Retinal Oxygen Metabolism During Normoxia and Hyperoxia in Healthy Subjects

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Sufficient oxygenation is an important prerequisite for adequate retinal function. In the human retina, oxygen is delivered via two independent vascular beds, the retinal and the choroidal vascular system. The retinal vasculature receives its supply from the central retinal artery.1,2 The inner retina receives oxygen mainly via the retinal circulation, while the outer retina receives oxygen via the choroidal circulation.3–6 Although several animal studies3–4 were directed toward oxygen metabolism in different species, little is known about the situation in humans. Most of the available techniques such as microelectrodes7–12 and phosphorescence quenching13 cannot be applied in humans because of their invasive character.

Fundus reflectometry was suggested as a tool for measuring oxygen saturation in retinal vessels more than 40 years ago,14–16 and techniques have been improved since then.17–25 Given the technical development, particularly in the field of charge-coupled device cameras, several techniques have recently become commercially available that measure oxygen saturation in retinal arteries and veins. Using these techniques, alterations in retinal vessel oxygenation have been reported in patients with glaucoma,24,25 AMD,26 diabetes mellitus,27,28 and retinal vascular occlusions.29,30 In addition, alterations in retinal vessel oxygenation occur during stimulation with flickering light,27 as well as during inhalation of gas mixtures with different fractional inspired oxygen concentrations.31 However, interpretation of data is difficult because none of these studies have measured blood flow in the retina. In fact, oxygen extraction depends on both arteriovenous oxygen difference and blood flow. As such, a decrease in arteriovenous oxygen may indicate reduced oxygen extraction if blood flow is unchanged or may indicate unchanged or even increased oxygen extraction if blood flow is increased.

In the present study, we aimed to characterize the metabolic response of the retina during 100% oxygen breathing, leading to systemic hyperoxia, using noninvasive methods. The response in retinal oxygen saturation in retinal arteries and veins was measured using spectrophotometric reflectometry. The response of retinal blood flow was evaluated by combining laser Doppler velocimetry (LDV) to measure retinal blood velocities with fundus camera-based measurement of retinal vessel diameters. Using these techniques, we hypothesized that...
it may be possible to gain information on retinal oxygenation in the human retina during systemic hyperoxia.

**METHODS**

**Subjects**

The study protocol was approved by the ethics committee of the Medical University of Vienna and followed the guidelines set forth in the Declaration of Helsinki. Forty-six healthy male and female subjects aged between 18 and 35 years were included in this study. All subjects signed written informed consent and passed a screening examination before the study day, including physical examination, 12-lead electrocardiogram, assessment of visual acuity, slitlamp biomicroscopy, funduscopy, and measurement of IOP. Exclusion criteria were ametropia of 3 diopter (D) or higher, anisometropia of 3 D or higher, other ocular abnormalities, and any clinically relevant illness, blood donation, or intake of any medication in the 3 weeks before the study. Participants had to abstain from beverages containing alcohol or caffeine for 12 hours before the study day.

**Protocol**

After instillation of 1 drop tropicamide (Mydriatikum; Agepha, Vienna, Austria) into the study eye, a resting period of at least 20 minutes was scheduled. The diameters of one major retinal vein was calculated by combining measurements of retinal vessel diameters using the Dynamic Vessel Analyzer (DVA) (Imedos GmbH, Jena, Germany) and retinal blood velocity using bidirectional LDV. In addition, baseline measurements of systemic hemodynamic parameters were taken. The reactivity of retinal hemodynamic parameters to systemic hyperoxia was investigated during 100% oxygen breathing (Messer Group GmbH, Vienna, Austria). For this purpose, an oxygen breathing period of 30 minutes was scheduled, and retinal hemodynamic measurements were begun 15 minutes after the start of inhalation. The oxygen was delivered through a partially expanded reservoir bag at atmospheric pressure using a two-valve system to prevent rebreathing.

**Measurement of Retinal Vessel Diameters and Oxygen Saturation**

The diameters of one major temporal retinal artery ($D_{art}$) and one major temporal vein ($D_{vein}$) within 1 to 2 disc diameters from the center of the optic disc, as well as the arterial and venous retinal oxygen saturation levels, were obtained using the DVA (Imedos GmbH) coupled to an oxygen module (Imedos GmbH). This system comprises a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany), a high-resolution digital video camera, and a personal computer with analyzing software (all provided by Imedos GmbH). This technique was described previously in detail.\(^{22,32}\) The evaluation of oxygen saturation in retinal vessels is based on spectral analysis of light that has been reflected at the ocular fundus at selected wavelengths. In the present system, two fundus pictures with wavelengths of 610 and 545 nm, respectively, were taken. Extraction of retinal oxygen saturation is based on the fact that oxyhemoglobin has different light absorption characteristics compared with deoxyhemoglobin. The isobestic point at a wavelength of 548 nm is defined as the point in the light spectrum where oxyhemoglobin and deoxyhemoglobin show identical absorption. In contrast, oxyhemoglobin is almost transparent if it is illuminated with light at a wavelength of 610 nm. Quantifying

\[
\text{Oxygen Saturation} = \sum \frac{A(OHb) - A(DeO2Hb)}{A(OHb) - A(DeO2Hb)} \times 100\% 
\]

