Acta Ophthalmologica

Acta Ophthalmologica 2022

Effect of hyperoxia and hypoxia on retinal vascular parameters assessed with optical coherence tomography angiography

Nikolaus Hommer,[1] Martin Kallab,[1] Yin Ci Sim,[2] Ashe XY Lee,[2] Jacqueline Chua,[2,3,4] Bingyao Tan,[2,4,5] Andreas Schlatter,[1,6] René M. Werkmeister,[7] Doreen Schmidl,[1] Leopold Schmetterer[1,2,3,5,7,8] and Gerhard Garhöfer[1]

[1]Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria
[2]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore
[3]Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Medical School, Singapore, Singapore
[4]SERI-NTU Advanced Ocular Engineering (STANCE), Singapore, Singapore
[5]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, Singapore
[6]VIROS - Vienna Institute for Research in Ocular Surgery - Karl Landsteiner Institute, Hanusch Hospital, Vienna, Austria
[7]Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria
[8]Institute of Clinical and Experimental Ophthalmology, Basel, Switzerland

ABSTRACT.

Purpose: **To investigate the response of the superficial and deep capillary plexuses to hyperoxia and hypoxia using optical coherence tomography angiography (OCT-A) and retinal vessel analyzer.**

Methods: **Twenty-four healthy volunteers participated in this randomized, double-masked, cross-over study. For each subject, two study days were scheduled: on one study day, hyperoxia was induced by breathing 100% oxygen whereas on the other study day, hypoxia was induced by breathing a mixture of 88% nitrogen and 12% oxygen. Perfusion density was calculated in the superficial vascular plexus (SVP) and the deep capillary plexus (DCP), using OCT-A before (normal breathing) and during breathing of the gas mixtures. Retinal vessel calibres in major retinal vessels were measured using a dynamic vessel analyzer.**

Results: **During 100% oxygen breathing, a significant decrease in DCP perfusion density from 41.7 ± 2.4 a.u to 35.6 ± 3.1 a.u. ($p < 0.001$) was observed, which was accompanied by a significant decrease in vessel diameters in major retinal arteries and veins ($p < 0.001$ each). No significant change in perfusion density in the SVP occurred ($p = 0.33$). In contrast, during hypoxia, perfusion density in the SVP significantly increased from 34.4 ± 3.0 a.u. to 37.1 ± 2.2 a.u. ($p < 0.001$), while it remained stable in the DCP ($p = 0.25$). A significant increase in retinal vessel diameters was found ($p < 0.01$). Systemic oxygen saturation correlated negatively with perfusion density in the SVP and the DCP and retinal vessel diameters ($p < 0.005$ each).**

Conclusion: **Our results show that systemic hyperoxia induces a significant decrease in vessel density in the DCP, while hypoxia leads to increased vessel density limited to the SVP. These results indicate that the retinal circulation shows the ability to adapt its blood flow to metabolic changes with high local resolution dependent on the capillary plexus.**

Key words: healthy subjects – hyperoxia – hypoxia – optical coherence tomography angiography – retinal blood vessel diameter – retinal oxygen saturation

ClinicalTrials.gov registry: NCT04094285

This work was funded by grants from the Austrian Science Foundation (FWF Project No. KLI 721), the National Medical Research Council (CG/C010A/2017; OFIRG/0048/2017; OFLCG/004c/2018; and TA/MOH-000249-00/2018), the National Research Foundation Singapore, A*STAR (A20H4b0141), the Singapore Eye Research Institute & Nanyang Technological University (SERI-NTU Advanced Ocular Engineering (STANCE) Program) the Duke-NUS Medical School (Duke-NUS-KP(Coll)/2018/0009A), the SERI-Lee Foundation (LF1019-1) Singapore.

Acta Ophthalmol. 2022: 100: e1272–e1279

© 2021 The Authors. Acta Ophthalmologica published by John Wiley & Sons Ltd on behalf of Acta Ophthalmologica Scandinavica Foundation.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

doi: 10.1111/aos.15077

Background

Adequate oxygen supply is essential for retinal function. Previous studies have shown that retinal blood flow is regulated in response to oxygen saturation changes to keep its oxygen supply constant (Pournaras et al. 2008; Palkovits et al. 2014a; Palkovits et al. 2014c). Systemic hyperoxia leads to a pronounced retinal vasoconstrictor response accompanied by reduced retinal blood flow (Riva et al. 1983; Fallon et al. 1985; Luksch et al. 2002; Jean-Louis et al. 2005; Werkmeister et al. 2012; Palkovits et al. 2014a; Klefter et al. 2015; Cheng et al. 2016) whereas hypoxia leads to retinal vasodilatation and an increase in retinal blood flow (Palkovits et al. 2014c). However, this autoregulatory response has only been investigated in larger retinal feeder vessels (Werkmeister et al. 2012; Palkovits et al. 2014a; Palkovits et al. 2014c; Klefter et al. 2015).

Even though the capillaries are a main site of flow resistance, their metabolic regulation is largely unknown. Evidence from other vascular beds such as those of the heart or the brain suggests that small resistance vessels respond differently compared to larger arterioles or feeder vessels

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(DeFily & Chilian 1995; Kulik et al. 2008). Furthermore, it has been hypothesized that in complex neural tissues, such as the retina, microvascular responses to metabolic stimuli are not distributed uniformly but vary locally with high spatial and temporal resolution (Kornfield & Newman 2014; Kallab et al. 2021).

Recently developed imaging techniques such as optical coherence tomography angiography (OCT-A) provide quantitative parameters for characterization of the perfusion of biological tissues and allow gaining information regarding the regulatory response in the retinal microvasculature with high spatial and temporal resolution. Small open studies in healthy subjects using OCT-A report that hyperoxia leads to a decrease in retinal vessel density, especially in the deep capillary plexus (DCP) and that hypoxia increases retinal vessel density (Pechauer et al. 2015; Hagag et al. 2018; Sousa et al. 2018, 2019).

