Retinal arteriolar diameter, blood velocity, and blood flow response to an isocapnic hyperoxic provocation

Edward D. Gilmore, Chris Hudson, David Preiss and Joe Fisher

Am J Physiol Heart Circ Physiol 288:H2912-H2917, 2005. First published 11 February 2005;
doi: 10.1152/ajpheart.01037.2004

Am J Physiol Heart Circ Physiol 288: H2912–H2917, 2005.
First published February 11, 2005; doi:10.1152/ajpheart.01037.2004.

Retinal arteriolar diameter, blood velocity, and blood flow response to an isocapnic hyperoxic provocation

Edward D. Gilmore,[1] Chris Hudson,[1] David Preiss,[2] and Joe Fisher[2]

[1]*Multi-Disciplinary Laboratory for the Research of Sight-Threatening Diabetic Retinopathy, Department of Ophthalmology and Vision Science, University of Toronto, and School of Optometry, University of Waterloo, Ontario; and* [2]*Department of Anesthesiology, Toronto General Hospital, Toronto, Ontario, Canada*

Submitted 12 October 2004; accepted in final form 8 February 2005

Gilmore, Edward D., Chris Hudson, David Preiss, and Joe Fisher. Retinal arteriolar diameter, blood velocity, and blood flow response to an isocapnic hyperoxic provocation. *Am J Physiol Heart Circ Physiol* 288: H2912–H2917, 2005. First published February 11, 2005; doi:10.1152/ajpheart.01037.2004.—The aim of this study was to simultaneously quantify the magnitude and response characteristics of retinal arteriolar diameter and blood velocity induced by an isocapnic hyperoxic provocation in a group of clinically normal subjects. The sample comprised 10 subjects (mean age, 25 yr; range, 21–40 yr). Subjects initially breathed air for 5–10 min, then breathed O_2 for 20 min, and then air for a final 10-min period via a sequential rebreathing circuit (Hi-Ox; Viasys) to maintain isocapnia. Retinal arteriolar diameter and blood velocity measurements were simultaneously acquired with a Canon laser blood flowmeter (CLBF-100). The response magnitude, time, and lag of diameter and velocity were calculated. In response to hyperoxic provocation, retinal diameter was reduced from control values of 111.6 (SD 13.1) to 99.8 (SD 10.6; $P < 0.001$) μm and recovered after withdrawal of hyperoxia. Retinal blood velocity and flow concomitantly declined from control values of 32.2 (SD 6.4) mm/s and 9.4 (SD 2.5) μl/min to 20.7 (SD 3.4) mm/s and 5.1 (SD 1.3) μl/min, respectively ($P < 0.001$ for both velocity and flow), and recovered after withdrawal of hyperoxia. The response times and response lags were not significantly different for each parameter between effect and recovery or between diameter and velocity. We conclude that arteriolar retinal vascular reactivity to hyperoxic provocation is rapid with a maximal vasoconstrictive effect occurring within a maximum of 4 min. Although there was a trend for diameter to respond before velocity to the isocapnic hyperoxic provocation, the response characteristics were not significantly different between diameter and velocity.

vascular reactivity; laser Doppler velocimetry; isocapnic hyperoxia

THE BLOOD SUPPLY TO THE INNER retina is derived from the central retinal artery, whereas the choriocapillaris supplies the outer retina and photoreceptors. The retinal tissue is one of the most metabolically active in the body and, correspondingly, an uninterrupted nutrient supply is essential (50). The inner retinal blood vessels (i.e., past the lamina cribrosa) are thought to be unique due to the absence of an autonomic nerve supply to regulate vascular tone (53). Blood supply to the inner retina is regulated via local feedback signals that alter retinal perfusion in response to changes in systemic blood pressure or the concentration of certain metabolites (11, 18). In particular, retinal blood flow is strongly dependent on the partial pressure of oxygen (P_{O_2}; Refs. 14, 25, 31, 42, 48).

The retinal vasculature can be noninvasively visualized and, consequently, its hemodynamic parameters quantified. Impairment of vascular reactivity has been demonstrated in the pathogenesis of various ocular diseases including diabetic retinopathy (13, 20, 25, 32). Administration of O_2 has previously been employed as a stimulus to provoke and assess the magnitude of the retinal vascular response. Vasoconstriction of retinal vessels (9, 24) and the resulting reduction of retinal hemodynamic parameters has been demonstrated using a variety of measurement techniques (4, 14, 22, 25, 26, 31, 33, 34, 37–39, 42, 43, 46, 48). However, none of these studies has utilized a technique that is capable of absolute quantification of retinal blood flow.

The aim of this study was to quantify the magnitude and response characteristics of retinal arteriolar diameter, blood velocity, and blood flow induced by a hyperoxic provocation in a group of clinically normal subjects. There are two unique aspects to this study. First, we used a technique that allows the simultaneous quantification of vessel diameter and centerline blood velocity to calculate retinal blood flow in microliters per minute. Second, we used a unique system validated in our laboratory (21, 45) to administer isocapnic hyperoxia. This overcomes the drawbacks of previous studies that were due to inadequate control of P_{CO_2} when hyperoxia was implemented. The precise sequence of hemodynamic events underlying retinal vascular reactivity can be elucidated by simultaneously investigating the changes in diameter and velocity relative to the onset of the stimulus and to one another.

