CLINICAL SCIENCE

Effect of inhalation of different mixtures of O₂ and CO₂ on retinal blood flow

A Luksch, G Garhöfer, A Imhof, K Polak, E Polska, G T Dorner, S Anzenhofer, M Wolzt, L Schmetterer

Br J Ophthalmol 2002;86:1143–1147

See end of article for authors' affiliations

Correspondence to:
Dr L Schmetterer,
Department of Clinical
Pharmacology, Währinger
Gürtel 18-20, A-1090
Vienna, Austria;
leopold.schmetterer@
univie.ac.at

Accepted for publication
29 April 2002

Aim: To determine the effects of various mixtures of O₂ and CO₂ on retinal blood flow in healthy subjects.

Methods: A randomised, double masked, four way crossover trial was carried out in 12 healthy male non-smoking subjects. Gas mixtures (100% O₂, 97.5% O₂ + 2.5% CO₂, 95% O₂ + 5% CO₂, and 92% O₂ + 8% CO₂) were administered for 10 minutes each. Two non-invasive methods were used: laser Doppler velocimetry (LDV) for measurement of retinal blood velocity and fundus imaging with the Zeiss retinal vessel analyser (RVA) for the assessment of retinal vessel diameters. Arterial pH, pCO₂, and pO₂ were determined with an automatic blood gas analysis system. Retinal blood flow through a major temporal vein was calculated.

Results: Retinal blood velocity, retinal vessel diameter, and retinal blood flow decreased during all breathing periods ($p < 0.001$ each). Administration of 92% O₂ + 8% CO₂ significantly increased SBP, MAP, and PR ($p < 0.001$ each, versus baseline), whereas the other gas mixtures had little effect on systemic haemodynamics. Addition of 2.5%, 5%, and 8% CO₂ to oxygen caused a marked decrease in pH and an increase in pCO₂ ($p < 0.001$ versus pure oxygen).

Conclusions: Breathing of pure oxygen and oxygen in combination with carbon dioxide significantly decreases retinal blood flow. Based on these data the authors speculate that hyperoxia induced vasoconstriction is not due to changes in intravascular pH and cannot be counteracted by an intravascular increase in pCO₂.

Elevated arterial blood oxygen tension (pO₂) results in vasoconstriction and a pronounced decrease in retinal blood flow.^{1–3} The mechanisms underlying O₂ induced vasoconstriction are not completely clear. We have previously shown that endothelin receptor mediated vasoconstriction plays a part in hyperoxia induced vasoconstriction in healthy subjects.⁴ This is in keeping with several animal experiments,^{5–6} in which thromboxane A₂, 20-HETE, and cytochrome P-450 were identified as other mediators of hyperoxia induced vasoconstriction. Another factor which may contribute to the reduction in retinal blood flow during hyperoxia is intracellular and extracellular alkalosis. In vitro experiments indicate that the contractile tone of pericytes strongly depends on the pH.^{7–9} Pericytes are, however, assumed to play a part in the regulation of vascular resistance in the retina. Hence, one may speculate that pericytes participate in the regulation of retinal capillary blood flow in response to changes in the pH.

In the present study we examined the effects of various mixtures of O₂ and CO₂ (hyperoxia-hypercapnia) on retinal blood flow in healthy subjects. Previous studies investigating this topic were contradictory.^{10–14} This may be related to the fact that none of these trials was masked or randomised. Moreover, in none of these clinical studies in healthy subjects was retinal perfusion assessed continuously during the inhalation periods and time dependent effects may therefore have been missed.

METHODS

Subjects

The study was performed in adherence to the guidelines of the Declaration of Helsinki and the good clinical practice (GCP) guidelines. After approval of the study protocol by the ethics committee of the Vienna University School of Medicine and after written informed consent was obtained, 12 healthy male

non-smoking subjects (sample size calculation according to Stolley and Strom¹⁵: a study in 12 volunteers has the statistical power of 80% ($\beta = 1$) to detect a 2% difference in vessel diameter), were included (age 21–35 years, mean 29.0 (SD 4.8)). The participants were selected by the department of clinical pharmacology. All subjects passed a prestudy screening during the 4 weeks before the first study day, which included a physical examination and medical history, 12 lead electrocardiogram, urine analysis, haematological status (haemoglobin, haematocrit, APTT, thrombin time) and an ophthalmic examination. Subjects with normal findings in the screening examinations and ametropia of less than 3 dioptres were included in the trial. In all subjects the right eye was studied.