The present study set out to investigate the effect of systemic hyperoxia and hypoxia on both microvascular changes and larger retinal feeder vessels in healthy subjects in a randomized double-masked, cross-over study design. In contrast to previous studies, we removed larger retinal vessels from OCT-A analysis, to specifically obtain data from the microvasculature, since larger vessels contribute disproportionally to the OCT-A signal and therefore could conceal more minor changes in microcirculation. This offers the possibility to differentiate between changes in the micro- and the macrovasculature. Further, we investigated whether there is a correlation between systemic oxygen saturation and retinal vascular parameters. Measurements of the outcome variables were performed at baseline during breathing room air and afterwards during breathing 100% oxygen or 88% nitrogen in 12% oxygen.

Methods

Subjects

The study protocol was approved by the Ethics Committee of the Medical University of Vienna and was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the European Union. Written informed consent was obtained from all study participants. Subjects were selected by the Department of Clinical Pharmacology at the Medical University of Vienna. All included subjects passed a screening examination in the four weeks before the first study day consisting of pregnancy testing in women of childbearing potential, blood pressure and heart rate measurement, physical examination including 12-lead ECG, assessment of visual acuity, slit lamp biomicroscopy, indirect funduscopy and measurement of intraocular pressure (IOP) with the Goldmann applanation tonometry.

Subjects were excluded if any clinically significant abnormality was found, including a history of drug or alcohol abuse, regular use of medication during the three weeks before the start of the study (except contraceptives), symptoms of a relevant illness and blood donation in the 3 weeks before the first study day, ametropia of more than 6 dioptres and smoking.

Study design

The study was conducted in a randomized, double-masked, cross-over design. A member of the Department of Clinical Pharmacology not involved in the study procedures generated a randomization list using a computer software (http://www.randomization.com). According to this list, a randomization envelope for each subject was prepared. All subjects started with breathing ambient air followed by breathing one of two gas mixtures in a consecutive way in the randomized order. The randomization list contained the allocated sequence for each subject. Covering of the gas containers ensured blinding during the measurement. The specific assignment was documented on the randomization list. The masking of the gas containers and the storage of the randomization list were done by a study nurse.

Subjects arrived after a light meal between 8:00 a.m. and 2:00 p.m. at our department and had to abstain from alcohol and stimulating beverages containing xanthine derivatives (tea, coffee, cola-like drinks) 12 hr before the study day. At the beginning of the first study day, a pregnancy test was performed in females of childbearing potential and one drop of 0.5% tropicamide (Mydriaticum 'Agepha', Agepha, Vienna, Austria) was instilled in the eye. For all subjects, the right eye was determined as the study eye. After a resting period of 20 min, Dynamic Vessel Analyzer (DVA) measurements were performed followed by OCT measurement while breathing of ambient room air. Then, capillary blood for blood gas analysis was drawn from the arterialized earlobe. Afterwards, a 30-min breathing period with the first gas mixture was performed in accordance with the randomization list. During the last 15 min of this period, the measurements of DVA followed by OCT-A were repeated, while subjects were still breathing the assigned gas mixture through the mask. Heart rate and peripheral oxygen saturation were monitored continuously by an unmasked investigator not involved in any other study procedures. This unmasked investigator also performed the analysis of the capillary blood samples before the end of the breathing period in order to contain masked conditions. Blood pressure was recorded every 5 min during the breathing periods. The second study day was scheduled within 14 days after the first study day. The same schedule for baseline measurement and the breathing period was performed for the second gas mixture on the second study day.

Investigational medical products

Oxygen: SAUERSTOFF medizinisch, Messer GmbH, Industriestrasse 5, 2352 Gumpoldskirchen, Austria.

Dose: 100%, breathing for 30 min.

Nitrogen: STICKSTOFF medizinisch, Messer GmbH, Industriestrasse 5, 2352 Gumpoldskirchen, Austria.

Dose: 88% nitrogen in 12% oxygen, breathing for 30 min.

Methods

Non-invasive measurement of systemic haemodynamics: Measurements of systemic haemodynamics were performed on the upper arm by an automated oscillometric device (Infinity Delta; Dräger, Vienna, Austria). The same device recorded systolic, diastolic and mean arterial pressures (SBP, DBP, MAP), pulse rate (HR) and peripheral oxygen saturation using a fingertip pulse oximeter.

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Intraocular pressure: A slit lamp-mounted Goldmann applanation tonometer was used to assess intraocular pressure. One drop of oxybuprocainhydrochloride combined with sodium fluorescein was used for anaesthesia of the cornea before each measurement.

Gas breathing period: Oxygen and nitrogen were delivered using a high concentration oxygen mask (HUDSON RCI, Teleflex Medical, Morrisville, USA).

Blood gas analysis: Blood gas analysis was performed after spreading the earlobe with Finalgon® (Boehringer-Ingelheim RCV GmbH & Co KG, Vienna, Austria) paste to induce capillary vasodilatation. A thin glass capillary tube was used to collect arterialized blood from a lancet incision. Arterial pH, pCO_2, pO_2, oxygen saturation (sO_2) and blood glucose level were determined with an automatic blood gas analysis system (ABL 800, FLEX, Drott Medizintechnik GmbH, Wiener Neudorf, Austria).

Optical coherence tomography angiography (OCT-A): Non-invasive measurement of retinal vasculature was performed using the OCT-A module of a commercially available spectral-domain OCT device (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany). This optical imaging modality enables the visualization in a full depth resolved and label-free manner of blood vessels contrasting against static tissue. High resolution scans were performed at the macula (512 B-scans, 512 A-scans/B-scan, 10° × 10°). Each measurement took approximately 5 minutes (including set-up and positioning of the subjects).