The contrast at these wavelengths enables determination of the relationship between oxygenated and total hemoglobin and calculation of oxygen saturation.

The measuring procedure and the subsequent image analysis were performed as published previously.\(^{22,32}\) Briefly, fundus images with the optic nerve head in the center were taken. Oxyhemoglobin saturation was measured in the selected vein ($SO_2_{vein}$) and the adjacent artery ($SO_2_{art}$) in a peripapillary annulus, with an inner radius of 1 disc diameter and an outer radius of 1.5 disc diameters. The arteriovenous oxygen difference was calculated as the difference between arterial and venous oxygen saturation levels. Retinal arterial ($D_{art}$) and venous ($D_{vein}$) diameters were measured at the same positions using the DVA (Imedos GmbH).\(^{33}\)

**Laser Doppler Velocimetry**

For measurement of retinal venous blood velocity, we used a fundus camera–based system (LDV-5000; Oculix, Inc., Arbaz, Switzerland). Measurements were performed in retinal veins at the same location as diameter and oxygen saturation measurements. The principle of LDV is based on the optical Doppler effect. Laser light of a single-mode laser diode with a wavelength of 670 nm is scattered by moving erythrocytes, leading to a broadened and shifted frequency spectrum. The frequency shift in the Doppler shift power spectrum is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte velocity ($V_{max}$).\(^{34}\) The Doppler shift power spectra are recorded simultaneously for two directions of the scattered light in the image plane of the fundus camera. This scattering plane can be rotated and adjusted in alignment with the direction of $V_{max}$, which enables absolute velocity measurements.\(^{35}\) We have recently shown that, after calculation of the absolute blood velocity, the angle of incidence can be calculated based on the data of both channels.\(^{36}\) This enables a quality control for the measurements. Only if the angle of incidence as calculated from the two channels is equal can the measurements be considered accurate. In the present study, we considered the measurements adequate if the agreement was with 0.5 rad. If this criterion was not reached, data were not considered accurate. Considering a parabolic flow profile, the mean blood velocity $vel$ in retinal vessels can be calculated from $V_{max}$ as follows:

\[
vel = \frac{V_{max}}{2}
\]

Blood flow in the retinal vein under study was calculated as follows:

\[
flow = vel \cdot D_{vein} \cdot \frac{\pi}{4}
\]

**Measurement of Systemic Hemodynamics**

Systolic blood pressure and diastolic blood pressure, as well as the mean arterial pressure, were measured on the upper arm by an automated oscillometric device (Infinity Delta; Dräger, Vienna, Austria). The same device automatically recorded pulse rate and systemic oxygen saturation by a finger pulse oximeter.

**Blood Gas Analysis and Calculation of Oxygen Content**

Arterialized capillary blood from the earlobe was collected from a lancet incision into a thin glass capillary tube. Arterial pH, PCO$_2$, and PO$_2$ were determined with an automatic blood
Retinal Oxygen Metabolism in Humans

TABLE. Outcome Variables at Baseline and During 100% Oxygen Breathing (n = 41)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>100% Oxygen Breathing</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114.0 ± 9.5</td>
<td>111.4 ± 9.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>67.4 ± 8.5</td>
<td>66.6 ± 8.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>85.0 ± 8.7</td>
<td>83.6 ± 8.3</td>
<td>0.81</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>68.4 ± 10.8</td>
<td>65.8 ± 9.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Arterial diameter, μm</td>
<td>122.8 ± 14.3</td>
<td>107.9 ± 13.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venous diameter, μm</td>
<td>153.3 ± 18.0</td>
<td>153.3 ± 17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial oxygen saturation, %</td>
<td>92.5 ± 3.9</td>
<td>96.4 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venous oxygen saturation, %</td>
<td>61.8 ± 4.4</td>
<td>73.9 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arteriovenous oxygen difference, %</td>
<td>30.5 ± 7.9</td>
<td>22.4 ± 10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC velocity, cm/s</td>
<td>1.73 ± 0.45</td>
<td>0.98 ± 0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC flow, µL/min*</td>
<td>20.1 ± 9.7</td>
<td>8.6 ± 4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.423 ± 0.019</td>
<td>7.459 ± 0.022</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood PO₂, mm Hg</td>
<td>90.2 ± 7.7</td>
<td>371.3 ± 92.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood PCO₂, mm Hg</td>
<td>37.0 ± 3.5</td>
<td>34.4 ± 4.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs. RBC, red blood cell.
* Blood flow through one specific vein and not total retinal blood flow.

