The Impact of Flash Intensity on Retinal Vessel Oxygen Saturation Measurements Using Dual Wavelength Oximetry

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Purpose. To establish the optimal flash settings for retinal vessel oxygen saturation parameters using dual-wavelength imaging in a multiethnic group.

Methods. Twelve healthy young subjects (mean age 32 years [SD 7]; three Mediterranean, two South Asian, and seven Caucasian individuals) underwent retinal vessel oxygen saturation measurements using dual-wavelength oximetry, noncontact tonometry, and manual sphygmomanometry. In order to evaluate the impact of flash intensity, we obtained three images (fundus camera angle 30°, ONH centered) per flash setting. Flash settings of the fundus camera were increased in steps of 2 (initial setting of 6 and the final of 22), which reflect logarithmic increasing intensities from 13.5 to 214 Watt seconds (Ws).

Results. Flash settings below 27 Ws were too low to obtain saturation measurements, whereas flash settings of more than 214 Ws resulted in overexposed images. Retinal arteriolar and venular oxygen saturation was comparable at flash settings of 27 to 76 Ws (arterioles' range: 85%–92%; venules' range: 45%–53%). Higher flash settings lead to increased saturation measurements in both retinal arterioles (up to 110%) and venules (up to 92%), with a more pronounced increase in venules.

Conclusions. Flash intensity has a significant impact on retinal vessel oxygen saturation measurements using dual-wavelength retinal oximetry. High flash intensities lead to supranormal oxygen saturation measurements with a magnified effect in retinal venules compared with arteries. In addition to even retinal illumination, the correct flash setting is of paramount importance for clinical acquisition of images in retinal oximetry. We recommend flash settings between 27 to 76 Ws.

Keywords: oximetry, dual wavelength, photography, ethnicity, pigmentation

Retinal vessel oximetry using dual wavelength imaging is a technology which assesses the metabolic function of the human retina in vivo using photography. The measurement principle is based upon the assessment of the absorption spectra of oxygenated and deoxygenated hemoglobin. The oxygen saturation of a given retinal blood vessel can be determined from two images each at a different wavelength, where one wavelength is at the isosbestic point of hemoglobin and the other one chosen such that the absorption coefficients of oxygenated and deoxygenated hemoglobin yield the greatest difference. The optical density ratio can be calculated from the optical densities at these two wavelengths.[1] Images are acquired using a double bandpass filter inserted into the illumination path of a fundus camera (Zeiss FF450+; Carl Zeiss Meditec, Dublin, CA), with transmitted light at the wavelength 548 nm ± 10 nm full width at half maximum and 610 nm ± 10 nm. A more detailed description of the technique and instrumentation can be found elsewhere.[2]

Retinal vessel oximetry offers great potential for clinical use as it noninvasively assesses metabolic function within the eye. However, the measurements may be confounded by varying fundus pigmentation and pupil size, requiring adjustment of flash intensity in order to obtain images with sufficient brightness and contrast. Although increasing the flash setting can increase brightness in dark fundi, it potentially influences the measured optical density since the intensity of reflected light of the immediate vessel surroundings is a constituent of the equation used to calculate oxygen saturation.[2] One could assume that theoretically, the increasing intensity of the reflected light of the vessel itself and its surrounding by increasing flash intensity is equal and hence the optical density ratio (ODR) remains unchanged. Practically, however, this is questionable as the ODR is dependent not only on color but also on light scattering and sample cuvette; (where the cuvette in this case is represented by the vessel wall). Therefore, there is a possibility that the reflected intensities are not increased by the same amount leading to a change in ODR obtained.

Further increasing light levels via the flash intensity leads to overexposure (i.e., very bright images with numerous reflections along retinal vessels). Low pigmentation areas (bright areas) surrounding the vessels will be more overexposed at lower flash intensities than the vessel itself, leading to variability of the measurement. The change in reflectance intensity ultimately influences both, the ODR and vessel diameter measurements, as they are dependent upon even illumination at sufficient brightness and contrast. Palsson et al. demonstrated the impact of direction of gaze and image area on retinal vessel oxygen parameters,[3] showing a statistically significant change in

oxygen saturation measurements resulting from changes in gaze but also within a single image depending on vessel location. In particular, changes of gaze reported in this paper showed an increase in saturation for downward gaze and no change for upward gaze, presuming the angle for upward and downward gaze was identical, and the changes are of technical origin (measurement principle and calculation), one could expect these changes to be of the same direction.