Study design

Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial days and were studied after an overnight fast. The study was performed in a randomised, double masked, four way crossover design. The following gas mixtures were administered for 10 minutes each: 100% O₂, 97.5% O₂ + 2.5% CO₂, 95% O₂ + 5% CO₂ (known as carbogen), and 92% O₂ + 8% CO₂ (AGA, Vienna, Austria, certified gases for human use). All gases were delivered, after preparation by an unblinded study nurse, through a partially expanded reservoir bag at atmospheric pressure using a two valve system to prevent rebreathing. The odourless gas mixture was not identifiable for volunteers or assessing physicians.

Description of study days

Given that two different instruments were applied for measuring retinal vessel diameter and retinal blood velocity, the study had to be performed on two separate study days for technical reasons. Both study days followed an identical protocol. Four inhalation periods with different mixtures of oxygen and carbon dioxide were scheduled. Subjects were randomised at

Table 1 Effect of inhalation of different mixtures of O₂ and CO₂ on blood pressure and pulse rate (n=12; means (SD) on both study days

	SBP (mm Hg)		DBP (mm Hg)		MAP (mm Hg)		PR (beats/min)	
	Baseline	End of breathing period	Baseline	End of breathing period	Baseline	End of breathing period	Baseline	End of breathing period
RVA, study day								
100% O ₂	116 (9)	116 (12)	64 (9)	64 (9)	81 (8)	81 (8)	69 (8)	68 (11)
97.5% O ₂ + 2.5% CO ₂	121 (12)	121 (10)	68 (13)	65 (9)	86 (12)	84 (9)	68 (11)	72 (13)
95% O ₂ + 5% CO ₂	118 (12)	121 (11)	65 (9)	63 (11)	83 (9)	82 (10)	66 (11)	73 (12)
92% O ₂ + 8% CO ₂	119 (12)	133 (9)*	68 (9)	67 (17)	85 (8)	89 (13)*	67 (11)	79 (13)*
LDV, study day								
100% O ₂	116 (13)	116 (10)	65 (12)	64 (10)	82 (10)	82 (9)	70 (12)	67 (6)
97.5% O ₂ + 2.5% CO ₂	114 (12)	120 (10)	67 (12)	69 (12)	83 (10)	86 (11)	66 (13)	66 (9)
95% O ₂ + 5% CO ₂	115 (9)	122 (8)	67 (11)	68 (6)	83 (10)	86 (5)	71 (11)	74 (13)
92% O ₂ + 8% CO ₂	120 (12)	133 (13)*	68 (11)	74 (8)	86 (9)	94 (7)*	67 (9)	74 (11)*

*p<0.05 versus 100% O₂.

study entry according to block randomisation with respect to inhalation of four different mixtures of oxygen and carbon dioxide and with respect to the two different study days for either measurements with the Zeiss retinal vessel analyser (RVA) or the laser Doppler velocimetry (LDV). A period of at least one day was scheduled between the two study days.

All subjects were studied with pupil dilated after instillation with tropicamide (Mydriaticum "Agepha"). An initial 20 minute resting period ensured stable haemodynamic conditions. Then systemic and retinal haemodynamic baseline values were measured. After completion an inhalation period of an O₂/CO₂ gas mixture was started for 10 minutes and retinal haemodynamic measurements were performed continuously throughout the inhalation period. Blood pressure and pulse rate were measured every 2 minutes. The minimum time span between two inhalation periods was 30 minutes to re-establish baseline conditions. After this resting period new baseline values were measured and the next O₂/CO₂ gas mixture was administered according to the randomisation list. Before and at the end of each breathing period capillary blood gas values were determined from the arterialisated earlobe (Finalgon ointment, Thomae, Biberach, Germany) to assess pH, pCO₂, and pO₂.

Methods

Systemic haemodynamics

Systolic, diastolic, and mean blood pressures (SBP, DBP, MAP) were measured on the upper arm by an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger pulse oximetric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA, USA).