MATERIALS AND METHODS

Sample. The sample comprised 10 clinically normal subjects (5 male and 5 female; mean age, 25; SD 6 yr). Only subjects who were 40 yr of age or younger and had no media opacities were included, i.e., those with nuclear opalescence <1, nuclear color <1, posterior subcapsular cataract <1, and cortical cataract <1 according to the Lens Opacity Classification System III (5). All subjects had a LogMAR (logarithm of the minimum angle of resolution) visual acuity of 0.00 or better. Subjects were excluded if they exhibited any eye disease, cardiovascular or respiratory disorders, a refractive error greater than ±6.00 diopters sphere or ±2.00 diopters cylinder, glaucoma, diabetes in a first-degree relative, or medications with known effects on blood flow (e.g., muscle relaxants or anticonvulsant or anti-inflammatory medications). None of the subjects smoked. All participants were asked to refrain from caffeine-containing drinks or snacks for at least 12 h before their study visit. The study was approved by the University of Waterloo Office of Research Ethics and

Address for reprint requests and other correspondence: C. Hudson, School of Optometry, Univ. of Waterloo, Waterloo, Ontario N2L 3G1, Canada (E-mail: chudson@scimail.uwaterloo.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

the University Health Network Research Ethics Board, Toronto. Informed consent was obtained from each subject after explanation of the nature and possible consequences of the study according to the tenets of the Declaration of Helsinki.

Gas-delivery system. The sequential rebreathing system comprised a fresh gas reservoir and an expiratory gas reservoir, each of which was connected to the patient with one-way valves. The inspiratory and expiratory limbs were interconnected by a single positive end-expiratory pressure valve that allowed exhaled gas to be rebreathed when the gas in the inspiratory limb was depleted. This system was assembled by adding a gas reservoir to the expiratory port of a commercial three-valve O_2 delivery system (Hi-Ox; Viasys Healthcare; Yorba Linda, CA). Flow from the gas tanks was controlled using standard rotometers as flowmeters. This method has been described in detail in previous publications (21, 45; Fig. 1).

Canon laser blood flowmeter. The principal underlying the Canon laser blood flowmeter (CLBF-100) is based on the Doppler effect. Laser light reflected from a moving particle is shifted in frequency (Δf) by an amount that is proportional to the velocity of the moving particle. A vessel that exhibits Poiseuille flow will have a range of velocities and thus a range of frequency shifts up to a maximum frequency shift (Δf_{max}), which corresponds to the maximum velocity of the blood moving at the center of the vessel (since resistance is developed at the vessel wall). Light scattered from stationary tissue is not shifted and acts as the reference frequency from which a relative change in retinal blood velocity is measured (16). With the use of two photomultipliers separated by a known angle, the maximum frequency shift is subtracted to allow the absolute quantification of centerline blood velocity irrespective of the angle between the moving particle and the reflected beam (15, 41). A red diode laser (675 nm, 80 × 50-μm oval) is used to measure velocity every 0.02 s across a 2-s measurement window, which results in a velocity-time trace. The CLBF-100 also uses a green diode vessel-tracking laser system (543 nm, 1,500 × 150-μm rectangle) that is used to stabilize and measure the diameter of the vessel of interest (7, 35). The vessel-tracking system stabilizes measurement-site position and allows rejection of velocity measurements that involve significant saccades. Diameter readings are acquired every 4 ms during the first and final 60 ms of the 2-s velocity-measurement window. Two sequential measurements using different optical paths (*paths 1* and *2*) are taken to ensure consistency and are averaged to yield one reading. In combination with the average velocity (V_{mean}) over a pulse cycle and diameter (D), flow through the vessel can be calculated as $\frac{1}{2} \times (\pi/4) \times V_{mean} \times 60 \times D^2$ [for technical summary, see Kida and co-workers (30)]. Magnification effects associated with refractive and axial components of ametropia are corrected to provide absolute measurements of diameter (in μm), velocity (in mm/s), and flow (in μl/min). The technological principles used in this device have been described in detail elsewhere (3, 15, 30, 41). In addition, this device has been extensively evaluated on clinically normal subjects (19, 23) and those with various types of retinal pathologies (30, 54).

Procedures. Each subject was seated for ≥5 min to allow stabilization of heart rate and blood pressure before measurements were started. An initial air-breathing period was employed to allow stabilization of baseline parameters, e.g., respiration rate, P_{O_2}, and P_{CO_2}. Retinal arteriolar diameter and centerline blood velocity measurements were simultaneously acquired from either the supero- or inferotemporal arteriole in one eye of each subject using the CLBF-100. A minimum of five baseline measurements were acquired while the subject breathed air (5–10 min). The isocapnic hyperoxic stimulus was then initiated and maintained for 20 min. Subsequently, air was readministered for an additional 10 min to maintain isocapnia at baseline levels. Retinal blood flow measurements were acquired every minute during the study.

Gas analysis and systemic responses. A rapid-response critical care gas analyzer (Cardiocap5; Datex-Ohmeda) was used to quantify the relative concentrations of O_2 and CO_2 in both the inspired and expired gases on a breath-by-breath basis. The relative O_2 and CO_2 concentrations were sampled continuously by the gas analyzer, whereas the inspired and end-tidal O_2 and CO_2 concentrations were downloaded to a personal computer every 5 s (S5 Collect software; Datex-Ohmeda). In addition, finger O_2 saturation, respiration rate, and pulse rate were also recorded continuously. The fractional concentration of O_2 in the expired breath ($F_{E_{O_2}}$) was chosen as the parameter that most closely reflects the change in arterial P_{O_2}. Gas data were analyzed using box plots that depicted the median, upper 25th and lower 75th percentiles, and outliers of end-tidal gas concentrations. Data points lying outside the upper 25th and lower 75th percentiles were excluded from the analysis, because all of these values were found to be erroneous; i.e., these points resulted from inappropriate interpretation of tidal waveforms by the gas monitor. Blood pressure was measured noninvasively once every 3 min during the experiment (Cardiocap5).