Although the change in saturation reported in this experiment is within the standard deviation of that reported for repeated saturation measurements, changes of gaze might contribute to further measurement variability, a point which is important to research should this technique be applied for diagnostic and monitoring purposes. Although these findings highlight the need for even illumination, they further emphasize standardization of image acquisition in order to gain clinically reliable and useful results for future comparison between studies.

The aim of this study was to evaluate whether there is an optimal range of flash settings within which reproducible results of retinal vessel oxygen saturation may be acquired. This is particularly important when imaging multiethnic groups with varying fundus pigmentation.

Materials and Methods

Subjects

Twelve healthy young subjects (mean age 32 years [SD 7]; three Mediterranean, two South Asian, and seven Caucasian individuals) were recruited from staff and students at Aston University, Birmingham, UK. Written informed consent was received from all subjects participating in the study. The study was designed and conducted in accordance with the tenets of the Declaration of Helsinki. All subjects were instructed to refrain from consuming caffeinated products, smoking, and drinking alcohol on the study day. Measurements were performed between 12 PM and 2 PM on all subjects. The following measurements were performed in one unselected eye of each participant; IOP measurement using a noncontact tonometer (Keeler Pulsair; Keeler, Windsor, UK) prior to pupil dilation with 1% tropicamide (Bausch & Lomb, London, UK) followed by manual sphygmomanometry (Digital BP Monitor UA-767EX-C; PMS Instruments, Albany, OR) to ensure stable blood pressure. Once pupils were fully dilated, oximetry images were taken using a fundus camera (Carl Zeiss Meditec) with a dual-wavelength filter (Imedos Systems, Jena, Germany).

Image Acquisition and Data Analysis

Thirty degree images with the optic nerve head (ONH) centered were acquired for all participants. Three images per flash setting (6, 8, 10, 12, 14, 16, 18, 20, and 22) corresponding to 13.5, 19, 27, 38, 53.5, 76, 107, 151, and 214 Ws, respectively, underwent further analysis (Fig. 1). Images at each setting were taken consecutively (with usually up to a 5-second interval; this is required to have the stated flash output according to the manufacturer). Between increasing intensities, subjects closed their eyes for a minimum of 2 to 3 minutes. Care was taken during image acquisition to ensure that the alignment of the camera and the eye produced even retinal illumination.

ODR (ODR = $\log (I_{610out}/I_{610in})/ \log(nI_{610out}/I_{548in})$; where n is the ratio of the intensities measured at an ideal white reflector [Spectralon; Labsphere, Inc., North Sutton, NH]); vessel diameter (D) and oxygen saturation (SO_2 calculated according to the formula detailed by Hammer and colleagues in reference 2) of one arteriole and venule one-half disc diameter (DD) away from the ONH and one-half DD in length for each of the three images per setting were analyzed and averaged for further comparison between individual flash intensities.

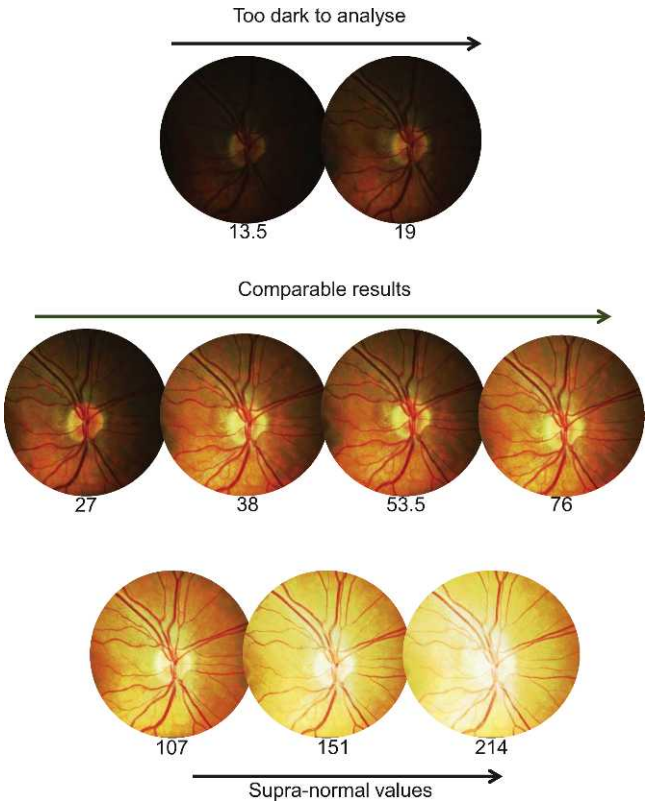

Figure 1. Example images from a Caucasian subject obtained at all flash intensities (Ws).