Blood gas analysis

Arterialisated capillary blood from the earlobe was collected from a lancet incision into a thin glass capillary tube. Arterial pH, pCO₂, and pO₂ were determined with an automatic blood gas analysis system (AVL 995-Hb, Graz, Austria).

Bidirectional laser Doppler velocimetry (LDV)

The principle of red blood cell velocity measurement with LDV is based on the optical Doppler effect. Laser light, which is scattered by moving erythrocytes is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centre line erythrocyte velocity (V_{max}).¹⁶ Using bidirectional laser Doppler velocimetry the absolute velocity in the retinal vessels can be obtained.¹⁷

In the present study we used a fundus camera based system with a single mode laser diode at a centre wavelength of 670 nm (Oculix Sarl, Arbaz, Switzerland). The Doppler shift power spectra were recorded simultaneously for two directions of the

scattered light. The scattered light was detected 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. LDV provides a reliable and reproducible technique for retinal blood velocity measurement.¹⁶

In the present study V_{max} was determined in a main inferior or superior temporal retinal vein. All measurement locations were within one to two disc diameters from the centre of the optic disc. Before data were analysed, spikes due to micro movements and blinks were removed.

Diameter measurement using Zeiss retinal vessel analyser (RVA)

The vessel diameters at the same measurement locations (D_i) were determined from retinal images recorded with a fundus camera based system. In this study the anatomical marker was either the main superior or the inferior temporal vein, in both measurements (RVA and LDV) the same measurement point was selected. The Zeiss RVA is a commercially available system which comprises a fundus camera (Zeiss FF 450, Jena, Germany), a video camera, a real time monitor, and a personal computer with an analysing software for the accurate determination of retinal arterial and venous diameters.¹⁸ Every second a maximum of 25 readings of vessel diameter can be obtained. For this purpose the fundus is imaged onto the charge coupled device chip of the video camera. The consecutive fundus images are digitised using a frame grabber. In addition, the fundus image can be inspected on the real time monitor and, if necessary, stored on a video recorder. Evaluation of the retinal vessel diameters can either be done online or offline from the recorded video tapes. Because of the absorbing properties of haemoglobin each blood vessel has a specific transmittance profile. Measurement of retinal vessel diameters is based on adaptive algorithms using these specific profiles. Whenever a vessel profile is recognised in the region of interest, the RVA can follow this vessel as long as it appears within the measurement window. The system is therefore able to correct automatically for alterations in luminance as induced for instance by small eye movements. If the requirements for the assessment of retinal vessel diameters are not fulfilled anymore, as it occurs during blinks, the system automatically stops the measurement of vessel diameter. As soon as an adequate fundus image is achieved again, measurement of vessel diameters restarts automatically. Our previous data revealed excellent reproducibility of measurements with the Zeiss retinal vessel analyser.¹⁹

Calculation of retinal blood flow (Q)

Blood flow through an individual major retinal vein was calculated from the results of the measurements of blood flow

Table 2 Effect of inhalation of different mixtures of O₂ and CO₂ on systemic pH, pCO₂, and pO₂ (n=12; means (SD)) on both study days

	RVA, study day		LDV, study day	
	Baseline	End of breathing period	Baseline	End of breathing period
pH				
100% O ₂	7.38 (0.02)	7.39 (0.03)	7.35 (0.08)	7.37 (0.09)†
97.5% O ₂ + 2.5% CO ₂	7.38 (0.03)	7.37 (0.03)*	7.34 (0.09)	7.33 (0.09)*
95% O ₂ + 5% CO ₂	7.38 (0.02)	7.34 (0.02)*†	7.36 (0.07)	7.32 (0.08)*†
92% O ₂ + 8% CO ₂	7.38 (0.02)	7.27 (0.04)*†	7.34 (0.07)	7.23 (0.09)*†
pCO ₂				
100% O ₂	40 (2)	38 (2)†	40 (2)	37 (2)†
97.5% O ₂ + 2.5% CO ₂	40 (3)	42 (2)*	40 (3)	41 (2)*
95% O ₂ + 5% CO ₂	40 (2)	46 (3)*†	40 (6)	45 (6)*†
92% O ₂ + 8% CO ₂	40 (2)	55 (7)*†	40 (3)	58 (5)*†
pO ₂				
100% O ₂	85 (7)	425 (69)†	85 (7)	413 (92)†
97.5% O ₂ + 2.5% CO ₂	86 (7)	390 (96)†	84 (8)	386 (111)†
95% O ₂ + 5% CO ₂	86 (6)	413 (98)†	87 (10)	414 (101)†
92% O ₂ + 8% CO ₂	85 (6)	406 (102)†	88 (9)	443 (53)†