Statistical Analysis

Percentage changes were calculated for each subject individually. Data are presented as means ± SDs. Paired t-tests were applied to detect statistically significant changes. P < 0.05 was considered statistically significant.

RESULTS

In three subjects, no sufficient LDV data could be obtained, and in two other subjects oxygen extraction could not be evaluated. After omitting these subjects, data presented in this article are from 41 healthy subjects (male and female) with a mean age of 25.4 ± 3.7 years. The mean hemoglobin concentration in this population was 14.1 ± 1.2 g/dL, and the mean systemic arterial SO₂ as measured with finger plethysmography was 98.4% ± 1.2%. During 100% oxygen breathing, systemic arterial SO₂ increased to 99.8% ± 0.1% (P < 0.001). The outcome variables at baseline and during 100% oxygen breathing are summarized in the Table. Systemic hyperoxia did not alter systemic blood pressure or pulse rate.

As expected, 100% oxygen breathing caused a pronounced decrease in retinal vessel diameters (−12.1% ± 4.0% for arteries and −13.0% ± 4.5% for veins), in retinal blood velocity (−43.4% ± 7.7%), and in retinal blood flow (−57.0% ± 5.7%) (P < 0.001 for all). These changes were accompanied by an increase in SO₂arter and SO₂vein, which was more pronounced in the retinal veins (+19.6% ± 6.2%) than in the retinal arteries (+4.4% ± 2.3%) (P < 0.001 for both). Accordingly, the arteriovenous oxygen saturation difference decreased significantly (−29.4% ± 19.5%). Analysis of arterialized blood samples revealed a pronounced increase in PO₂, together with a decrease in PCO₂ and an increase in pH.

The results for oxygen content and oxygen extraction are shown in the Figure. Oxygen content increased in retinal arteries (+9.1% ± 3.0%) and retinal veins (+19.6% ± 6.2%) (P < 0.001 for both) during 100% oxygen breathing. Accordingly, we observed a significant decrease in arteriovenous oxygen content difference (−12.6% ± 15.0%, P < 0.001) between baseline and inhalation of 100% oxygen. Oxygen extraction decreased by as much as 62.5% ± 9.5% (P < 0.001) during hyperoxia compared with normoxia.

DISCUSSION

The data herein indicate that 100% oxygen breathing causes complex changes in retinal oxygen metabolism in humans. Consistent with previous studies, we observed a pronounced reduction in retinal vessel diameters, retinal blood velocities, and retinal blood flow during 100% oxygen breathing. We also observed an increase in SO₂ in both retinal arteries and retinal veins, with a more pronounced effect in the latter, which is again comparable to previous data. Based on these measurements, we estimated oxygen content in retinal arteries and veins, oxygen difference between arteries and veins, and oxygen extraction. Our data indicate that during 100% oxygen breathing the retinal oxygen extraction from the retinal circulation strongly decreases.

Although to the best of our knowledge there are no other data on oxygen extraction during 100% oxygen breathing in humans, our data are compatible with a variety of previous studies in experimental animals. Most of our knowledge on retinal oxygenation comes from studies using oxygen microelectrodes. Such studies have been performed in cats, rats, pigs, and monkeys and provided consistent results in all species. Differences were observed between light and dark.
dark conditions. Herein, we will focus on light conditions because all the techniques used in our study use light illumination. In the outer retina, PO$_2$ falls steeply between the choriocapillaris and the photoreceptor inner segments, indicating the high oxygen consumption of the photoreceptors. Oxygen in the outer retina is all delivered from the choroidal vessels. In the inner retina, one usually observes several peaks, reflecting the proximity of the microelectrode to retinal vessels. In different species, inner retinal PO$_2$ values between 18 and 30 mm Hg have been reported. In cat, inner retinal PO$_2$ shows an increase of 20 to 40 mm Hg in the vitreous and inner retina caused by systemic hyperoxia. No data in humans are available to date. The PO$_2$ of the inner retina shows better regulation than that of the outer retina during systemic hyperoxia, which is due to the pronounced retinal vasoconstriction and decrease in retinal blood flow. However, in the choroid there is a pronounced increase in PO$_2$, reaching values of more than 200 mm Hg because of the increase in systemic PO$_2$ during 100% oxygen breathing. This is due to the fact that the choroid shows almost no blood flow response to 100% oxygen breathing.