The length of one DD was chosen and kept constant in order to enable comparison with previous studies using the same location and segment length.

In brief, fundus images were taken using a customized dual-wavelength filter (transmission bands at 548 and 610 nm; bandwidth 10 nm each). Optical densities of the vessels were measured as the logarithmic ratio of the fundus reflection at the vessel center and its surrounding. The ODR at 610 and 548 nm is proportional to the vessel hemoglobin oxygen saturation[2] when compensating for the vessel diameter and fundus pigmentation.[2]

Statistical Analysis

All analyses were carried out using a commercial software program (STATISTICA version 6.0; StatSoft, Tulsa, OK). All parameters yielded normal distribution. Analyses of differences were sought by repeated measures ANOVA and Bonferroni's post hoc test. Statistical significance was defined at the level of $P < 0.05$.

Results

Participants' mean IOP was 12 (SD 3) mm Hg with a mean arterial BP of 85 (SD 8) mm Hg and average heart rate of 69 (SD 10) beats per minute. Images taken with a flash setting below 27 Ws were too dark—due to underexposure and oxygen saturation analysis—could not be initiated by the vessel analysis software. Conversely, images taken at more than 214

Table 1. Sample Characteristics Showing Sex, Skin Tone Classification, Iris Color, and Ethnicity

Subject No.	Sex	Skin Pigmentation (Fitzpatrick Skin Tone Scale)	Iris Color	Ethnic Background
S1	M	2	Blue/green	CA
S2	M	1	Grey	CA
S3	M	6	Dark brown	SA
S4	M	3	Brown	M
S5	F	3	Brown	SA
S6	M	1	Blue	CA
S7	M	2	Blue/grey	CA
S8	M	2	Blue/green	CA
S9	F	2	Blue/grey	CA
S10	F	2	Blue	CA
S11	F	3	Hazel	M
S12	F	3	Hazel	M

CA, Caucasian; SA, South Asian; M, Mediterranean.

Ws were too bright due to overexposure and could not be analyzed by the software. All subjects underwent pigmentation classification for skin pigmentation (according to the Fitzpatrick Skin Tone Scale)[4] and iris pigmentation. Pigmentation and ethnicity data of each subject can be found in Table 1. There was no interaction between pigmentation parameters and the effect of increased flash intensities in this sample.

Retinal arteriolar and venular diameters did not change significantly as a result of increasing flash intensity. However, ODR decreased significantly and consequently SO_2 of both arterioles and venules increased significantly with increasing flash intensity. ODR and SO_2 were comparable between flash settings of 27, 38, 53.5, and 76 Ws in both retinal arterioles and venules. Flash settings higher than 76 Ws resulted in significantly decreased values for ODR and increased SO_2 (Table 2, Figs. 2–4).

Retinal arteriolar and venular diameters were comparable at all flash settings (repeated measures ANOVA $P > 0.05$). ODR and SO_2 measurements were comparable over a range of flash settings up to 76 Ws (setting 16). Flash intensities of more than 76 Ws yielded below normal ODR and supranormal SO_2 readings (repeated measures ANOVA $P < 0.05$), in both retinal arterioles and venules. Further post hoc testing using Bonferroni's test revealed arterial ODR measured at 107, 151, and 214 Ws were incrementally decreased and significantly lower than those measured at 27 Ws ($P = 0.011$, $P = 0.005$, and $P = 0.032$, respectively) and 53.3 Ws ($P = 0.061$, $P = 0.030$, and $P = 0.142$, respectively). The difference in ODR measurements was more pronounced in veins as measured at increasing flash intensities, showing that measurements taken at 27, 38, 53.5, and 76 Ws were not statistically significantly different from each other (as were those at 107, 151, and 214 Ws), but significantly higher than those measured at 107 Ws ($P = 0.004$, $P = 0.001$, and $P = 0.064$, respectively); 151 Ws ($P = 0.0001$, $P = 0.0002$, and $P = 0.00006$, $P = 0.003$, respectively); and 214 Ws ($P = 0.0001$, $P = 0.0003$, $P = 0.0001$ and $P = 0.002$, respectively).

