009$).

Eye Laterality

The oxygen saturation of arterioles was 92.5 ± 3.6% in right eyes and 92.8 ± 3.4% in left eyes (paired *t*-test, $P = 0.30$). In venules, the oxygen saturation was 56.4 ± 5.7% in right eyes and 55.6 ± 5.6% in left eyes ($P = 0.07$, Fig. 6).

Quadrants

In retinal arterioles and venules, the inferotemporal quadrant had lower oxygen saturation measurements compared to the other three quadrants. In arterioles, oxygen saturation was

Table 3. Comparison of Retinal Vessel Oxygen Saturation in Different Retinal Quadrants ($n = 85$) for Arterioles, Venules, and AV Difference

	Superonasal	Inferonasal	Inferotemporal
Arterioles:			
Superotemporal	4.9 ± 6.5*	2.5 ± 5.9†	−2.5 ± 6.5†
	CI: 3.1 to 6.6	CI: 0.8 to 4.3	CI: −4.3 to −0.8
Superonasal		−2.3 ± 6.0†	−7.4 ± 6.8*
		CI: −4.1 to −0.6	CI: −9.1 to −5.6
Inferonasal			−5.1 ± 5.6*
			CI: −6.8 to −3.3
Venules:			
Superotemporal	−1.0 ± 7.4	−0.9 ± 8.4	−10.0 ± 9.8*
	CI: −3.5 to 1.6	CI: −3.4 to 1.6	CI: −12.5 to −7.5
Superonasal		0.1 ± 8.1	−9.0 ± 9.7*
		CI: −2.5 to 2.6	CI: −11.6 to −6.5
Inferonasal			−9.1 ± 10.2*
			CI: −11.6 to −6.6
AV difference:			
Superotemporal	5.8 ± 8.4*	3.4 ± 8.9	7.5 ± 11.7*
	CI: 2.1 to 9.5	CI: −0.3 to 7.2	CI: 3.7 to 11.2
Superonasal		−2.4 ± 9.3	1.7 ± 12.1
		CI: −6.1 to 1.3	CI: −2.1 to 5.4
Inferonasal			4.0 ± 11.7‡
			CI: 0.3 to 7.8

There is a significant variation between quadrants ($P < 0.0001$) by ANOVA for arterioles, venules, and AV difference, and the table shows the results of Tukey post-tests where the row is subtracted from the column. All numbers are retinal oxygen saturation percentages (mean ± SD and 95% CI).

* $P < 0.001$, statistically significant difference.

† $P < 0.01$, statistically significant difference.

‡ $P < 0.05$, statistically significant difference.

TABLE 4. The Results of the Multivariate Analysis using Multiple Linear Regression Models for (a) arterioles, (b) venules, and (c) AV difference (n = 116)

Variable	Estimate	SE	*P* Value
(a) Arterioles			
(Intercept)	58.8 (x_0)	27.5	0.035
Age (years)	−0.057 (x_1)	0.033	0.086
Sex (males = 0; females = 1)	−0.66 (x_2)	2.03	0.74
Current smoker (nonsmoker = 0; smoker = 1)	−1.30 (x_3)	1.02	0.20
Finger pulse oximetry (pulseox, %)	0.31 (x_4)	0.28	0.27
Ocular perfusion pressure (perfus, mm Hg)	0.094 (x_5)	0.041	0.024
Age:sex (years; males = 0; females = 1)	0.069 (x_6)	0.041	0.094
(b) Venules			
(Intercept)	−12.7 (x_0)	43.9	0.77
Age (years)	−0.20 (x_1)	0.052	0.0003
Sex (males = 0; females = 1)	−1.32 (x_2)	3.25	0.69
Current smoker (nonsmoker = 0; smoker = 1)	0.17 (x_3)	1.62	0.92
Finger pulse oximetry (pulseox; %)	0.71 (x_4)	0.45	0.12
Ocular perfusion pressure (perfus; mm Hg)	0.12 (x_5)	0.065	0.075
Age:sex (years; males = 0; females = 1)	0.12 (x_6)	0.065	0.063
(c) AV difference			
(Intercept)	70.8 (x_0)	39.7	0.77
Age (years)	0.11 (x_1)	0.034	0.0015
Sex (males = 0; females = 1)	−1.85 (x_2)	0.99	0.064
Current smoker (nonsmoker = 0; smoker = 1)	−1.52 (x_3)	1.46	0.30
Finger pulse oximetry (pulseox; %)	−0.38 (x_4)	0.41	0.36
Ocular perfusion pressure (perfus; mm Hg)	−0.026 (x_5)	0.059	0.66