*p<0.05 versus 100% O₂; †p<0.05 versus baseline.

velocity and retinal vessel diameter. The mean retinal blood velocity (V_{mean}), in the measured vein was calculated as¹⁶:

$$V_{\text{mean}} = V_{\text{max}}/1.6$$

Blood flow through this retinal vein was calculated as:

$$Q = V_{\text{mean}} \times \pi \times D^2/4.$$

Data analysis

From continuous retinal vessel diameter and retinal blood velocity measurements the mean over 2 minutes was calculated for these parameters. Accordingly, six values of retinal blood velocity or vessel diameter were obtained from each breathing period, one at baseline and five during the 10 minute inhalation period.

Baseline values of all outcome variables were compared by one way ANOVA. The effect of breathing different gas mixtures on retinal haemodynamic parameters was calculated as percentage change from baseline values. Percentage changes as presented in the results section represent the maximum deviation from baseline. Relative data of retinal vessel diameter, retinal blood velocity, and retinal blood flow were used for statistical analysis. The effects of breathing different gas mixtures were assessed with four way ANOVA for repeated measures. Post hoc analysis was performed by comparing the effect of the different O₂/CO₂ mixtures versus 100% O₂ by using

two way ANOVA for repeated measures. In order to calculate the effects of the individual doses of O₂/CO₂ versus baseline a one way ANOVA was performed. For blood pressure, pulse rate, and blood gas parameters the statistical analyses were performed using the absolute values.

Data are presented as means (SD). A p value of < 0.05 was considered the level of significance.

RESULTS

Two subjects reported transient headache after breathing 92% O₂ + 8% CO₂, no other adverse events were observed during the study. In all subjects blood pressure and pulse rate returned to baseline levels immediately after cessation of the breathing periods.

Baseline values and systemic effects of different mixtures of O₂ and CO₂ breathing on blood pressure and pulse rate are shown in Table 1. There were no significant differences between the baseline values of systemic haemodynamics on the two trial days. Administration of 92% O₂ + 8% CO₂ significantly increased SBP (+15% (9%)) during RVA measurements and 14% (8%), during LDV measurements p < 0.001 each), MAP (RVA: +11% (8%); LDV: +11% (13%), p < 0.001 each) and PR (RVA: +19% (11%); LDV: +13% (19%), p < 0.001 each versus baseline) on both trial days. This effect of 92% O₂ + 8% CO₂ was also significant versus 100% O₂ breathing alone for SBP (p < 0.001), MAP (p = 0.009), and PR (p < 0.001), but not for DBP. The other gas mixtures had no consistent effects on blood pressure or PR.

Baseline values of all blood gas parameters were comparable on both study days. The same holds true for the effects of pH, pCO₂, and pO₂, which are shown in Table 2. As expected, a pronounced increase in pO₂ (p < 0.001 versus baseline) was observed during all breathing periods. However, this response to hyperoxia was comparable during all inhalation periods.

The effect of inhaling different gas mixtures on pH and pCO₂ was dose dependent (Table 2).

Addition of 2.5%, 5%, and 8% CO₂ caused a marked decrease in pH (p < 0.001 each) and an increase in pCO₂ (p < 0.001). Pure oxygen tended to increase pH, but this effect was only significant on the LDV study day (p = 0.009).

PCO₂ was significantly increased by addition of 2.5%, 5%, and 8% CO₂ (p < 0.001 each) versus pure oxygen.