Quantitative OCT-A analysis: The superficial vascular plexus (SVP) and the deep capillary plexus (DCP) were the layers of interest in this investigation. Quality checks were performed to filter images with poor quality. Scans from 3 subjects were removed because excessive motion artefacts and/or shadow artefacts. The volumetric OCT-A scans were imported into MATLAB (v2021a, MathWorks, VA) for image post-processing and the assessments of the vessel and perfusion densities. Non-rigid registration was applied to the set of scans with the baseline for nitrogen breathing as the reference image to adjust for perspective shifts across each examination. From the four images recorded (two baseline images, one image with oxygen and one image with nitrogen) an average was calculated as:

$$I_{avg} = \frac{I_{baseline1} + I_{baseline2} + I_{oxygen} + I_{nitrogen}}{4}$$

Where I_{avg}, $I_{baseline}$, I_{oxygen} and $I_{nitrogen}$ represent the averaged, baseline, oxygen and nitrogen images, respectively. This was done in an effort to reduce the background noise, resulting a more reliable large vessel segmentation. Large vessels were then removed using an intensity threshold of 130.

The centre of the fovea was automatically determined, based on which two circles with radii (1 and 2.5 mm) were generated. Based on the annulus created between the two circles, the mean intensity within the annulus was computed as the background threshold. No vessel enhancement filters were used to retain the original decorrelation information. Figure 1 shows an example of the image processing steps.

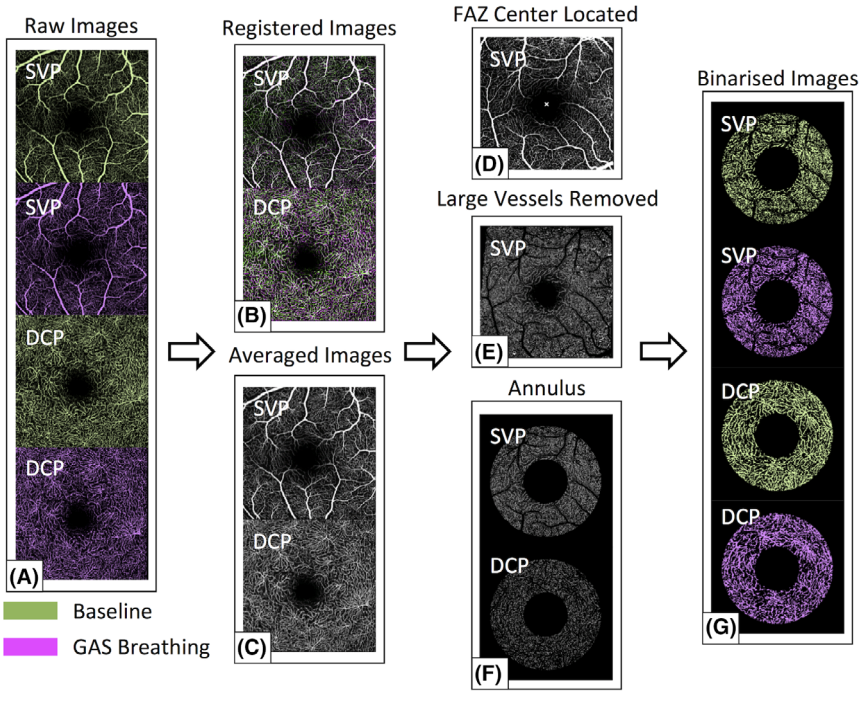

Fig. 1. Image processing steps of the OCT-A images. (A) Raw 3 × 3 mm OCT-A images from the Nitrogen experiment, with overlapped regions coloured in white. The top two images are the superficial vascular plexus (SVP) and the bottom two images are the deep capillary plexus (DCP). (B) Images registered at baseline before nitrogen breathing for both the SVP and the DCP. (C) Registered images for both the Nitrogen and Oxygen experiments were added to generate an overlapped mean. (D) Foveolar avascular zone (FAZ) center located and fovea marked with a cross. (E) Large vessels removed from SVP. (F) An annulus was generated centred at the FAZ center, with an inner diameter of 1mm and an outer diameter of 2.5 mm. (G) Images from (F) were binarized using the mean from the annulus of the overlapped mean as the background intensity. Green: before nitrogen breathing; Magenta: during nitrogen breathing.

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

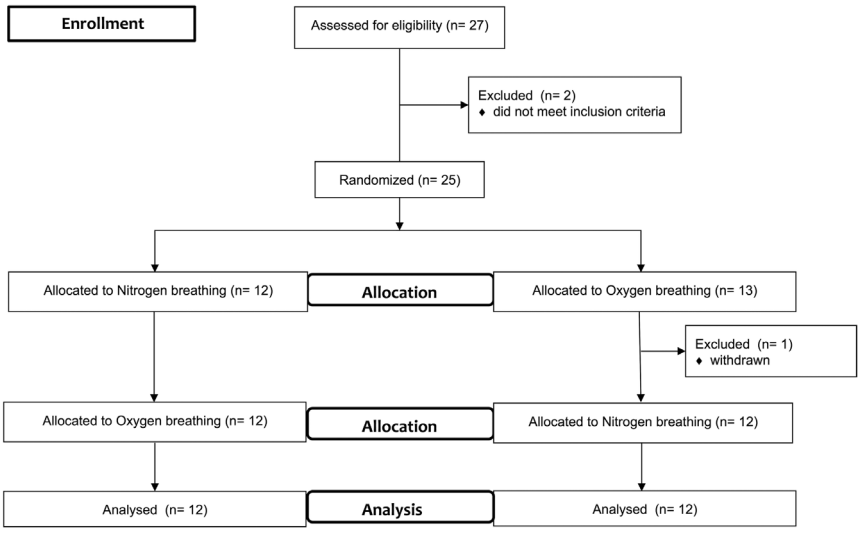

Fig. 2. Study flow chart

Dynamic Vessel Analyzer (DVA): The DVA system (IMEDOS, Jena, Germany) allowed the evaluation of the diameters of one temporal retinal artery and vein between 1 and 2 disc diameters from the margin of the optic disc. A fundus camera (FF 450; Carl Zeiss Meditec, Jena, Germany) was used for the determination of retinal vessel diameters by analysing digital pictures from a continuous video over 1 min of the respective vessels (Garhofer et al. 2010). Each measurement took approximately 5 min (including set-up and positioning of the subjects).