Multiple linear regression of all variables with interaction between age and sex for (a) arterioles and (b) venules, but the interaction was not included for (c), the AV difference model. $Oxygen\ saturation = x_0 + x_1 \times age + x_2 \times sex + x_3 \times smoker + x_4 \times pulseox + x_5 \times perfus + x_6 \times age \times sex$. Adjusted R^2 was 0.15 for arterioles, 0.26 for venules, and 0.16 for AV difference.

88.2 ± 5.9%, 90.7 ± 5.3%, 93.3 ± 4.9%, and 95.6 ± 6.2% in the inferotemporal, superotemporal, inferonasal, and superonasal quadrants, respectively. In venules, the corresponding saturation values in the same quadrants were 47.4 ± 9.4%, 57.4 ± 8.0%, 56.5 ± 6.9%, and 56.5 ± 7.1%, respectively. The AV difference was 40.8 ± 11.5%, 33.3 ± 8.7%, 36.7 ± 7.9%, and 39.1 ± 8.9%, respectively. There is a significant variation between quadrants and the intraretinal quadrant comparison with the Tukey post-test results (Table 3).

Age and Sex

In a simple linear regression with retinal oxygen saturation in relation to age and sex separately, there was a significant decrease in venular oxygen saturation with increasing age in males (slope = –0.19 ± 0.06%/year [mean ± SEM], $r^2 = 0.19$, 95% CI –0.31 to –0.07, $P = 0.003$) and a similar trend in females (slope = –0.07 ± 0.04%/year, $r^2 = 0.047$, 95% CI –0.14 to –0.01, $P = 0.068$, Fig. 7a). In arterioles, there was no significant change in retinal oxygen saturation with age in both males (slope = –0.04 ± 0.04%/year, $r^2 = 0.025$, 95% CI –0.11–0.04, $P = 0.30$) and females (slope = 0.03 ± 0.02%/year, $r^2 = 0.021$, 95% CI –0.02–0.08, $P = 0.23$, Fig. 7a). The AV difference increased significantly with increasing age in males (slope = 0.15 ± 0.05%/year, $r^2 = 0.18$, 95% CI 0.05–0.25, $P = 0.0043$) and females (slope = 0.098 ± 0.035%/year, $r^2 = 0.10$, 95% CI 0.03–0.17, $P = 0.0067$, Fig. 7b).

A multivariate analysis using multiple linear regression was performed with the following variables included: age, sex,

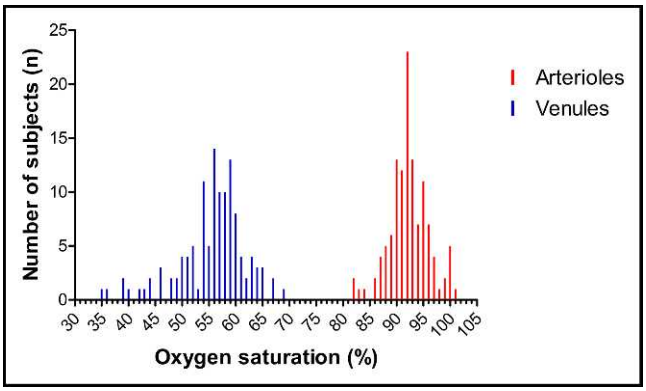

FIGURE 5. Distribution of mean retinal oxygen saturation value (%) in venules (*blue*) and arterioles (*red*, n = 120). The retinal arteriolar oxygen saturation values were distributed normally (D'Agostino & Pearson omnibus normality test $K^2 = 5.2$, $P = 0.07$). The distribution of venous saturation was skewed to the left and did not pass a normality test ($K^2 = 9.5$, $P = 0.009$).