Function fitting. Arteriolar diameter and velocity data were fit using a double sigmoidal function of the form

$$y = ((t < 20) \times \{[(\alpha - \beta)/(1 + \eta^{t-\gamma})] + \beta\}) + ((t > 20) \times \{[(\beta - \delta)/(1 + \theta^{t-\varepsilon})] + \delta\})$$

where y is the magnitude of the hemodynamic parameter (i.e., diameter, velocity, or flow) at a certain time (t) from the initial measurement ($t = 0$). An arbitrary time point ($t = 20$; i.e., approximately midway through the procedure) was used to divide the data into two

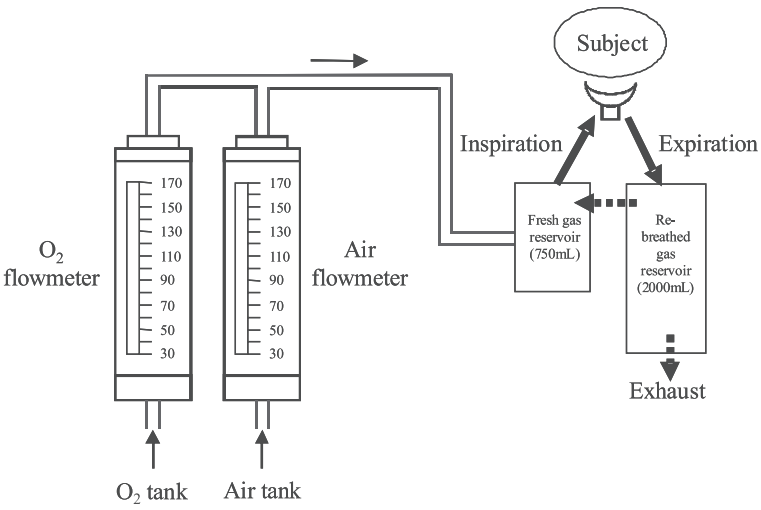

Fig. 1. Components of the sequential rebreathing system.

sections. The exponents η and θ were constrained so that the inflexion points of the function could not occur before the O_2 had been turned on (effect phase) or before the O_2 had been turned off (recovery phase), respectively. For $t < 20$, α and β are the upper and lower asymptotes, respectively; γ is the value of t that corresponds to a value halfway between α and β; i.e., the midpoint of the effect phase of the function. For $t > 20$, β is set as the lower asymptote and δ is the upper asymptote (independent of α); ϵ is the value of t that corresponds to a value halfway between β and δ; i.e., the midpoint of the recovery phase of the function. The values for α, β, γ, δ, and ϵ were varied using the "nonlinear regression" module in Statistica software (StatSoft) to produce a least-squares fit. As a result, the same mathematical model was used for all subjects and all hemodynamic parameters, but the coefficients of the model varied between subjects and between hemodynamic parameters of a given subject. An example of the function fitting is shown in Fig. 2.

Goodness of fit (r value) was determined. Fitted functions with r values <0.6 were excluded from the analysis. The velocity data from two subjects and diameter data from one subject were not included in the analysis due to either a low r value (<0.60) or inappropriate fit of the data. The magnitudes of the retinal vascular responses of diameter and velocity were calculated (i.e., α − β for the effect phase and δ − β for the recovery phase). The time interval between the 5th and 95th percentiles of the changes in diameter and velocity were quantified and are referred to as the retinal vascular "response time." The time interval between the onset (or cessation) of the hyperoxic stimulus and the midpoint of the effect (i.e., γ) or recovery (i.e., ϵ) phase of the function was determined and is referred to as the "response lag."

RESULTS

There were abrupt reductions in vessel diameter, blood velocity, and blood flow on initiation of hyperoxia. All parameters returned to control levels when hyperoxia was discontinued (Figs. 2 and 3). The group mean retinal arteriolar diameter was 111.6 (SD 13.1; range, 85–129) μm before isocapnic hyperoxic provocation; it decreased to 99.8 (SD 10.6) μm during provocation (two-tailed paired t-test; $P < 0.001$) and recovered to 109.9 (SD 11.7) μm on removal of the stimulus (Fig. 3). The group mean retinal blood velocity was 32.2 (SD 6.4; range, 22–42) mm/s before provocation; it decreased to 20.7 (SD 3.4) mm/s during provocation ($P < 0.001$) and recovered to 33.3 (SD 5.0) mm/s (Fig. 3). The group mean retinal blood flow was 9.4 (SD 2.5; range, 5.4–13.4) μl/min before provocation; it decreased to 5.1 (SD 1.3) μl/min during provocation ($P = 0.001$) and recovered to 9.2 (SD 1.7) μl/min.

The group mean response times of diameter were 2.37 (SD 0.46) and 2.22 (SD 0.60) min for the effect (i.e., after initiation of hyperoxia) and recovery (i.e., after cessation of hyperoxia) phases, respectively (Fig. 3). The group mean response times of velocity were 2.68 (SD 0.55) and 2.55 (SD 0.53) min for the effect and recovery phases, respectively (Fig. 3). The group mean response times of flow were 2.54 (SD 0.56) and 2.28 (SD 0.43) min for the effect and recovery phases, respectively (Fig. 3). The response times during the effect phase were not significantly different from the response times during the recovery phase for both diameter and velocity (two-tailed paired t-test). Also there was no significant difference between the response times of diameter vs. velocity.