Statistical analysis

Data are presented as means ± SD. Paired *t*-tests were applied to detect statistical significance between baseline and breathing periods for normally distributed variables as confirmed by the Shapiro-Wilk test using IBM SPSS Statistics (Version 26, IBM, Armonk, New York, USA). A Wilcoxon test was used for variables that were not normally distributed (peripheral oxygen saturation, pO_2, sO_2 and pH). In addition, correlation analyses (Pearson) were carried out to identify the influence of parameters of systemic oxygenation (pO_2, sO_2 and pCO_2) on retinal vascular parameters (OCT-A parameters and retinal arterial and venous diameters). For this purpose, all values (baseline and during breathing of different gas mixtures) were taken together. A p-value < 0.05 was considered the level of significance.

Results

Twenty-five healthy subjects, of which 11 were female, aged between 21 and 33 years were included. For safety reasons, one female study participant was withdrawn from the study due to decreased arterial blood pressure during oxygen breathing and was replaced according to the randomization list. All other 24 subjects (mean age: 26 ± 3 years) tolerated the breathing periods and study procedures well and finished the study according to the protocol. Mean refractive error (spherical equivalent) of the study population was −1.0 ± 1.8 dpt. A study flow chart is provided in Fig. 2. Baseline values for both study days for the 24 subjects included in the analyses are given in Table 1. No significant differences in baseline parameters were observed on the two study days.

Effect of oxygen breathing

During breathing of 100% oxygen, no changes in SBP or MAP were observed ($p > 0.05$ each), while heart rate showed a slight but significant decrease from 71.9 ± 10.3 bpm^{-1} to 68.8 ± 9.0 bpm^{-1} ($p = 0.04$) and DBP slightly increased from 73.5 ± 5.7 mmHg to 76.2 ± 8.2 mmHg ($p = 0.05$). As expected, an increase in peripheral oxygen saturation from 98.1 ± 1.3% to 99.0 ± 0.8% was observed ($p = 0.004$). Arterialized capillary blood samples showed a significant increase in pO_2 from 93.3 ± 15.1 mmHg to 203.3 ± 58.5 mmHg and in sO_2 from 98.0 ± 0.9% to 99.8 ± 0.4% during oxygen breathing period ($p < 0.001$ each). pCO_2 showed a tendency to decrease from 36.7 ± 4.2 mmHg to 35.9 ± 3.6 mmHg ($p = 0.18$), while pH did not change (7.418 ± 0.020 versus 7.423 ± 0.017, $p = 0.35$).

Breathing of 100% oxygen induced a significant decrease in retinal perfusion density in the DCP (from 41.7 ± 2.4 a.u. to 35.6 ± 3.1 a.u., $p < 0.001$), while no changes in the SVP were observed (35.2 ± 2.0 a.u. at baseline versus 35.8 ± 2.3 a.u. during O_2 breathing, $p = 0.33$). Retinal vessel calibres in major retinal vessels decreased significantly in response to oxygen breathing by 7.7 ± 3.6% from 121.8 ± 15.0 µm to 112.5 ± 14.5 µm ($p < 0.001$) in arteries and by 7.0 ± 4.4% from 150.9 ± 21.4 µm to 140.3 ± 20.8 µm ($p < 0.001$) in veins. Figure 3 provides an overview of relative changes in all retinal parameters assessed.

Table 1. Baseline values for both study days during breathing of room air ($n = 24$) for systemic and retinal perfusion parameters (perfusion density in the superficial vascular plexus (SVP) and deep capillary plexus (DCP), retinal arterial diameter (Diaart) and retinal venous diameter (Diavein))

	Hyperoxia day	Hypoxia day	p-Value
Systolic blood pressure (mmHg)	120 ± 9	120 ± 10	0.99
Diastolic blood pressure (mmHg)	74 ± 6	76 ± 9	0.21
Mean arterial pressure (mmHg)	91 ± 7	93 ± 9	0.34
Heart rate (bpm^{-1})	72 ± 10	71 ± 12	0.76
Blood glucose level (mg/dl)	101 ± 15	101 ± 11	0.84
Peripheral oxygen saturation (%)	98 ± 1	98 ± 1	0.55
SVP perfusion density (a.u.)	35.2 ± 2.0	34.3 ± 3.0	0.23
DCP perfusion density (a.u.)	41.7 ± 2.4	40.8 ± 2.5	0.27
Diaart (µm)	121.8 ± 15.0	121.1 ± 16.6	0.89
Diavein (µm)	150.9 ± 21.4	150.0 ± 20.7	0.89

Data are presented as mean ± SD.

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

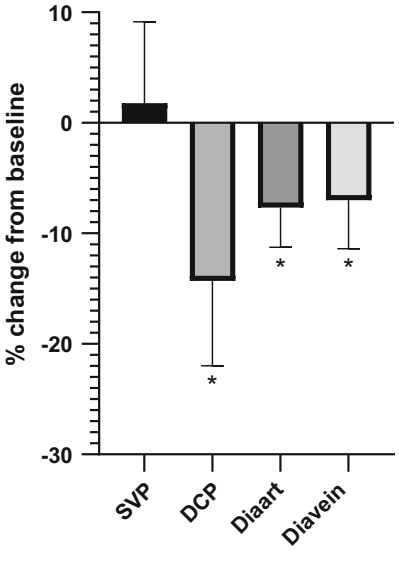

Fig. 3. Relative change from baseline in perfusion density in the superficial vascular plexus (SVP) and the deep capillary plexus (DCP), retinal arterial diameter (Diaart) and retinal venous diameter (Diavein), during breathing of 100% oxygen. *Indicates significant changes versus baseline. Data are presented as mean ± SD.

Effect of nitrogen breathing

Breathing of nitrogen had no effect on systemic haemodynamics (SBP, MAP and HR, $p > 0.05$ each) except for DBP, where a slight decrease from 76.3 ± 8.9 mmHg to 73.0 ± 7.6 mmHg was observed ($p = 0.03$). Peripheral oxygen saturation did not change (98.3 ± 1.1% versus 98.0 ± 1.6%, $p = 0.26$). In arterialized capillary samples, a statistically significant decrease in pO_2 from 92.5 ± 7.9 mmHg to 62.9 ± 6.1 mmHg and in sO_2 from 98.0 ± 0.7% to 93.7 ± 2.2% was found ($p < 0.001$ each). pCO_2 did not change during breathing of nitrogen (36.9 ± 3.5 mmHg versus 35.3 ± 4.2 mmHg, $p = 0.07$). A small but statistically significant increase in pH from 7.416 ± 0.016 to 7.426 ± 0.022 ($p = 0.04$) was seen.