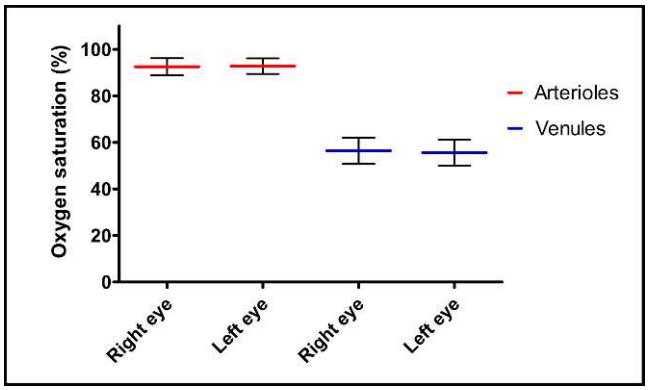

FIGURE 6. Mean oxygen saturation (%) in retinal arterioles (*red*) and venules (*blue*) in right and left eyes. *Error bars*: standard deviation.

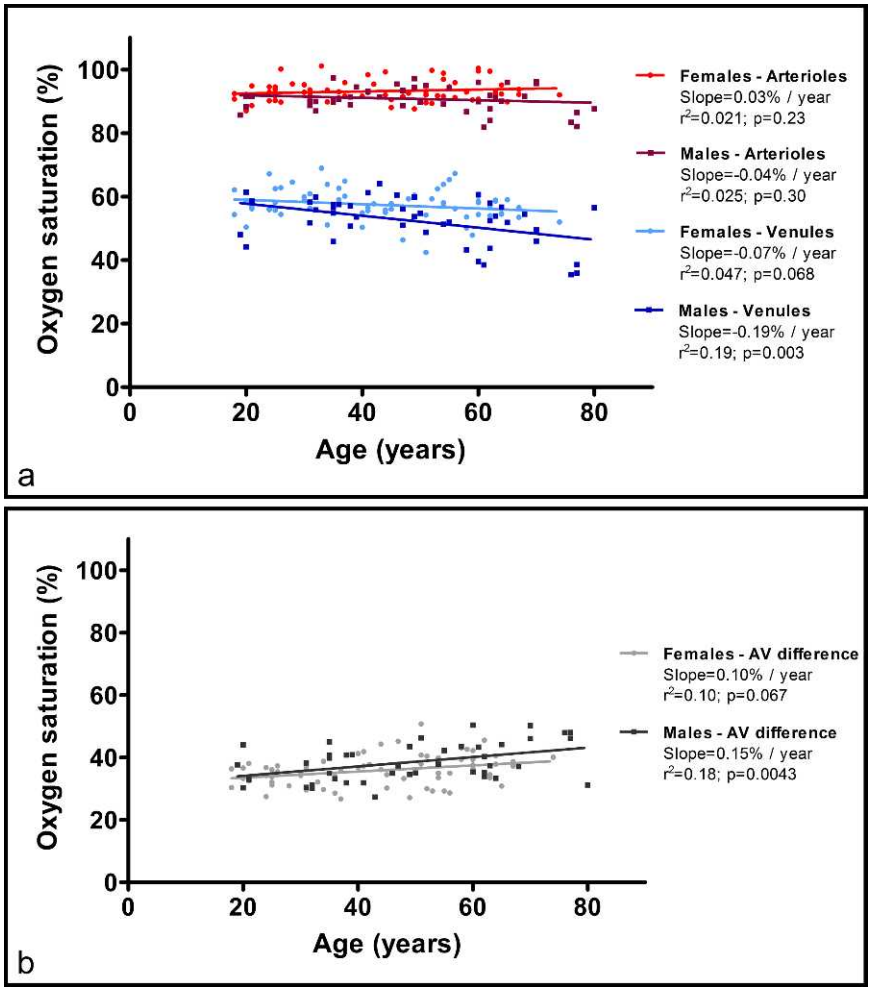

Figure 7. (**a**) Oxygen saturation (%) with age in retinal vessels: arterioles in males (*dark-red squares*), arterioles in females (*red dots*), venules in males (*dark-blue squares*), and venules in females (*light-blue dots*). (**b**) The AV difference (%) with age in males (*dark-gray squares*) and females (*light-gray dots*).

current smoking status, finger pulse oximetry, and ocular perfusion pressure (Table 4).