The slight increase in inner retinal PO$_2$ is consistent with the increase of approximately 12% in SO$_2$venin as observed in the present study. How can it then be explained that both arteriovenous oxygen difference and retinal blood flow decrease during systemic hyperoxia? The most likely explanation is that the retinal veins pick up oxygen that is delivered from the choroid because of the pronounced increase of choroidal PO$_2$. Hence, our data do not indicate reduced oxygenation of the inner retina during systemic hyperoxia but rather a shift of the proportion of oxygenation arising from the retinal and choroidal vasculatures.

A number of limitations need to be considered when discussing the present data. In retinal arteries, SO$_2$ was below systemic arterial SO$_2$ as assessed with finger plethysmography. In addition, retinal arterial SO$_2$ did not reach 100% during 100% oxygen breathing, which might have been expected. Two reasons may be responsible for this observation. On the one hand, it might be that there is some countercurrent exchange between the central retinal artery and vein that keeps retinal arterial SO$_2$ lower than systemic arterial SO$_2$. In this case, it may also be that arterial PO$_2$ is lower than assumed from our earlobe measurements. However, the error introduced from this limitation is small given that even under systemic hyperoxia the amount of oxygen that is hemoglobin bound exceeds the amount of free oxygen by far. On the other hand, it may also be that the lower retinal arterial SO$_2$ levels are related to a calibration error of the system. We and others have shown that in patients with systemic hypoxia due to either Eisenmenger syndrome or chronic obstructive pulmonary disease reduced retinal arterial PO$_2$ values are measured that correlate with the level of systemic hypoxia. Whether fully oxygen-saturated hemoglobin concentrations in retinal arterial vessels indeed result in values of 100% SO$_2$ in retinal arteries when measured with the present system is not established. However, previous data do not indicate that this is because of the limited reproducibility of the technique.

In the equation using Henry's law, we estimated several parameters that we were not able to measure directly from systemic parameters. First, PO$_2$ in retinal arteries was
estimated from values in the earlobe. Second, hemoglobin concentrations in the retina were estimated from peripheral blood samples. Third, PO$_2$ in veins was measured from previously published curves. Given that the present study dealt with healthy subjects, these errors are considered very small and do not exceed 1%.

Another limitation relates to the point that blood flow was measured in only one retinal vein, and SO$_2$ was also only measured in the same vessel and the adjacent retinal artery. This results in a number of limitations, which are discussed on a point-to-point basis. It is not entirely clear whether the response of approximately 60% reduction of retinal blood flow during 100% oxygen breathing is representative of the entire retina. Indeed, studies$^{40,69}$ indicate regional differences in this respect. However, there is general agreement that systemic hyperoxia leads to pronounced vasoconstriction in all parts of the human retina. In principle, such data could be obtained by measuring all retinal vessels entering the optic nerve head,$^{61,62}$ but such an approach is extremely time-consuming. Alternatively, one could also apply Doppler optical coherence tomography approaches.$^{63-69}$ Our approach assumes that the response in retinal blood flow to 100% oxygen breathing is the same in retinal arteries and retinal veins. Although this has not formally been proven to date, it is expected in an end organ like the retina. Related to this point is the fact that we cannot ensure that all the blood supplied through the measured artery was also drained through the adjacent vein. Indeed, little is known about this issue in the human retina. Our regimen of delivering oxygen induced a pronounced increase in PO$_2$, as well as a small decrease in PCO$_2$, which may have resulted in a slightly more pronounced vasoconstriction than hyperoxia alone. Gilmore and coworkers$^{41,42}$ used a sequential rebreathing circuit and were able to induce isocapnic hyperoxia. Based on our previous results administering gases with variable amounts of oxygen and carbon dioxide and quantifying retinal blood flow,$^{59}$ we assume that this effect is small. It would have been interesting to also measure blood flow in retinal arteries during hyperoxia in the present study, but we omitted this approach to reduce the burden among the participating subjects to a minimum. Nevertheless, all these limitations do not affect the major conclusion of this study that in humans calculated oxygen extraction from retinal vessels shows a pronounced decrease during 100% oxygen breathing. However, values shown in the Figure should not be taken as absolute values for the entire human retina, especially for oxygen extraction.

In conclusion, the present study indicates that during 100% oxygen breathing there is a pronounced decrease in retinal oxygen supply and consumption in the macaque retina, Am J Physiol Heart Circ Physiol. 2007;293:H1696–H1704.


**References**