The group mean response lags of diameter were 2.00 (SD 1.07) and 1.38 (SD 0.45) min for the effect and recovery phases, respectively. The group mean response lags of velocity were 2.60 (SD 1.19) and 2.29 (SD 0.87) min for the effect and recovery phases, respectively. The group mean response lags

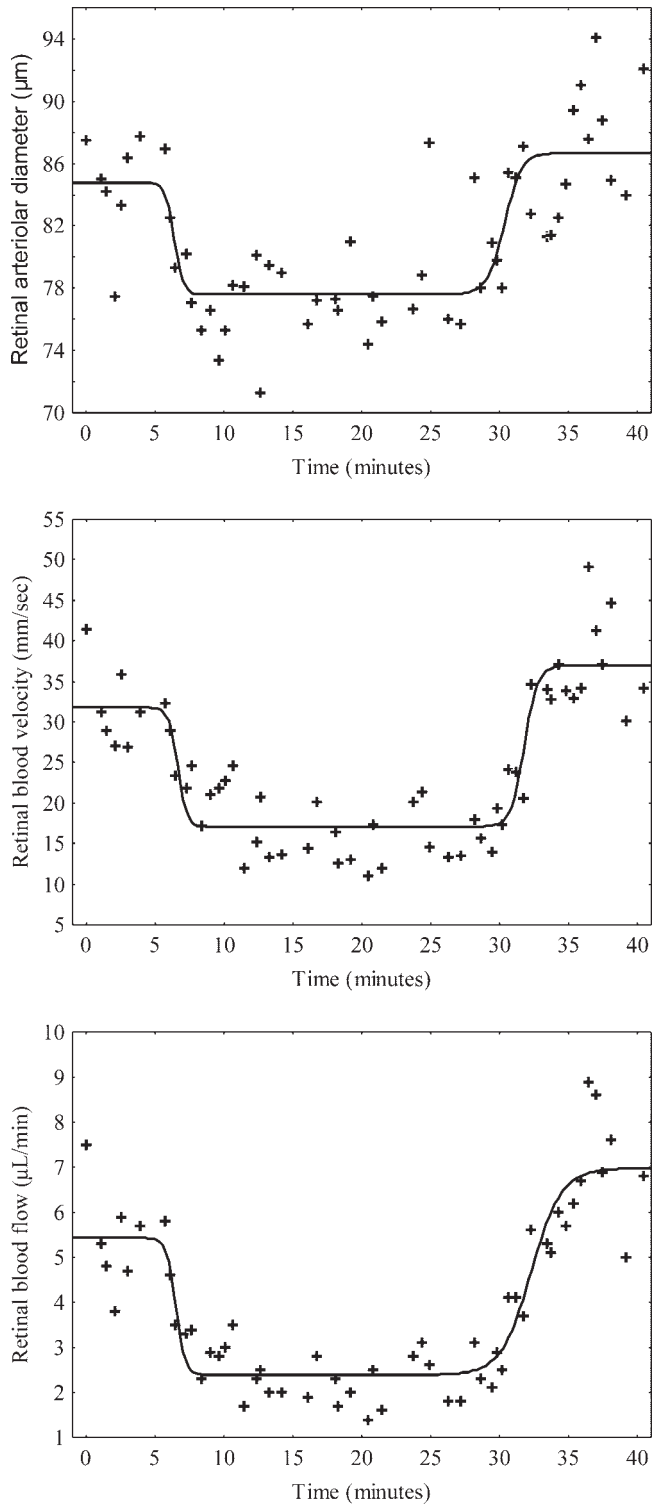

Fig. 2. Changes for a single participant in retinal arteriolar diameter (*top*), blood velocity (*middle*), and blood flow (*bottom*) induced by isocapnic hyperoxia delivered using the sequential rebreathing circuit. Data were fit using a sigmoidal function. The r values for the fitted functions were 0.77, 0.89, and 0.92 for diameter, velocity, and flow, respectively. Concentrations of expired CO_2 were 4.53, 4.47, and 4.30% during initial air, O_2, and final air breathing periods, respectively.