The decrease in venular oxygen saturation with age still was significant ($P = 0.0003$). There also was a statistically significant difference between sexes after adjusting for age ($P = 0.0001$; see Table S1b in Supplementary Material, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9912/-/DCSupplemental). However, this difference disappeared ($P = 0.69$) when the interaction between age and sex was included in the model, which was borderline significant ($P = 0.06$, Table 4b). This implies that the difference in oxygen saturation between sexes was dependent partly on age as indicated in the simple linear regression model where the slopes become further apart with increasing age (Fig. 7a).

As in the simple linear regression model for arterioles, retinal arteriolar oxygen saturation did not change with age after adjusting for sex and the other variables (Table 4a, $P = 0.086$). There is a significant difference between sexes in arterioles when adjusted for age ($P = 0.0006$, Table S1a in Supplementary Material, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9912/-/DCSupplemental), but as in venules, this sex difference disappeared ($P = 0.74$, Table 4a) when corrected for the interaction between age and sex, which showed a trend towards significance, as the sex difference is noteworthy mainly in older age groups (Fig. 7a).

In the multiple linear regression, the AV difference increased significantly with increasing age (Table 4c, $P = 0.0015$) as in the simple model. There also was a trend towards significant difference between the sexes ($P = 0.06$), but there

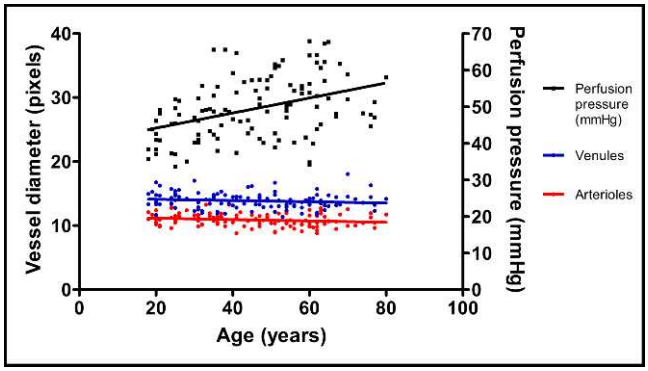

Figure 8. Retinal vessel diameter (*pixels; left y-axis*) of arterioles (*red*) and venules (*blue*) with increasing age ($n = 120$). Also, ocular perfusion pressure (mm Hg; *right y-axis*) with increasing age ($n = 117$). The slope was –0.01 ± 0.005 pixel/year ($r^2 = 0.033$, $P = 0.046$) for arterioles, –0.01 ± 0.007 pixel/year ($r^2 = 0.017$, $P = 0.16$) for venules, and 0.21 ± 0.044 mm Hg/year ($r^2 = 0.16$, $P < 0.0001$) for perfusion pressure.

was no interaction between age and sex ($P = 0.37$, Table S1c in Supplementary Material, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9912/-/DCSupplemental). Therefore, the model without the interaction was chosen for the presentation of the AV difference.

Cardiovascular Parameters

Perfusion pressure increased by 0.21 ± 0.044 mm Hg/year (Fig. 8, $P < 0.0001$). For every 1 mm Hg increase in ocular perfusion pressure, the oxygen saturation in arterioles increased by 0.09 ± 0.04% (mean ± SEM, Table 4a, $P = 0.024$) and in venules by 0.12 ± 0.07% (Table 4b, $P = 0.075$). No correlation was seen between ocular perfusion pressure and AV difference (Table 4, $P = 0.66$).

Systolic and diastolic blood pressure as well as intraocular pressure were included in the calculation of ocular perfusion pressure, and therefore were not included independently in the statistical models.

No difference was seen in retinal oxygen saturation in smokers compared to nonsmokers in either arterioles (Table 4a, $P = 0.20$), venules (Table 4b, $P = 0.92$), or the AV difference (Table 4c, $P = 0.30$). No correlation was seen between finger pulse oxygen saturation and retinal oxygen saturation in arterioles (Table 4a, $P = 0.27$) and venules (Table 4b, $P = 0.12$), nor in the AV difference (Table 4c, $P = 0.36$).