Similarly, arterial SO_2 was not statistically significantly different at flash intensities of 27, 38, 53.5, and 76 Ws (as were at 107, 151, and 214 Ws), but lower than compared with those at 107 Ws ($P = 0.013$, $P = 0.067$, and $P = 0.021$, respectively); 151 Ws ($P = 0.004$, $P = 0.022$, and $P = 0.007$, respectively); and 214 Ws ($P = 0.011$; $P = 0.048$, and $P = 0.022$, respectively). Venous SO_2 results obtained at flash intensities of 27, 38, 53.5, and 76 Ws were not statistically significantly different (as were those at 107, 151, and 214 Ws), but significantly higher than those measured at 107 Ws ($P = 0.027$, $P = 0.008$, $P = 0.001$, and $P = 0.063$, respectively); 151 Ws ($P = 0.001$, $P = 0.0002$, $P = 0.00003$, and $P = 0.003$, respectively); and 214 Ws ($P = 0.0007$, $P = 0.0002$, $P = 0.00004$, and $P = 0.001$, respectively).

Discussion

In addition to the individual retinal vessel diameter, retinal vessel oxygen saturation measurements rely on the brightness of the vessel and its immediate surrounding tissue.[2] Increasing flash intensity is a possible factor altering the contrast profile used to determine the vessel margins by increasing light scatter which induces additional reflections along the vessel margins making accurate vessel diameter measurements difficult. When under or overexposure of images occurs, artifacts in the clinical validity of the measurements of oxygen saturation are introduced. We observed that higher flash intensities cause a decrease in ODR, which leads to supranormal oxygen saturation values in both retinal arterioles and venules. This effect was more pronounced in venules, leading to erroneous venous oxygen saturation values as high as those normally measured in arteries. Since not only the ODR but also the vessel diameter are constituents of the equation used to calculate saturation, it is important to establish the effect of changing light levels on both. Although with increasing flash intensity ODR values decreased and subsequently SO_2 values were increased, diameter measurements were comparable at all settings. The trend of smaller diameters measured with increasing flash intensity was not statistically significant. This finding indicates that the increased saturation values originate

Table 2. ODRs, SO_2, and Diameter Measurements as Obtained at Different Flash Intensities

Flash Setting and Intensity for the Fundus Camera Flash Setting: Flash Energy (Ws)	SO_2 (%), Mean (SD)		ODR, Mean (SD)		D (au), Mean (SD)	
	Arterial	Venous	Arterial	Venous	Arterial	Venous
6: 13.5	–	–	–	–	–	–
8: 19	–	–	–	–	–	–
10: 27	85 (11)	45 (16)	0.07 (0.03)	0.18 (0.04)	104 (15)	124 (20)
12: 38	89 (11)	49 (14)	0.06 (0.03)	0.17 (0.04)	99 (15)	113 (20)
14: 53.5	88 (10)	44 (14)	0.06 (0.03)	0.17 (0.04)	98 (15)	111 (19)
16: 76	92 (13)	53 (16)	0.05 (0.04)	0.15 (0.04)	97 (14)	111 (19)
18: 107	103 (9)	74 (20)	0.03 (0.02)	0.10 (0.05)	97 (15)	110 (19)
20: 151	105 (10)	83 (25)	0.03 (0.02)	0.08 (0.05)	93 (14)	106 (15)
22: 214	110 (3)	92 (16)	0.02 (0.01)	0.06 (0.04)	95 (17)	107 (17)
Repeated measures ANOVA (P)	0.000024	<0.000001	0.00017	<0.000001	0.203	0.689

au, arbitrary units.