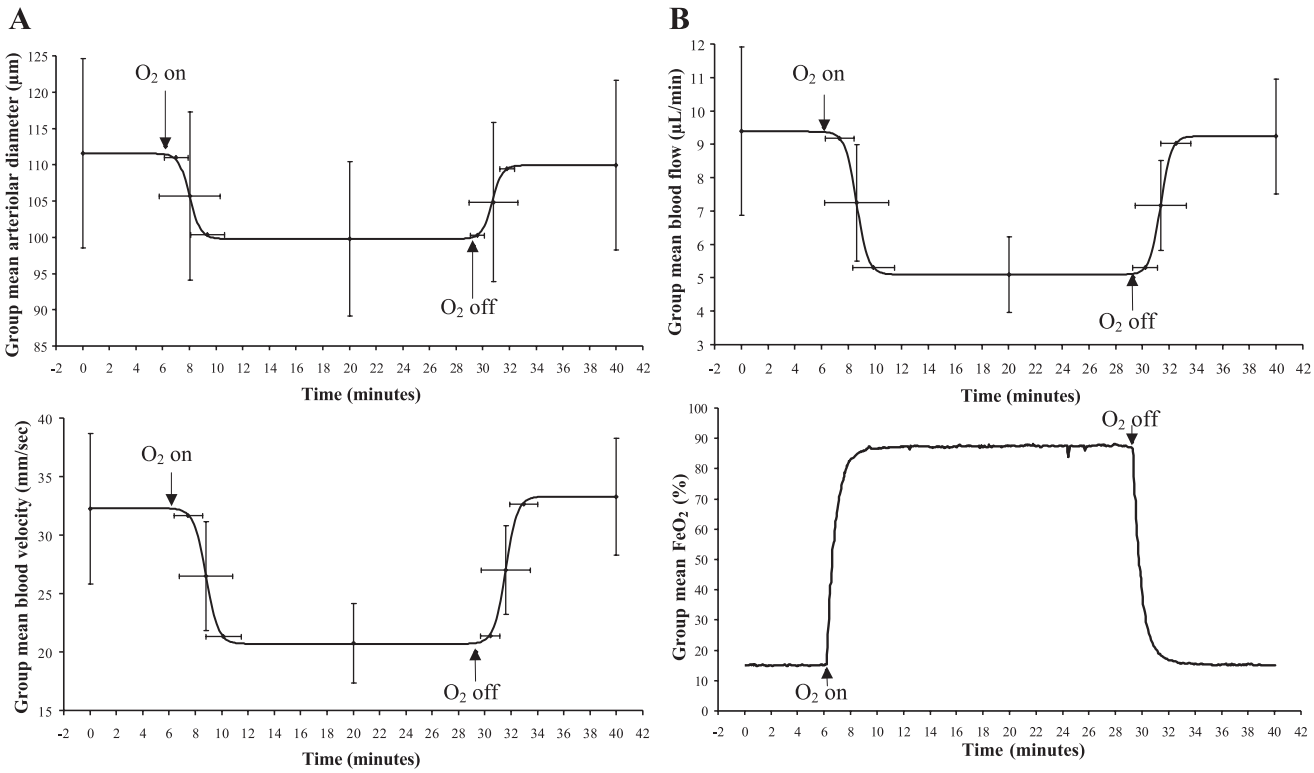

Fig. 3. *A*: group mean magnitudes of retinal arteriolar diameter (*top*) and blood velocity (*bottom*) before, during, and after isocapnic hyperoxic provocation. *B*: group mean magnitudes of retinal blood flow (*top*) and fractional concentration of O_2 in expired gas (FeO_2; *bottom*) before, during, and after isocapnic hyperoxic provocation. "O_2 on" and "O_2 off" points are coincident for all participants. Diameter, velocity, and flow data were fit using a sigmoidal function. Time points detailed include (from *left* to *right*) group mean baseline magnitude, 5% point, midpoint, and 95% point (effect function); group mean magnitude during hyperoxia, 5% point, midpoint, and 95% point (recovery function); and final group mean magnitude. Error bars represent ±1 SD.

of flow were 2.44 (SD 1.32) and 2.03 (SD 1.02) min for the effect and recovery phases, respectively. The response lags during the effect phase were not significantly different from the response lags during the recovery phase for both diameter and velocity (two-tailed paired *t*-test). The diameter response lags were not significantly different than the velocity response lags for the effect and recovery phases.

A correction factor was calculated because the change in arterial P_{O_2} was not a square wave, i.e., the time from the onset (or cessation) of O_2 until 50% of the observed change in $F_{E_{O_2}}$ had taken place. The group mean correction factors were 0.50 min for effect and 0.49 min for recovery and were not significantly different. The magnitude of the correction factor relates to group mean response lags of 1.7 min for diameter and 2.6 min for velocity (means of effect and recovery phases).

The group mean *r* values for diameter and velocity of the fitted functions were 0.843 and 0.700, respectively.

The inspired and end-tidal gas parameters and relevant systemic measures for initial air, isocapnic hyperoxia, and final air are detailed in Table 1. Only heart rate, fractional inspired CO_2 and O_2 fractions ($F_{I_{CO_2}}$ and $F_{I_{O_2}}$, respectively), and $F_{E_{O_2}}$ values changed significantly as a result of the hyperoxic provocation. The group mean values for mean arterial blood pressure [2/3(diastolic BP) + 1/3(systolic BP)] were 81.7 (SD 10.1) before hyperoxic provocation, 80.9 (SD 6.0) during provocation, and 81.8 (SD 7.9) mmHg after provocation. There were no significant differences in mean arterial pressure across the stimulus conditions.

DISCUSSION

Retinal blood flow varies inversely with arterial P_{O_2} to maintain retinal oxygenation at a relatively constant level (25, 51) and also varies directly with arterial P_{CO_2} (34). Numerous studies have investigated retinal vascular reactivity using a hyperoxic stimulus. Some have investigated changes of retinal vessel diameter (9, 24, 26), whereas others have measured changes in aspects of hemodynamics using a variety of tech-

Table 1. *Parameter values measured before and after hyperoxic provocation*

Breathing Sequence	O_2, %		CO_2, %		Respiration Rate, breaths/min	Heart Rate, beats/min	Blood O_2 Saturation, %
	Inspired	End Tidal	Inspired	End Tidal			
Air, 5–10 min	19.84±0.34	15.10±0.58	0.62±0.31	5.10±0.50	17.22±1.59	67.30±7.81	97.80±1.46
O_2, 20 min	91.69±7.90	86.23±3.29	0.90±0.57	4.98±0.45	17.61±2.45	63.99±6.48	98.38±1.05
Air, 10 min	19.88±0.43	16.25±0.53	0.67±0.38	5.00±0.46	17.40±2.79	68.89±8.16	97.72±2.11

Values are group means ± SD; $n = 10$ subjects.

niques (4, 14, 25, 33, 43, 46, 48). Alternatively, others have measured retinal vessel diameter and separately employed a bidirectional laser Doppler system to measure centerline retinal blood velocity to calculate flow (22, 31, 34, 37–39, 42). The technique used in this study measured centerline retinal blood velocity using a bidirectional photodetector and *simultaneously* acquired retinal vessel diameter measurements for the absolute quantification of retinal blood flow. All of these studies have employed 100% O_2 or coadministered O_2 (>90%) and CO_2 (~5%). None has used a truly isocapnic hyperoxic stimulus. Administration of enriched O_2 concentrations typically results in the reduction of arterial P_{CO_2} (1). The change of end-tidal CO_2 concentration (the maximum concentration of CO_2 during each expiration) reflects the change in arterial P_{CO_2} (40). The method of gas delivery used in this study minimized alterations in systemic P_{CO_2} concentrations (45). We have previously shown (21) that this delivery system results in stabilization of end-tidal CO_2 during hyperoxic provocation and thereby isolates the retinal vascular reactivity response to O_2 alone. Additionally, a recent report (17) has demonstrated separate vasoconstrictive effects of hyperoxia (i.e., O_2 mediated) and arterial hypocapnia (i.e., CO_2 mediated) in the cerebral vasculature.