Retinal perfusion density significantly increased in the SVP (from 34.4 ± 3.0 a.u. to 37.1 ± 2.2 a.u., $p < 0.001$), while it remained stable in the DCP (40.8 ± 2.5 a.u. versus 40.2 ± 1.9 a.u., $p = 0.25$). Retinal vessel diameters of major arteries and veins increased significantly during breathing of nitrogen by 2.3 ± 4.1% from 121.1 ± 16.6 µm to 123.8 ± 16.5 µm ($p = 0.01$) and by 3.0 ± 2.8% from 150.0 ± 20.7 µm to 154.6 ± 22.5 µm ($p < 0.001$), respectively. Relative changes in all retinal parameters are presented in Fig. 4.

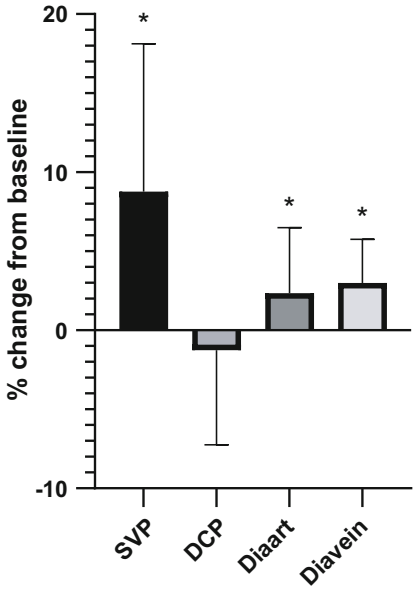

Fig. 4. Relative change from baseline in perfusion density in the superficial vascular plexus (SVP) and the deep capillary plexus (DCP), retinal arterial diameter (Diaart) and retinal venous diameter (Diavein) during breathing of 88% nitrogen in 12% oxygen. *indicates significant changes versus baseline. Data are presented as mean ± SD.

Correlation analyses

Table 2 shows the correlations between systemic parameters of oxygenation (sO_2, pO_2 and pCO_2 from blood gas analysis) and perfusion parameters (perfusion density and vessel diameters. sO_2 significantly negatively correlated with all perfusion parameters ($p < 0.05$ each), while a significant negative correlation was found between pO_2 and retinal vessel diameters ($p < 0.001$ each), as well as with perfusion density in the DCP ($p < 0.001$). No significant correlation was found between pCO_2 and any of the perfusion parameters ($p > 0.09$ each).

Discussion

The results of the present study confirm the ability of the retinal microcirculation to adapt its blood flow in response to both hyperoxia and hypoxia with high local resolution. Whereas hyperoxia mainly leads to a constriction in the deep capillary plexus of the retina, hypoxia leads to vasodilation mainly in the superficial layers. Further, our data show that there is a correlation between systemic oxygen partial pressure and microvascular changes as measured with OCT-A.

The relationship between perfusion in major retinal vessels and changes in blood oxygen saturation is well described. Using fundus imaging-based approaches, it has been reported that breathing of 100% oxygen leads to a pronounced and almost immediate decrease in vessel calibres in the range of 10% (Kiss et al. 2002; Luksch et al. 2002; Palkovits et al. 2014a), accompanied by a decrease of retinal blood flow of more than 50% (Kiss et al. 2002; Palkovits et al. 2014a; Werkmeister et al. 2015). This strong response has generally been interpreted as a counter-regulatory mechanism to avoid excess oxygen supply to the retina, which may be toxic to neurons. Such measurements in large vessels do, however, not clarify to what extent the microcirculation at the different levels of the retina contributes to this response.

Our results are in line with findings of a previous study by Hagag et al. (2018) that investigated the effect of hyperoxia on OCT-A parameters in 9 healthy subjects. The authors reported a decrease in DCP vessel density, while no significant changes in vessel density in the other layers, as well as in total retinal vessel density, were found

Table 2. Results of the correlation analysis for systemic parameters of oxygenation (sO_2, pO_2 and pCO_2) and retinal perfusion parameters (perfusion density in the superficial vascular plexus (SVP) and the deep capillary plexus (DCP), retinal arterial diameter (Diaart) and retinal venous diameter (Diavein)

	sO_2	pO_2	pCO_2
SVP perfusion density	**$r = -0.217$**	$r = 0.054$	$r = 0.069$
	$p = 0.048$	$p = 0.629$	$p = 0.534$
DCP perfusion density	**$r = -0.308$**	**$r = -0.484$**	$r = 0.011$
	$p = 0.004$	**$p < 0.001$**	$p = 0.922$
Diaart	**$r = -0.337$**	**$r = -0.331$**	$r = 0.141$
	$p < 0.001$	**$p < 0.001$**	$p = 0.171$
Diavein	**$r = -0.358$**	**$r = -0.348$**	$r = 0.175$
	$p < 0.001$	**$p < 0.001$**	$p = 0.089$

Statistically significant values are indicated in bold.

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(Hagag et al. 2018). While the study from Hagag et al. found no significant change in total retinal vessel density (all layers combined), two other studies report a significant decrease in this parameter during hyperoxia (Pechauer et al. 2015; Xu et al. 2016). However, these studies cannot be directly compared with our experimental approach since in the study by Xu et al. (2016) other oxygen mixtures (80% oxygen in contrast to 100% oxygen in the present study) were used, and the aim was to compare the macular and parapapillary region, but not different retinal layers. In the study by Pechauer et al. (2015), measurements were performed only in the parapapillary region and again, only total retinal vessel density and not vessel density in different retinal layers was assessed. Therefore, it cannot be excluded that the observed reduction in total retinal vessel density during hyperoxia in these studies might be mainly due to vasoconstriction in the DCP.