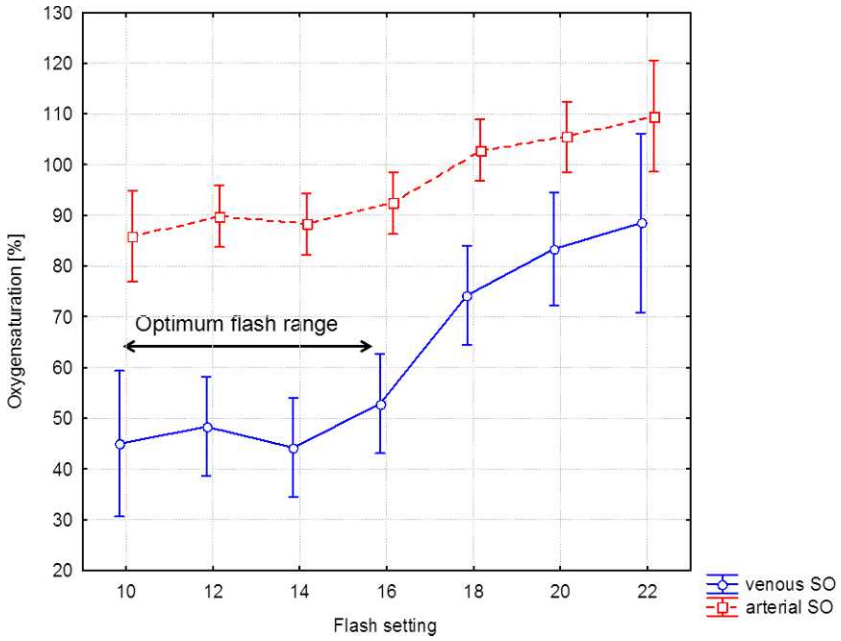

FIGURE 2. Oxygen saturation of retinal arterioles (*red*) and venules (*blue*) as taken at increasing flash settings.

mainly from changes in optical density measurements rather than diameter measurements. The basis of the saturation calculation is reflected light intensity from inside and outside of the blood vessel under observation. Thus, any increase in flash intensity will subsequently change the reflected intensity. Nevertheless, the reflected intensity is not only dependent upon the intensity of the camera flash, but more so on the absorption properties (i.e., optical density, cuvette) and transmission of the tissue, which reflects back the light used to image it. What we measure as a darker color absorbs more visible light than those colors we measure as light or brighter.

The effect of flash intensity was more pronounced in veins than arteries, the reason for this, we hypothesize, might lie in the fact that many charge-coupled devices tend to be less reliable at low absorbance values as too little light is reaching the detector. Another factor could be the sample cuvette (here the vessel wall); arterial vessel walls are thicker and morphologically different to those of veins therefore the light scatter and/or reflection contribution of the arteriolar and venular walls might be different from each other as well as being different from the light reflected back from the surrounding tissue.

Veins have a higher ODR because the difference in OD at 610 nm and 548 nm is less than those in arteries. Increasing flash intensity caused the venous ODR to decrease substantially more than arteriolar ODR as demonstrated in this study.

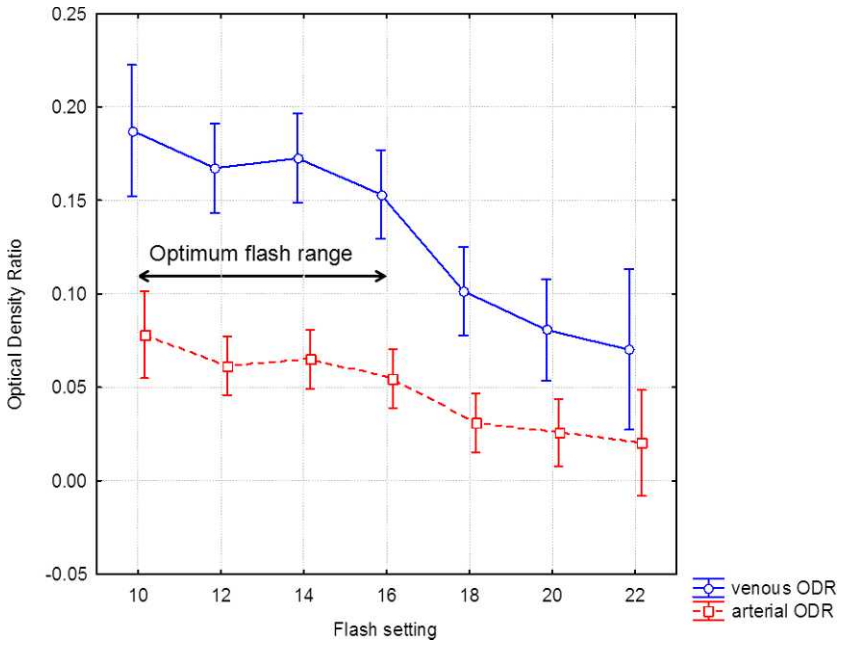

FIGURE 3. Optical density ratios of retinal arterioles (*red*) and venules (*blue*) as taken at increasing flash settings.