In general, previous studies using 100% O_2 to assess retinal vascular reactivity have tended to find a greater magnitude of vasoconstriction, which we attribute to a compounded effect of elevated arterial O_2 and reduced CO_2 (22, 37, 38, 42). Most of these studies have measured vascular reactivity in venules, possibly because the derived velocity profile was nonpulsatile. Arterioles were used in this study because they are thought to be primarily responsible for the vascular reactivity response and to obey Poiseuille flow principles to a greater extent (given their more circular cross section).

In three previously published studies (22, 24, 34), researchers have investigated the time course of the changes in diameter or velocity using a hyperoxic stimulus (referred to in this report as response time and response lag). To the best of our knowledge, this is the first time that the response characteristics of arteriolar diameter and blood velocity have been simultaneously quantified due to hyperoxic provocation. In addition, the characteristics of the effect and recovery phases (i.e., after onset and cessation of the hyperoxic stimulus, respectively) have not been investigated concomitantly. The response characteristics of the retinal arterioles reported in this study are comparable to those of previous studies. There was a trend for diameter changes to occur before velocity responses to the hyperoxic stimulus, but neither the response time nor the response lag values were significantly different between diameter and velocity. Nagaoka and co-workers (36) found that retinal arteriolar velocity responded ~1.3 min before diameter in response to cold pressor provocation. When considered with the results reported in this report, different response characteristics of the retinal vasculature to transmural pressure-mediated autoregulation as opposed to metabolic-mediated vascular reactivity are suggested.

The changes in arterial P_{O_2} were not square waves, and as a result, a correction factor was calculated to compensate for this effect. A finite time is required for O_2 to reach the retinal vasculature owing to physiological delay of gas exchange in the lungs and lung-to-eye circulation time. The corrected response lag was therefore the measured response lag of diameter and velocity reported above, less the influence of the correction factor. Nevertheless, the impact of the correction factor does not influence the differential relationship between diameter and velocity.

Homeostatic O_2 supply is primarily maintained during hyperoxia by a reduction in vessel diameter (51), although the exact governing mechanism has yet to be fully elucidated. Microelectrode animal studies indicate that inner retinal P_{O_2} is well regulated during hyperoxia (52). Various biochemical factors that may be responsible for retinal hyperoxia-induced vasoconstriction include endothelin-1 (6, 27, 29, 49, 57) and prostanoids (55). In addition, other mechanisms have been investigated in the cerebral vasculature that involve superoxide generation and nitric oxide (2, 8, 44, 56) or red blood cell physiological changes (10, 12, 28, 47).

In summary, this study is novel in that it used an isocapnic hyperoxic stimulus to provoke retinal vascular reactivity. Previous studies have been unable to avoid a concomitant reduction in P_{CO_2} during hyperoxia. In addition, the measurement technique used to assess retinal hemodynamics provided the unique ability to simultaneously quantify retinal blood velocity and vessel diameter for the absolute quantification of retinal blood flow. Although there was a trend for diameter to respond before velocity to the hyperoxic stimulus, neither the response times nor the response lag values were significantly different between diameter and velocity.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Trefford Simpson for help with the function-fitting technique.

GRANTS

This work was funded by an Operating Grant and a New Investigator Award from the Canadian Institutes of Health Research and a Premier's Research Excellence Award (to C. Hudson).

REFERENCES

1. **Becker HF, Polo O, McNamara SG, Berthon-Jones M, and Sullivan CE.** Effect of different levels of hyperoxia on breathing in healthy subjects. *J Appl Physiol* 81: 1683–1690, 1996.
2. **Boveris A and Chance B.** The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J* 134: 707–716, 1973.
3. **Brein KR and Riva CE.** Laser Doppler velocimetry measurement of pulsatile blood flow in capillary tubes. *Microvasc Res* 24: 114–118, 1982.
4. **Chung HS, Harris A, Halter PJ, Kagemann L, Roff EJ, Garzozi HJ, Hosking SL, and Martin BJ.** Regional differences in retinal vascular reactivity. *Invest Ophthalmol Vis Sci* 40: 2448–2453, 1999.
5. **Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, Friend J, McCarthy D, and Wu SY.** The lens opacities classification system. III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol* 111: 831–836, 1993.
6. **Dallinger S, Dorner GT, Wenzel R, Graselli U, Findl O, Eichler HG, Wolzt M, and Schmetterer L.** Endothelin-1 contributes to hyperoxia-induced vasoconstriction in the human retina. *Invest Ophthalmol Vis Sci* 41: 864–869, 2000.
7. **Delori FC, Fitch KA, Feke GT, Deupree DM, and Weiter JJ.** Evaluation of micrometric and microdensitometric methods for measuring the width of retinal vessel images on fundus photographs. *Graefes Arch Clin Exp Ophthalmol* 226: 393–399, 1988.
8. **Demchenko IT, Oury TD, Crapo JD, and Piantadosi CA.** Regulation of the brain's vascular responses to oxygen. *Circ Res* 91: 1031–1037, 2002.
9. **Deutsch TA, Read JS, Ernest JT, and Goldstick TK.** Effects of oxygen and carbon dioxide on the retinal vasculature in humans. *Arch Ophthalmol* 101: 1278–1280, 1983.