As demonstrated in Table 3, baseline values of RVA or LDV were similar before each breathing period. Figure 1 shows the time course of retinal venous diameter, retinal blood velocity, and retinal blood flow during the breathing periods. Inhalation of all gas mixtures induced a decrease of retinal vessel

Table 3 Baseline values of retinal blood flow (RBF), vessel diameter (D), and retinal blood velocity (Vel) (n=12; means (SD)) before inhalation of different mixtures of O₂ and CO₂

	Baseline
RBF* (μl/min)	
100% O ₂	10.5 (4.5)
97.5% O ₂ + 2.5% CO ₂	10.2 (4.3)
95% O ₂ + 5% CO ₂	10.8 (4.1)
92% O ₂ + 8% CO ₂	9.7 (4.0)
D (RVA) (μm)	
100% O ₂	158 (15)
97.5% O ₂ + 2.5% CO ₂	156 (17)
95% O ₂ + 5% CO ₂	156 (16)
92% O ₂ + 8% CO ₂	155 (13)
Vel (LDV) (cm/s)	
100% O ₂	1.67 (0.44)
97.5% O ₂ + 2.5% CO ₂	1.67 (0.53)
95% O ₂ + 5% CO ₂	1.77 (0.49)
92% O ₂ + 8% CO ₂	1.62 (0.47)

*Denotes RBF through one specific retinal vein and not total RBF.

Figure 1 Time course of retinal venous diameter, retinal blood velocity, and retinal blood flow during breathing of different mixtures of O₂/CO₂. Data are presented as means (SD) (n=12). Asterisks indicate significant differences versus pure oxygen breathing.

diameter during the first 6 minutes of breathing. During the following 4 minutes retinal vessel calibres remained stable. Retinal blood velocities also decreased during the first 6 minutes of breathing and then remained stable during all breathing periods. Inhalation of all gases significantly decreased retinal venous diameters, retinal blood velocity, and retinal blood flow versus baseline ($p < 0.001$ each). This decrease was significantly higher during pure oxygen or gas mixtures with lower partial pressure of CO₂ than during breathing 92% O₂ + 8% CO₂ for retinal vessel diameter ($p = 0.015$) and retinal blood flow ($p = 0.029$).

DISCUSSION

The present study shows that breathing of pure oxygen as well as oxygen in combination with carbon dioxide significantly decreases retinal vessel diameter, retinal blood velocity, and retinal blood flow. Consequently neither 5% nor 8% CO₂ abolishes the oxygen induced vasoconstriction in the retina. The observed reduction in blood flow with 8% CO₂ + 92% O₂ was significantly smaller compared to breathing of pure O₂ or gas mixtures with smaller amounts of CO₂.

Previous results on the effects of hyperoxia-hypercapnia on retinal blood flow are highly contradictory. The present study, which was the first to monitor retinal haemodynamic parameters continuously during a 10 minute inhalation period, precludes that this is the result of the time course of retinal blood flow during combined O₂ and CO₂ breathing. Our results confirm the findings of Deutsch *et al*¹⁰ and Pakola *et al*.¹² However, in contrast with these previous findings our study was double masked and investigated a dose-response relation. The results

of other studies, however, indicated retinal vasodilation during carbogen breathing. Arend and co-workers¹³ observed an increase in mean dye velocity and a reduction in arteriovenous passage time in the human retina by using video fluorescein angiography. While this may be indicative for increased retinal blood speed it does not indicate an increase in retinal blood flow during pronounced vasoconstriction. Other investigators implied retinal vasoconstriction based on the observation that 8% CO₂ + 92% O₂ reduced resistance index as assessed with colour Doppler imaging,¹⁴ but we have recently shown that this index is not an adequate measure of vascular resistance in the retina.²⁰ Finally, an increase in retinal blood flow during carbogen breathing has been proposed from measurements using the blue field entoptic technique.¹¹ One has to be careful, however, to draw any conclusions on retinal blood flow from this study, because leucocyte rather than erythrocyte movement is assessed with this technique. Our results are, however, compatible with the idea that hypercapnia without hyperoxia induces vasodilation as evidenced from a variety of studies.^{21, 22}

Interpreting our results during 8% CO₂ + 92% O₂ breathing one has to consider the increase in blood pressure during this stimulus. Whereas the retina is autoregulated over a wide range of perfusion pressures,^{23–25} hypercapnia may narrow the autoregulatory plateau as it does in the brain.²⁶ Hence, the increase in ocular perfusion pressure during 8% CO₂ + 92% O₂ may well contribute to the response in retinal blood flow during this stimulus.