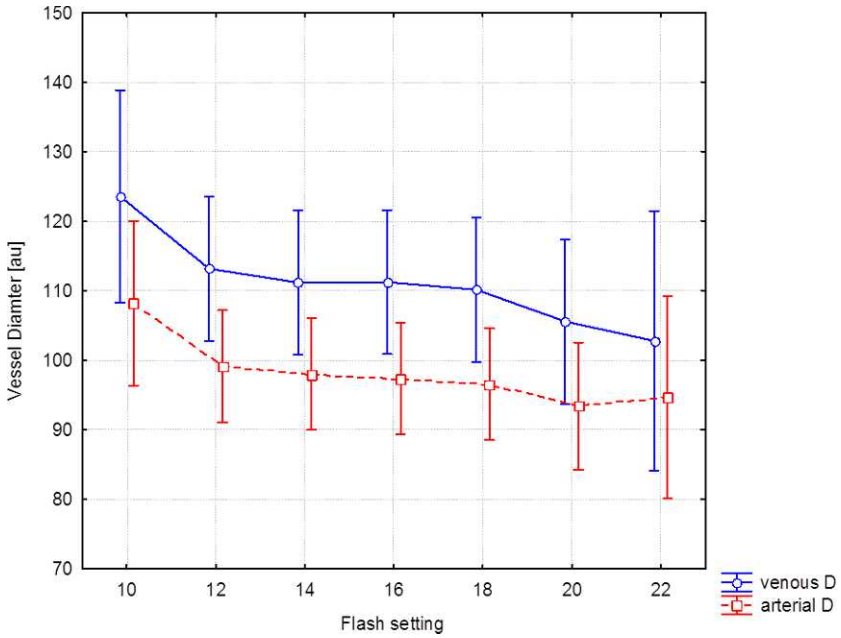

FIGURE 4. Diameter measurements of retinal arterioles (*red*) and venules (*blue*) as taken at increasing flash settings.

We believe the reason that this effect is more magnified in veins than in arteries is due to a nonlinear relationship in reflectance change across the color spectrum—that is, for two given objects (one lightly colored and one darkly colored), their change in measured absorbance (density) when illuminated with the same color light and the same increase in its intensity will not yield a same rate change. The more pronounced change in the measured reflected light in veins compared with arteries could also lie in the size of the vein carrying a larger volume of blood, thus causing a larger shift in the ODR with increasing flash intensity.

Often, the arteriovenous SO_2 difference is quoted in preference to individual retinal arteriolar and venular saturation values. For images taken at too bright flash settings, this will lead to a lower SO_2 difference being reported and in multiethnic samples, may lead to a high variance across the group under observation. This investigation provides further evidence that retinal oximetry imaging needs to be standardized, as highlighted by Palsson and colleagues,[3] which showed the impact of image location and angular image acquisition and suggesting these saturation changes are nonphysiological.

Our findings support that in order to improve the clinical utility of retinal oximetry when undertaking analyses, it is essential to obtain images with sufficient brightness and contrast over the whole image area undergoing analysis, as changes in illumination due to eye pupil size, angle of gaze, or by increasing flash intensity can lead to erroneous values. From the presented data, it is not possible to conclude if it is advisable to deploy a higher flash setting in highly pigmented fundi or if this introduces artificially increased saturation values. On the basis of these results, we recommend flash settings of between 27 and 76 Ws (flash settings 10 and 16) are used with the commercial oximeter (Imedos Systems). Whether darker fundi require generally higher flash settings or a correction factor, comparing different ethnic groups will need further evaluation in a larger cohort. Until this factor is further explored, it is advisable to at least record the flash setting at which the images were taken.

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