10. **Dietrich HH, Ellsworth ML, Sprague RS, and Dacey RG Jr.** Red blood cell regulation of microvascular tone through adenosine triphosphate. *Am J Physiol Heart Circ Physiol* 278: H1294–H1298, 2000.
11. **Dumskyj MJ, Eriksen JE, Dore CJ, and Kohner EM.** Autoregulation in the human retinal circulation: assessment using isometric exercise, laser Doppler velocimetry, and computer-assisted image analysis. *Microvasc Res* 51: 378–392, 1996.
12. **Ellsworth ML.** The red blood cell as an oxygen sensor: what is the evidence? *Acta Physiol Scand* 168: 551–559, 2000.
13. **Evans DW, Harris A, Danis RP, Arend O, and Martin BJ.** Altered retrobulbar vascular reactivity in early diabetic retinopathy. *Br J Ophthalmol* 81: 279–282, 1997.
14. **Fallon TJ, Maxwell D, and Kohner EM.** Retinal vascular autoregulation in conditions of hyperoxia and hypoxia using the blue field entoptic phenomenon. *Ophthalmology* 92: 701–705, 1985.
15. **Feke GT, Goger DG, Tagawa H, and Delori FC.** Laser Doppler technique for absolute measurement of blood speed in retinal vessels. *IEEE Trans Biomed Eng* 34: 673–680, 1987.
16. **Feke GT and Riva CE.** Laser Doppler measurements of blood velocity in human retinal vessels. *J Opt Soc Am A* 68: 526–531, 1978.
17. **Floyd TF, Clark JM, Gelfand R, Detre JA, Ratcliffe S, Guvakov D, Lambertsen CJ, and Eckenhoff RG.** Independent cerebral vasoconstrictive effects of hyperoxia and accompanying arterial hypocapnia at 1 ATA. *J Appl Physiol* 95: 2453–2461, 2003.
18. **Funk RH.** Blood supply of the retina. *Ophthalmic Res* 29: 320–325, 1997.
19. **Garcia JP Jr, Garcia PT, and Rosen RB.** Retinal blood flow in the normal human eye using the canon laser blood flowmeter. *Ophthalmic Res* 34: 295–299, 2002.
20. **Gasser P, Flammer J, Guthauser U, and Mahler F.** Do vasospasms provoke ocular diseases? *Angiology* 41: 213–220, 1990.
21. **Gilmore ED, Hudson C, Venkataraman ST, Preiss D, and Fisher J.** Comparison of different hyperoxic paradigms to induce vasoconstriction: implications for the investigation of retinal vascular reactivity. *Invest Ophthalmol Vis Sci* 45: 3207–3212, 2004.
22. **Grunwald JE, Riva CE, Petrig BL, Sinclair SH, and Brucker AJ.** Effect of pure O_2-breathing on retinal blood flow in normals and in patients with background diabetic retinopathy. *Curr Eye Res* 3: 239–241, 1984.
23. **Guan K, Hudson C, and Flanagan JG.** Variability and repeatability of retinal blood flow measurements using the Canon Laser Blood Flowmeter. *Microvasc Res* 65: 145–151, 2003.
24. **Hague S, Hill DW, and Crabtree A.** The calibre changes of retinal vessels subject to prolonged hyperoxia. *Exp Eye Res* 47: 87–96, 1988.
25. **Harris A, Arend O, Kopecky K, Caldemeyer K, Wolf S, Sponsel W, and Martin B.** Physiological perturbation of ocular and cerebral blood flow as measured by scanning laser ophthalmoscopy and color Doppler imaging. *Surv Ophthalmol Suppl* 38: 81–86, 1994.
26. **Hickam JB and Frayser RP.** Studies of the retinal circulation in man: observations on vessel diameter, arteriovenous oxygen difference, and mean circulation time. *Circulation* 33: 302–316, 1966.
27. **Higgins RD, Hendricks-Munoz KD, Caines VV, Gerrets RP, and Rifkin DB.** Hyperoxia stimulates endothelin-1 secretion from endothelial cells; modulation by captopril and nifedipine. *Curr Eye Res* 17: 487–493, 1998.
28. **Jia L, Bonaventura C, Bonaventura J, and Stamler JS.** *S*-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* 380: 221–226, 1996.
29. **Kawamura H, Oku H, Li Q, Sakagami K, and Puro DG.** Endothelin-induced changes in the physiology of retinal pericytes. *Invest Ophthalmol Vis Sci* 43: 882–888, 2002.
30. **Kida T, Harino S, Sugiyama T, Kitanishi K, Iwahashi Y, and Ikeda T.** Change in retinal arterial blood flow in the contralateral eye of retinal vein occlusion during glucose tolerance test. *Graefes Arch Clin Exp Ophthalmol* 240: 342–347, 2002.
31. **Kiss B, Polska E, Dorner G, Polak K, Findl O, Mayrl GF, Eichler HG, Wolzt M, and Schmetterer L.** Retinal blood flow during hyperoxia in humans revisited: concerted results using different measurement techniques. *Microvasc Res* 64: 75–85, 2002.
32. **Kohner EM, Patel V, and Rassam SM.** Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. *Diabetes* 44: 603–607, 1995.
33. **Langhans M, Michelson G, and Groh MJ.** Effect of breathing 100% oxygen on retinal and optic nerve head capillary blood flow in smokers and non-smokers. *Br J Ophthalmol* 81: 365–369, 1997.