Another important difference between our study and previously published reports is that we excluded larger retinal vessels in the OCT-A image from the analysis. As the current study was designed to investigate mainly the pre-capillary and capillary vasculature, vessels in the size of approximately 30 μm or more were removed during image processing as described previously (Tan et al. 2020; Kallab et al. 2021). This is of special importance, given that these larger vessels would contribute disproportionally to the OCT-A signal and may mask subtle changes in the microcirculation.

Hyperoxia may selectively lead to vasoconstriction in the DCP due to the specific metabolism of the inner and outer retina. Retinal oxygen profiles during 100% oxygen breathing have previously been studied using oxygen microelectrodes in experimental animals (Pournaras et al. 1989; Braun et al. 1995; Linsenmeier & Zhang 2017). During systemic hyperoxia, the outer retina is flooded with oxygen because in contrast to the retinal circulation the choroid does not respond with vasoconstriction during 100% oxygen breathing (Schmetterer et al. 1996; Kergoat & Faucher 1999; Geiser et al. 2000). The DCP which also partially supplies the outer retina (Pournaras et al. 1989; Kur et al. 2012) therefore reacts with pronounced vasoconstriction, to counteract this excess oxygen. This is required because the photoreceptors are more sensitive to hyperoxia than the inner retinal neurons (Yu & Cringle 2005), which has also recently been proven in disease models for retinal degeneration (Roberts et al. 2018). Thus, the vasoconstrictor response of DCP as seen in the current study counteracts elevated oxygen tension due to diffusion from the choroid in the outer retina in order to reduce the risk of hyperoxygenation and oxidative stress (Werkmeister et al. 2015; Hagag et al. 2018). Our results also indicate that this vasoconstrictor response is not required in the SVP, which mainly supplies the retinal ganglion cells and the retinal nerve fibre layer (Pournaras et al. 1989).

During hypoxia, we found a significant increase in perfusion density in the SVP which was paralleled by an increase in retinal vessel diameters, but no changes in the DCP. One previous study inducing hypoxia by breathing 15% oxygen in nitrogen reported an increase in retinal vessel density during hypoxic conditions (Sousa et al. 2018). However, this study reported total vessel density only and no information for the different vascular plexuses was provided. Data from another experiment showed an increase in retinal vessel density in the SVP and the DCP during hypoxia (Sousa et al. 2019), which is in contrast to our findings. However, in this study, larger vessels were not removed from analysis, which may at least partially account for the differences.

Currently, the reason why hypoxia leads to an increase in vessel density mainly in the SVP remains unclear. It is known that even slight hypoxia changes the oxygen gradient between the choroidal capillaries and the outer retina, which in turn slows down the metabolic activity of the photoreceptors (Steinberg 1987; Yancey & Linsenmeier 1989; Lin et al. 2012). In contrast, the ganglion cell layer seems to be more vulnerable in response to hypoxia, which may trigger an autoregulatory response particularly in the superior vascular layers (Ugurlu et al. 2018; Karakahya et al. 2021). However, further studies are needed to clarify this issue.

In our study, a slight but significant negative correlation between sO_2 and perfusion parameters was observed. However, although pO_2 correlated negatively with retinal vessel diameters and with perfusion density in the DCP, no significant association was observed between SVP and pO_2. The reason for this difference is unclear, but given that the correlations between oxygen and perfusion parameters were generally weak, it may be speculated that the lack of a correlation between SVP and pO_2 may be related to the limited sample size of the study.

Our results also indicate that the microvessels of the SVP and the DCP vary considerably in terms of regulation of vascular tone. Little is known about the mechanisms that mediate vasoconstriction and vasodilatation in response to hyperoxia and hypoxia, respectively. A study in newborn pigs has shown that the arachidonic acid metabolites thromboxane and 20-HETE as well as endothelin contribute to hyperoxia-induced changes in vascular tone (Zhu et al. 1998). Results for endothelin were also confirmed for rats (Takagi et al. 1996) and humans (Dallinger et al. 2000). Little is known regarding the mediators of hypoxia, but the polyamine/ATP-sensitive potassium channel/Ca2+ influx/calcium-induced calcium release pathway has been shown to boost the lethality of hypoxia in retinal capillaries (Puro 2012). Our study indicates that there is a need to re-visit this topic in order to better understand the mechanisms on how hypoxia, which is an early sign of diseases such as diabetic retinopathy (Stitt et al. 2016; Fondi et al. 2017; Li et al. 2020), leads to neuronal and subsequent vision loss. In this context, it has been shown that in patients with type 1 diabetes and no signs of diabetic retinopathy the response of retinal capillaries to hypoxia is already diminished when compared with healthy controls (Sousa et al. 2020). Whether this pathological response of retinal capillaries to gas breathing may in future serve as an OCTA-based biomarker for disease development, and progression has yet to be investigated.

A major strength of the present study is the randomized, double-masked, two-way cross-over design, which is currently considered the gold standard for clinical trials. One of the key advantages of this study design is that every subject receives both interventions and therefore acts as its own

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

control, which considerably increases the study's power. Further, as described earlier, we have processed the OCT-A images to exclude larger vessels in the size of 30µm or more. Due to excluding larger vessels from the analysis of OCT-A, the observed changes in vessel density do only represent the retinal microvasculature and allow therefore direct conclusions regarding the microcirculation of the retina.

When discussing our study results, some limitations need to be mentioned. First, as OCT-A calculates the decorrelation of repeated OCT-scans, it does not directly provide information regarding volumetric blood flow. Thus, in principle, a change in vessel density may reflect either a change in capillary resistance or a change in blood velocity. The OCT-A technique currently available is not capable of distinguishing between these options. A further limitation is related to the technique itself and limited resolution of the OCTA systems currently available. Basically, the acquired OCTA image is a convolution of the object and the system point spread function (PSF). The reason why capillaries are still seen despite the limited resolution of OCTA is the considerable oversampling. This oversampling rate is, however, similar in both plexuses. Therefore, we think it is reasonable to conclude a differential response between the plexuses. Finally, our measurements were done in the macular region only with a limited scan width of approximately 10 degrees. Thus, we cannot entirely exclude that other areas of the retina react differently in response to hypoxia and hyperoxia.