A technical limitation of the present study is that red blood cell velocity can not be recorded simultaneously with vessel diameter because these parameters are evaluated by two different methods. Hence, we have measured retinal vessel diameter and retinal blood velocity on two different study days. At both study days the same subjects were studied and baseline conditions were comparable. In addition, comparable effects of the gas mixtures on systemic haemodynamics were achieved. To obtain adequate results with the methods employed, it is necessary that the subjects under study are concentrated and collaborate with the instructions of the investigator. As breathing higher carbon dioxide concentrations is exhausting, it was an advantage to schedule two study days in the present study.

The present study may be of interest with respect to the mechanism underlying hyperoxia induced vasoconstriction and indicates that alkalosis does not have a major role. On the one hand the effects of a more than fourfold elevation in pO₂ levels on pH were small, whereas pronounced vasoconstriction in the retina was achieved. On the other hand adding CO₂ to the inhalate induces acidosis but does not abolish hyperoxia induced retinal blood flow changes. In other words the degree of vasoconstriction was not coupled with changes in pH in the present study. This indicates that during systemic hyperoxia potent vasoconstrictors are produced in the retina, which clearly dominate the regulatory potential of acidosis.

These results, however, do not preclude that extravascular acidosis in the retina may counteract hyperoxia induced vasoconstriction. In the present study we measured blood gas values from capillary blood samples of the arteriatised earlobe and there is no reason to assume that pO₂, pCO₂, and pH differ largely between arteries in the ear and in the eye. There is, however, evidence that the pH of retinal tissue may considerably differ from intravascular pH in retina arteries. During normocapnia retinal pH in the pig is more acidic (7.22) than arterial pH (7.44) as evidenced by studies using microelectrodes.²⁷ CO₂ was considered the main factor affecting retinal pH in these experiments, because it easily diffused through retinal vessels to the locations where it is hydrolysed by carbonic anhydrase. In the same experiments it was also observed that the first part of the response slope to CO₂ breathing was steep and linear (after 3–4 minutes) reaching a plateau after 8–10 minutes. This indicates that the selected

duration of the breathing periods in our study was adequate to reach steady state conditions. Hence, the present data indicate that intravascular pH is not an important factor in mediating oxygen induced vasoconstriction in the human retina. Whether this is also true for pH in the inner retina can not be answered from the present study, because of the obvious methodological limitations of human trials.

In conclusion, the present study shows that breathing of pure oxygen and oxygen in combination with carbon dioxide significantly decreases retinal blood flow. Based on our data we speculate that hyperoxia induced vasoconstriction is not due to changes in intravascular pH and cannot be counteracted by an intravascular increase in pCO₂.

ACKNOWLEDGEMENTS

Financial support for the Zeiss retinal vessel analyser by the Fonds zur Förderung der Wissenschaftlichen Forschung (FWF, project number P14262), Vienna, Austria, is gratefully acknowledged.

Authors' affiliations

A Luksch, G Garhöfer, A Imhof, E Polska, S Anzenhofer, M Wolzt, L Schmetterer, Department of Clinical Pharmacology, Vienna University, Austria