34. **Luksch A, Garhofer G, Imhof A, Polak K, Polska E, Dorner GT, Anzenhofer S, Wolzt M, and Schmetterer L.** Effect of inhalation of different mixtures of O_2 and CO_2 on retinal blood flow. *Br J Ophthalmol* 86: 1143–1147, 2002.
35. **Milbocker MT, Feke GT, and Goger DG.** Laser Doppler velocimetry stabilized in one dimension. *IEEE Trans Biomed Eng* 38: 928–930, 1991.
36. **Nagaoka T, Mori F, and Yoshida A.** Retinal artery response to acute systemic blood pressure increase during cold pressor test in humans. *Invest Ophthalmol Vis Sci* 43: 1941–1945, 2002.
37. **Pakola SJ and Grunwald JE.** Effects of oxygen and carbon dioxide on human retinal circulation. *Invest Ophthalmol Vis Sci* 34: 2866–2870, 1993.
38. **Polska E, Kircher K, Ehrlich P, Vecsei PV, and Schmetterer L.** RI in central retinal artery as assessed by CDI does not correspond to retinal vascular resistance. *Am J Physiol Heart Circ Physiol* 280: H1442–H1447, 2001.
39. **Rassam SM, Patel V, Chen HC, and Kohner EM.** Regional retinal blood flow and vascular autoregulation. *Eye* 10: 331–337, 1996.
40. **Rhoades R.** Respiratory physiology: gas transfer and transport. In: *Medical Physiology*, edited by Rhoades R and Tanner G. Boston, MA: Little, Brown, 1992, p. 386–398.
41. **Riva CE.** Bidirectional LDV system for absolute measurement of blood speed in retinal vessels. *Appl Opt* 18: 2301–2306, 1979.
42. **Riva CE, Grunwald JE, and Sinclair SH.** Laser Doppler velocimetry study of the effect of pure oxygen breathing on retinal blood flow. *Invest Ophthalmol Vis Sci* 24: 47–51, 1983.
43. **Roff EJ, Harris A, Chung HS, Hosking SL, Morrison AM, Halter PJ, and Kagemann L.** Comprehensive assessment of retinal, choroidal and retrobulbar haemodynamics during blood gas perturbation. *Graefes Arch Clin Exp Ophthalmol* 237: 984–990, 1999.
44. **Rubanyi GM and Vanhoutte PM.** Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol Heart Circ Physiol* 250: H822–H827, 1986.
45. **Sommer LZ, Iscoe S, Robicsek A, Kruger J, Silverman J, Rucker J, Dickstein J, Volgyesi GA, and Fisher JA.** A simple breathing circuit minimizing changes in alveolar ventilation during hyperpnoea. *Eur Respir J* 12: 698–701, 1998.
46. **Sponsel WE, DePaul KL, and Zetlan SR.** Retinal hemodynamic effects of carbon dioxide, hyperoxia, and mild hypoxia. *Invest Ophthalmol Vis Sci* 33: 1864–1869, 1992.
47. **Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, and Piantadosi CA.** Blood flow regulation by *S*-nitrosohemoglobin in the physiological oxygen gradient. *Science* 276: 2034–2037, 1997.
48. **Strenn K, Menapace R, Rainer G, Findl O, Wolzt M, and Schmetterer L.** Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in PO_2. *Br J Ophthalmol* 81: 360–364, 1997.
49. **Takagi C, King GL, Takagi H, Lin YW, Clermont AC, and Bursell SE.** Endothelin-1 action via endothelin receptors is a primary mechanism modulating retinal circulatory response to hyperoxia. *Invest Ophthalmol Vis Sci* 37: 2099–2109, 1996.
50. **Vanderkooi JM, Erecinska M, and Silver IA.** Oxygen in mammalian tissue: methods of measurement and affinities of various reactions. *Am J Physiol Cell Physiol* 260: C1131–C1150, 1991.
51. **Vucetic M, Jensen PK, and Jansen EC.** Diameter variations of retinal blood vessels during and after treatment with hyperbaric oxygen. *Br J Ophthalmol* 88: 771–775, 2004.
52. **Wangsa-Wirawan ND and Linsenmeier RA.** Retinal oxygen: fundamental and clinical aspects. *Arch Ophthalmol* 121: 547–557, 2003.
53. **Ye XD, Laties AM, and Stone RA.** Peptidergic innervation of the retinal vasculature and optic nerve head. *Invest Ophthalmol Vis Sci* 31: 1731–1737, 1990.
54. **Yoshida A, Feke GT, Mori F, Nagaoka T, Fujio N, Ogasawara H, Konno S, and McMeel JW.** Reproducibility and clinical application of a newly developed stabilized retinal laser Doppler instrument. *Am J Ophthalmol* 135: 356–361, 2003.
55. **Yu DY, Su EN, Cringle SJ, Schoch C, Percicot CP, and Lambrou GN.** Comparison of the vasoactive effects of the docosanoid unoprostone and selected prostanoids on isolated perfused retinal arterioles. *Invest Ophthalmol Vis Sci* 42: 1499–1504, 2001.
56. **Zhilyaev SY, Moskvin AN, Platonova TF, Gutsaeva DR, Churilina IV, and Demchenko IT.** Hyperoxic vasoconstriction in the brain is mediated by inactivation of nitric oxide by superoxide anions. *Neurosci Behav Physiol* 33: 783–787, 2003.
57. **Zhu Y, Park TS, and Gidday JM.** Mechanisms of hyperoxia-induced reductions in retinal blood flow in newborn pig. *Exp Eye Res* 67: 357–369, 1998.