Vessel Diameters

Vessel diameter was 10.9 ± 1.0 pixels (mean ± SD) for retinal arterioles and 13.9 ± 1.2 pixels for venules. With increasing age, retinal vessel diameter decreased in arterioles (slope = –0.01 ± 0.005 pixels/year [mean ± SEM], $r^2 = 0.033$, $P = 0.046$) and venules (slope = –0.01 ± 0.007 pixels/year, $r^2 = 0.017$, $P = 0.16$, Fig. 8).

Discussion

Retinal vessel oximetry values are reliable and relatively stable in a healthy Caucasian population. No difference is seen between left and right eyes.

Over the age span of 18–80 years, oxygen saturation values in retinal arterioles are stable. The measured retinal venular saturation decreases significantly with age in males, while there is a similar trend in females. The change in saturation per 10 years of life is 2% for men and nearly 1% for women, and must be taken into account in clinical research. When patient groups are compared to healthy control groups, it is necessary to standardize for age and sex.

Our study included relatively few individuals 70 years and older. The oldest individuals, especially males, showed declining venous saturation, which requires further evaluation. It also will be necessary to accumulate more normative data in healthy individuals 70 to 90 years old for the study of age-related eye diseases in this age group. This is difficult as many individuals at that age have systemic and/or eye diseases, and lens opacities that reduce the retinal image quality.

Interestingly, multiple linear regression revealed significant differences in the venous saturation and AV difference between males and females. This difference has been attributed to lower saturations in the older male population. We found that in males, saturation decreased with age in venules at a rate faster than in females. There is a definite difference in life expectancy between the sexes and in Iceland women can expect to have an approximately 4-year longer lifespan compared to men.[40] It is interesting to speculate whether the males actually deteriorate faster and, therefore, may be physiologically "older" than females.

There also is the possibility that this decrease in venular saturation with age is due to an artifact. For instance, gradual cataract and worse pupil dilation is seen with increasing age, possibly causing worse image quality. However, all images were screened before analysis and those with poor image quality were excluded, but images with sufficient quality were included even though signs of early cataract existed. The fact that a comparable decrease in oxygen saturation is not seen in the arterioles with increasing age makes this possibility less likely, although it might be difficult to predict the final effect on the optical density ratio under these circumstances.

It has been shown that narrower retinal vessels show higher measurements of oxygen saturation.[10] For this reason, our measurements were corrected for vessel diameter. Moreover, it has been reported that retinal vessel diameter decreases with age,[41] which is in agreement with our results. Therefore, it is unlikely that changes in vessel diameter with age are causing the decrease in venular saturation, as we would rather expect opposite effects.

The AV difference correlates with the oxygen extraction of the retina and offers some inner control of the data as it corrects for those who have low arteriolar and venular retinal oxygen saturation measurements. The AV difference increases significantly with age for males and females, or approximately 1.5% and 1% for every 10 years of increasing age, indicating greater oxygen extraction per milliliter blood with increasing age. This implies that proportionally more oxygen is extracted from the blood with age. However, total oxygen delivery to the retina from the retinal circulation is the product of the AV difference and the retinal blood flow (RBF): $Oxygen\ delivery = \Delta AV \times RBF$. It is reasonable to assume that oxygen delivery and retinal oxygen consumption are closely related, even though the relative contribution of retinal and choroidal vasculatures may vary. We did not measure blood flow in our study, but we did measure a decrease in vessel diameter with age, which by itself might indicate decreased blood flow with age. However, we also saw an increase in ocular perfusion pressure with age, which might compensate for the increased resistance from the decrease in vessel diameter to some extent. As data on blood flow with age in published reports are conflicting, it is difficult to determine the overall effect of age on oxygen delivery or oxygen consumption in the retina, and further studies on this matter are needed.

Higher ocular perfusion pressure was correlated with increased arteriolar oxygen saturation and there was the same trend in venules as well. High perfusion pressure may correlate with higher blood flow and blood flow velocity, and thereby decrease the relative loss of oxygen from arterial blood by diffusion through arteriolar wall.