Further, for ethical reasons, experimentally induced hypoxia in our study was only mild. Although this mild hypoxia was well reflected in blood pO_2, which dropped from 92.5 ± 7.9 mmHg to 62.9 ± 6.1 mmHg, no significant change in peripheral oxygen saturation was detected. However, it is reasonable to suggest that the lack of change in oxygen saturation measured by pulse oximetry during mild hypoxia might lie within the used technique itself. As such, it has been reported that although pulse oximetry is non-invasive and easy to use, it may overestimate oxygen saturation during hypoxia (Amalakanti & Pentakota 2016). Thus, in particular during mild hypoxic conditions, blood gas analysis seems to be more sensitive to detect changes, which may account for this observed difference.

Further, to limit the exposure time and thus the burden of the subjects, the breathing period was restricted to 15 min before the start of the measurements. This time schedule has also been used in similar studies investigating the effect of hypoxia on retinal haemodynamics where similar changes in pO_2 have been reported (Palkovits et al. 2014a; Palkovits et al. 2014b; Palkovits et al. 2014c; Petersen & Bek 2015). However, we cannot exclude that a deeper hypoxia and a longer breathing period would have led to a more pronounced effect on our outcome parameters.

In summary, systemic hyperoxia induced a significant decrease in vessel density in the DCP, whereas systemic hypoxia led to an increase mainly in vessel density of the SVP. This indicates that during both hyperoxia and hypoxia, the retina has the ability to regulate its blood flow plexus specific in response to metabolic changes. The molecular mechanism underlying these regulatory processes are yet to be identified.

References

Amalakanti S & Pentakota MR (2016): Pulse oximetry overestimates oxygen saturation in COPD. Respir Care **61**: 423–427.

Braun RD, Linsenmeier RA & Goldstick TK (1995): Oxygen consumption in the inner and outer retina of the cat. Invest Ophthalmol vis Sci **36**: 542–554.

Cheng RW, Yusof F, Tsui E, Jong M, Duffin J, Flanagan JG, Fisher JA & Hudson C (2016): Relationship between retinal blood flow and arterial oxygen. J Physiol **594**: 625–640.

Dallinger S, Dorner GT, Wenzel R, Graselli U, Findl O, Eichler HG, Wolzt M & Schmetterer L (2000): Endothelin-1 contributes to hyperoxia-induced vasoconstriction in the human retina. Invest Ophthalmol vis Sci **41**: 864–869.

DeFily DV & Chilian WM (1995): Coronary microcirculation: autoregulation and metabolic control. Basic Res Cardiol **90**: 112–118.

Fallon TJ, Maxwell D & Kohner EM (1985): Measurement of autoregulation of retinal blood flow using the blue field entoptic phenomenon. Trans Ophthalmol Soc UK **104**(Pt 8): 857–860.

Fondi K, Wozniak PA, Howorka K et al. (2017): Retinal oxygen extraction in individuals with type 1 diabetes with no or mild diabetic retinopathy. Diabetologia **60**: 1534–1540.

Garhofer G, Bek T, Boehm AG et al. Blood Flow Research (2010): Use of the retinal vessel analyzer in ocular blood flow research. Acta Ophthalmol **88**: 717–722.

Geiser MH, Riva CE, Dorner GT, Diermann U, Luksch A & Schmetterer L (2000): Response of choroidal blood flow in the foveal region to hyperoxia and hyperoxia-hypercapnia. Curr Eye Res **21**: 669–676.

Hagag AM, Pechauer AD, Liu L, Wang J, Zhang M, Jia Y & Huang D (2018): OCT angiography changes in the 3 parafoveal retinal plexuses in response to hyperoxia. Ophthalmol Retina **2**: 329–336.

Jean-Louis S, Lovasik JV & Kergoat H (2005): Systemic hyperoxia and retinal vasomotor responses. Invest Ophthalmol vis Sci **46**: 1714–1720.

Kallab M, Hommer N, Tan B et al. (2021): Plexus-specific effect of flicker-light stimulation on the retinal microvasculature assessed with optical coherence tomography angiography. Am J Physiol Heart Circ Physiol **320**: H23–h28.

Karakahya RH, Korkmaz M & Korkmaz H (2021): Decreased retinal nerve fiber and choroidal thickness in chronic rhinosinusitis. Eur Arch Otorhinolaryngol **278**: 2863–2868.

Kergoat H & Faucher C (1999): Effects of oxygen and carbogen breathing on choroidal hemodynamics in humans. Invest Ophthalmol vis Sci **40**: 2906–2911.

Kiss B, Polska E, Dorner G et al. (2002): Retinal blood flow during hyperoxia in humans revisited: concerted results using different measurement techniques. Microvasc Res **64**: 75–85.

Klefter ON, Lauritsen A & Larsen M (2015): Retinal hemodynamic oxygen reactivity assessed by perfusion velocity, blood oximetry and vessel diameter measurements. Acta Ophthalmol **93**: 232–241.

Kornfield TE & Newman EA (2014): Regulation of blood flow in the retinal trilaminar vascular network. J Neurosci **34**: 11504–11513.

Kulik T, Kusano Y, Aronhime S, Sandler AL & Winn HR (2008): Regulation of cerebral vasculature in normal and ischemic brain. Neuropharmacology **55**: 281–288.

Kur J, Newman EA & Chan-Ling T (2012): Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. Prog Retin Eye Res **31**: 377–406.

Li HY, Yuan Y, Fu YH, Wang Y & Gao XY (2020): Hypoxia-inducible factor-1α: A promising therapeutic target for vasculopathy in diabetic retinopathy. Pharmacol Res **159**: 104924.

Lin YB, Liu JH & Chang Y (2012): Hypoxia reduces the effect of photoreceptor bleaching. J Physiol Sci **62**: 309–315.