K Polak, G T Dörner, Department of Ophthalmology

L Schmetterer, Institute of Medical Physics

REFERENCES

- Riva CE, Grunwald JE, Sinclair SH. Laser Doppler velocimetry study of the effect of pure oxygen breathing on retinal blood flow. *Invest Ophthalmol Vis Sci* 1983;**24**:47–51.
- Fallon TJ, Maxwell D, Kohner EM. Measurement of autoregulation of retinal blood flow using the blue field entoptic phenomenon. *Trans Ophthalmol Soc UK* 1985;**104**:857–60.
- Eperon G, Johnson M, David NJ. The effect of arterial PO₂ on relative retinal blood flow in monkeys. *Invest Ophthalmol Vis Sci* 1975;**14**:342–52.
- Dallinger S, Dörner GT, Wenzel R, et al. Endothelin-1 contributes to hyperoxia-induced vasoconstriction in the human retina. *Invest Ophthalmol Vis Sci* 2000;**41**:864–9.
- Takagi C, King GL, Takagi H, et al. Endothelin-1 action via endothelin receptors is a primary mechanism modulating retinal circulatory response to hyperoxia. *Invest Ophthalmol Vis Sci* 1996;**37**:2099–109.
- Zhu Y, Park TS, Gidday JM. Mechanisms of hyperoxia-induced reductions in retinal blood flow in newborn pig. *Exp Eye Res* 1998;**67**:357–69.
- Haefliger IO, Chen Q, Anderson DR. Effect of oxygen on relaxation of retinal pericytes by sodium nitroprusside. *Graefes Arch Clin Exp Ophthalmol* 1997;**235**:388–92.
- Anderson DR, Davis EB. Glaucoma, capillaries and pericytes. 5. Preliminary evidence that carbon dioxide relaxes pericyte contractile tone. *Ophthalmologica* 1996;**210**:280–4.
- Chen Q, Anderson DR. Effect of CO₂ on intracellular pH and contraction of retinal capillary pericytes. *Invest Ophthalmol Vis Sci* 1997;**38**:643–51.
- Deutsch TA, Read JS, Ernest JT, et al. Effects of oxygen and carbon dioxide on the retinal vasculature in humans. *Arch Ophthalmol* 1983;**101**:1278–80.
- Sponsel WE, DePaul KL, Zetlan SR. Retinal hemodynamic effects of carbon dioxide, hyperoxia, and mild hypoxia. *Invest Ophthalmol Vis Sci* 1992;**33**:1864–9.
- Pakola SJ, Grunwald JE. Effects of oxygen and carbon dioxide on human retinal circulation. *Invest Ophthalmol Vis Sci* 1993;**34**:2866–70.
- Arend O, Harris A, Martin BJ, et al. Retinal blood velocities during carbogen breathing using scanning laser ophthalmoscopy. *Acta Ophthalmol (Copenh)* 1994;**72**:332–6.
- Schuttauf F, Cobet U, Klemenz A, et al. [Duplex ultrasound examinations of retinal circulation after inhalation of various mixed respiratory gases.] *Ophthalmologe* 1998;**95**:225–8.
- Stolley PD, Strom BL. Sample size calculations for clinical pharmacology studies. *Clin Pharmacol Ther* 1986;**39**:489–90.
- Riva CE, Grunwald JE, Sinclair SH, et al. Blood velocity and volumetric flow rate in human retinal vessels. *Invest Ophthalmol Vis Sci* 1985;**26**:1124–32.
- Riva C, Grunwald J, Sinclair S, et al. Fundus camera based LDV. *Appl Opt* 1981;**20**:117–20.
- Blum M, Bachmann K, Wintzer D, et al. Noninvasive measurement of the Bayliss effect in retinal autoregulation. *Graefes Arch Clin Exp Ophthalmol* 1999;**237**:296–300.
- Polak K, Dörner G, Kiss B, et al. Evaluation of the Zeiss retinal vessel analyser. *Br J Ophthalmol* 2000;**84**:1285–90.
- Polska E, Kircher K, Ehrlich P, et al. RI in central retinal artery as assessed by CDI does not correspond to retinal vascular resistance. *Am J Physiol Heart Circ Physiol* 2001;**280**:H1442–7.
- Chung HS, Harris A, Halter PJ, et al. Regional differences in retinal vascular reactivity. *Invest Ophthalmol Vis Sci* 1999;**40**:2448–53.
- Tsacopoulos M, David NJ. The effect of arterial PCO₂ on relative retinal blood flow in monkeys. *Invest Ophthalmol Vis Sci* 1973;**12**:335–47.
- Riva CE, Grunwald JE, Petrig BL. Autoregulation of human retinal blood flow. An investigation with laser Doppler velocimetry. *Invest Ophthalmol Vis Sci* 1986;**27**:1706–12.
- Robinson F, Riva CE, Grunwald JE, et al. Retinal blood flow autoregulation in response to an acute increase in blood pressure. *Invest Ophthalmol Vis Sci* 1986;**27**:722–6.
- Dumskyj MJ, Eriksen JE, Dore CJ, et al. Autoregulation in the human retinal circulation: assessment using isometric exercise, laser Doppler velocimetry, and computer-assisted image analysis. *Microvasc Res* 1996;**51**:378–92.
- Raichle ME, Plum F. Hyperventilation and cerebral blood flow. *Stroke* 1972;**3**:566–75.
- Tsacopoulos M, Levy S. Intraretinal acid-base studies using pH glass microelectrodes: effect of respiratory and metabolic acidosis and alkalosis on inner-retinal pH. *Exp Eye Res* 1976;**23**:495–504.