One might assume that there would be a correlation between increased finger pulse oximeter readings and retinal oximetry readings. However, we did not find such a relationship, possibly due to the narrow range of pulse oximeter readings, but 9 of 10 individuals were within 95 to 98% in finger pulse oxygen saturation. This range of 4% is similar to the standard deviation of the retinal oximetry measurements.

Concerning the lack of influence of smoking on retinal oxygen saturation, in this study we only looked at current smokers compared to nonsmokers, not the smoking history. The reason for this was that we were interested mainly in the possible effects of carboxyhemoglobin on our results. Carboxyhemoglobin has a color very similar to oxygenated hemoglobin and may be elevated in current smokers. We believe the effects of smoking must be studied further in a paired study of groups of nonsmokers and smokers.

Persons with a medical history of systemic hypertension were not excluded from the study if they were well controlled on their antihypertensive therapy. There have been suspicions if systemic medications can affect retinal vessel diameter, but Wong et al. found no evidence of substantial effect of common medications on retinal vessel diameter in the Beaver Dam Eye Study.[42] Moreover, this normal population will be used as a control group for further studies on ocular and systemic diseases, and some patients in those study groups also will have systemic hypertension.

The inferotemporal quadrant had significantly lower retinal oxygen saturation measurements compared to the other three quadrants. This was seen in arterioles and venules, but the difference was more pronounced in venules. This is in agreement with a previous report by our group,[43] and expands that study. The reason for this is unclear and could be either of optical, artificial, or physiologic origin. Garhöfer et al. recently reported results on retinal blood flow using bidirectional laser Doppler velocimetry in young healthy males, and found topographic variance in retinal quadrants as well.[44] In their study, the retinal blood flow was highest in the inferotemporal quadrant followed by the superotemporal, inferonasal, and superonasal quadrants. In comparison, but without any explanations for it, our arteriolar oxygen saturation values were related inversely to these blood flow measurements, with the lowest saturation in the quadrant with the highest blood flow.

The calibration of the Oxymap T1 oximeter used in our study depended on mean oxygen saturation values obtained from retinal vessels in healthy individuals by a device designed by Schweitzer et al.,[6] which was calibrated by measuring oxygen saturation in whole blood samples in vitro. We assumed that their mean retinal vessel oxygen saturation values are as close to accurate as possible and, therefore, matched our optical density ratios to these saturation values, and obtained the values for the constants *a* and *b* used in our study. The calibration of the oximeter is crucial for comparison of oxygen saturation values between different studies.

To standardize the images and analyses, a detailed protocol was written by our study group and described in detail above (Table 2). The oximetry images were taken by the same person in a predetermined order and the image with the optic disc in the center was used for the analysis in our study. Previously, we have shown that vessel location within an oximetry image, depending on the gaze of the subject, has a significant effect on measured oxygen saturation in arterioles and venules.[43] Moreover, the same study showed that there is a significant variation of oxygen saturation between retinal quadrants in the same image,[43] and that has been confirmed in the present study. Therefore, we believe it is important to measure only a certain length of a vessel and as close to the optic disc as possible, as measurements near the edges of the images are not as reliable. Also, we believe that measuring all the vessels around the optic disc above approximately 70 μm will give a good representation of the oxygenation of the retina and smaller vessels have been excluded in our analyses. The mean retinal vessel oxygen saturation was measured by averaging all the data from the individually measured vessel segments. Of course there are some limitations to this method, where all the vessels are contributing equally into the mean without regards to their diameter and, thus, blood flow. Performing a weighted average might be an option to correct for this limitation, but as there is no consensus on what is the appropriate approach to adjust the data we decided to use a simple average.

In conclusion, we provided normative data for retinal oximetry in a Caucasian population. Retinal vessel oxygen saturation is relatively constant in healthy individuals. Arteriolar oxygen saturation is stable, while there is a significant decrease in venular oxygen saturation with age in males and a similar trend in females. AV difference increases significantly with age for both sexes, indicating greater oxygen extraction with increasing age.

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