Linsenmeier RA & Zhang HF (2017): Retinal oxygen: from animals to humans. Prog Retin Eye Res **58**: 115–151.

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Luksch A, Garhofer G, Imhof A et al. (2002): Effect of inhalation of different mixtures of O(2) and CO(2) on retinal blood flow. Br J Ophthalmol **86**: 1143–1147.

Palkovits S, Lasta M, Told R et al. (2014a): Retinal oxygen metabolism during normoxia and hyperoxia in healthy subjects. Invest Ophthalmol vis Sci **55**: 4707–4713.

Palkovits S, Told R, Boltz A, Schmidl D, Popa Cherecheanu A, Schmetterer L & Garhofer G (2014b): Effect of increased oxygen tension on flicker-induced vasodilatation in the human retina. J Cereb Blood Flow Metab **34**: 1914–1918.

Palkovits S, Told R, Schmidl D et al. (2014c): Regulation of retinal oxygen metabolism in humans during graded hypoxia. Am J Physiol Heart Circ Physiol **307**: H1412–1418.

Pechauer AD, Jia Y, Liu L, Gao SS, Jiang C & Huang D (2015): Optical coherence tomography angiography of peripapillary retinal blood flow response to hyperoxia. Invest Ophthalmol vis Sci **56**: 3287–3291.

Petersen L & Bek T (2015): Diameter changes of retinal arterioles during acute hypoxia in vivo are modified by the inhibition of nitric oxide and prostaglandin synthesis. Curr Eye Res **40**: 737–743.

Pournaras CJ, Riva CE, Tsacopoulos M & Strommer K (1989): Diffusion of O_2 in the retina of anesthetized miniature pigs in normoxia and hyperoxia. Exp Eye Res **49**: 347–360.

Pournaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH & Stefansson E (2008): Regulation of retinal blood flow in health and disease. Prog Retin Eye Res **27**: 284–330.

Puro DG (2012): Retinovascular physiology and pathophysiology: new experimental approach/new insights. Prog Retin Eye Res **31**: 258–270.

Riva CE, Grunwald JE & Sinclair SH (1983): Laser Doppler Velocimetry study of the effect of pure oxygen breathing on retinal blood flow. Invest Ophthalmol vis Sci **24**: 47–51.

Roberts PA, Gaffney EA, Whiteley JP, Luthert PJ, Foss AJE & Byrne HM (2018): Predictive mathematical models for the spread and treatment of hyperoxia-induced photoreceptor degeneration in retinitis pigmentosa. Invest Ophthalmol vis Sci **59**: 1238–1249.

Schmetterer L, Lexer F, Findl O, Graselli U, Eichler HG & Wolzt M (1996): The effect of inhalation of different mixtures of O2 and CO2 on ocular fundus pulsations. Exp Eye Res **63**: 351–355.

Sousa DC, Leal I, Moreira S et al.(2019): A protocol to evaluate retinal vascular response using optical coherence tomography angiography. Front Neurosci **13**: 566.

Sousa DC, Leal I, Moreira S et al. (2020): Retinal vascular reactivity in type 1 diabetes patients without retinopathy using optical coherence tomography angiography. Invest Ophthalmol vis Sci **61**: 49.

Sousa DC, Leal I, Moreira S, Dionísio P, Abegão Pinto L & Marques-Neves C (2018): Hypoxia challenge test and retinal circulation changes - a study using ocular coherence tomography angiography. Acta Ophthalmol **96**: e315–e319.

Steinberg RH (1987): Monitoring communications between photoreceptors and pigment epithelial cells: effects of "mild" systemic hypoxia. Friedenwald lecture. Invest Ophthalmol vis Sci **28**: 1888–1904.

Stitt AW, Curtis TM, Chen M et al. (2016): The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res **51**: 156–186.

Takagi C, King GL, Takagi H, Lin YW, Clermont AC & Bursell SE (1996): Endothelin-1 action via endothelin receptors is a primary mechanism modulating retinal circulatory response to hyperoxia. Invest Ophthalmol vis Sci **37**: 2099–2109.

Tan B, Chua J, Lin E et al. (2020): Quantitative microvascular analysis with wide-field optical coherence tomography angiography in eyes with diabetic retinopathy. JAMA Netw Open **3**: e1919469.

Ugurlu E, Pekel G, Altinisik G, Bozkurt K, Can I & Evyapan F (2018): New aspect for systemic effects of COPD: eye findings. Clin Respir J **12**: 247–252.

Werkmeister RM, Palkovits S, Told R, Groschl M, Leitgeb RA, Garhofer G & Schmetterer L (2012): Response of retinal blood flow to systemic hyperoxia as measured with dual-beam bidirectional Doppler Fourier-domain optical coherence tomography. PLoS One **7**: e45876.

Werkmeister RM, Schmidl D, Aschinger G et al. (2015): Retinal oxygen extraction in humans. Sci Rep **5**: 15763.

Xu H, Deng G, Jiang C, Kong X, Yu J & Sun X (2016): Microcirculatory responses to hyperoxia in macular and peripapillary regions. Invest Ophthalmol vis Sci **57**: 4464–4468.

Yancey CM & Linsenmeier RA (1989): Oxygen distribution and consumption in the cat retina at increased intraocular pressure. Invest Ophthalmol vis Sci **30**: 600–611.

Yu DY & Cringle SJ (2005): Retinal degeneration and local oxygen metabolism. Exp Eye Res **80**: 745–751.

Zhu Y, Park TS & Gidday JM (1998): Mechanisms of hyperoxia-induced reductions in retinal blood flow in newborn pig. Exp Eye Res **67**: 357–369.

Received on August 3rd, 2021.
Accepted on November 29th, 2021.

Correspondence:
Gerhard Garhöfer, MD
Department of Clinical Pharmacology
Medical University of Vienna
Währinger Gürtel 18-20
1090 Vienna
Austria
Tel: +43-1 40400 29810
Fax: +43-1 40400 29980
Email: gerhard.garhoefer@meduniwien.ac.at

17553768, 2022, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aos.15077 by Nanyang Technological University, Wiley Online Library on [28/11/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License