Retinal Arteriolar Diameter, Blood Velocity, and Blood Flow Response to an Isocapnic Hyperoxic Provocation in Early Sight-Threatening Diabetic Retinopathy

Edward D. Gilmore,1,2 Chris Hudson,1,2 Ravi K. Nrusimbadevara,1,2 Patricia T. Harvey,1 Mark Mandelcorn,1 Wai Ching Lam,1 and Robert G. Devenyi1

PURPOSE. To quantify the magnitude of retinal arteriolar vascular reactivity in diabetic patients stratified by severity of retinopathy and in age-matched control subjects. The sample comprised 21 nondiabetic control subjects (group 1), 19 patients with no clinically visible DR (group 2), 19 patients with mild-to-moderate nonproliferative DR and without clinically evident diabetic macular edema (DME) (group 3), and 17 patients with DME (group 4).

METHODS. Subjects initially breathed air, followed by oxygen, while isocapnia was maintained. Retinal arteriolar diameter and blood velocity measurements were acquired simultaneously.

RESULTS. Changes in blood velocity and wall shear rate (WSR) were significantly less in groups 3 and 4 (P < 0.0001 and P = 0.0002, respectively) than in groups 1 and 2. Change in blood flow was significantly less in group 4 (P < 0.004) than in groups 1 and 2. The change in maximum-to-minimum (max:min) ratio was significantly less in groups 2 and 4 than in group 1 (P = 0.001). There was a significant relationship between baseline objective edema indices and vascular reactivity. The magnitude of vascular reactivity in response to isocapnic hyperoxia was reduced in those individuals with clinically evident DR relative to subjects without diabetes.

CONCLUSIONS. The differences in vascular reactivity occurred in the absence of any difference in baseline hemodynamic values. Vascular reactivity is impaired in early sight-threatening DR, and this impairment is related to the objectively defined magnitude of retinal edema. (Invest Ophthalmol Vis Sci. 2007;48: 1744–1750) DOI:10.1167/iovs.06-1016

The inner retinal blood vessels are thought to be unique due to the absence of an autonomic nerve supply to initiate changes in vascular tone.1 Blood supply to the inner retina is regulated via local feedback signals that alter retinal perfusion.2–3 Vasocostriction of retinal vessels in response to hyperoxia4–6 and the resultant reduction of parameters that reflect flow have been demonstrated using a variety of measurement techniques.4,6–10 Vascular reactivity represents the response of the vasculature to a given stimulus, such as hyperoxia.4 Impairment of retinal vascular reactivity to hyperoxia in diabetic retinopathy, as reflected by a reduced hemodynamic response, has been demonstrated previously.7–11–13 However, previous studies are limited because many have not used simultaneous diameter and velocity measurements,7–11–13 all did not control for systemic variation in arterial CO2 during hyperoxic provocation,7,10–13 and few have specifically focused on changes associated with the development of early sight-threatening diabetic retinopathy. Our hypothesis was that the magnitude of the vascular reactivity response would be impaired in those individuals with more advanced diabetic retinopathy (DR).

The purpose of this study was to quantify the magnitude of change in retinal arteriolar diameter, blood velocity, and blood flow induced by an isocapnic hyperoxic provocation in diabetic patients clinically stratified by retinopathy status and in age-matched subjects without diabetes. In addition, volunteers underwent noninvasive, objective assessment of diabetic macular edema (DME) by the Macular Edema Module (MEM) of the Heidelberg Retina Tomograph II (HRT; Heidelberg Engineering, Heidelberg, Germany).14 There are four unique aspects to this study. First, we used a technique that allowed the simultaneous quantification of vessel diameter and center-line blood velocity to calculate volumetric retinal blood flow in microliters per minute. Second, we used a unique standardized system15 to administer isocapnic hyperoxia. Unlike previous studies, a larger and clinically defined sample with early and sight-threatening DR culminating in the development of DME was used in this study. Finally, we correlated the retinal hemodynamic response to isocapnic hyperoxia with the objective assessment of retinal edema.

RESEARCH DESIGN AND METHODS

Sample

Using previously published data from our laboratory,4 we found the vascular reactivity response of healthy young subjects in terms of change of retinal blood flow in response to isocapnic hyperoxia to be 4.3 μL/min and the standard deviation of the difference between baseline and recovery to be 0.85 μL/min. If we assume a 50% reduction in vascular reactivity response when comparing healthy subjects to our most advanced diabetic retinopathy group,13 the difference between groups would have to be 0.72 μL/min—that is, (50% of 4.3 μL/min)/3, to reach statistical significance. Therefore, the standardized effect size (difference between means/standard deviation) was calculated to be 0.85 and the resultant sample size, with an α of 0.05 and power of 0.9, was 16 per group. The sample comprised 21 nondiabetic, age-matched control subjects (group 1; mean age, 49 ± 10 [SD] years), 19 patients...
with no clinically visible DR (group 2; mean age, 52 ± 11 years), 17 patients with mild-to-moderate nonproliferative DR as defined by the ETDRS (Early Treatment Diabetic Retinopathy Study) and an absence of DME (group 3; mean age, 51 ± 12 years) and 17 patients with clinically manifest DME (group 4; mean age, 55 ± 8 years). The diabetic groups were stratified for increasing risk for the development of clinically significant DME (groups 2–4). The range of retinopathy features manifested by group 4 (apart from the presence of DME) was equivalent to that of group 3. The number of patients classified as having type 1 diabetes as a function of group was two, two, and one for groups 2, 3, and 4, respectively. Table 1 details group mean age, duration of diabetes, number treated with insulin, male-to-female ratio, glycosylated hemoglobin (A1c), and random glucose as a function of group.

Volunteers were allocated into groups according to their retinal status using dilated stereo fundus biomicroscopy by agreement of two clinicians. The clinicians agreed on the retinal status of each volunteer in all cases. All volunteers were aged between 30 and 70 years and had a logMAR (logarithm of the minimum angle of resolution) visual acuity (VA) of 0.3 or better. Volunteers were excluded if they exhibited any eye disease (apart from DR for groups 2, 3, and 4) or had undergone ocular surgery, any cardiovascular (except well controlled systemic hypertension) and respiratory (except treated asthma) disorders, a refractive error greater than ±6.00 DS or ±2.00 DC, and glaucoma in a first-degree relative. None of the volunteers were regular smokers or had undergone retinal laser treatment. All volunteers were asked to refrain from caffeine-containing drinks or snacks for at least 8 hours before the visit study. Lens clarity was graded according to the Lens Opacity Classification System III (LOCS III). The study was approved by the University Health Network Research Ethics Board, Toronto, and the University of Waterloo Office of Research Ethics. Informed consent was obtained from each volunteer after explanation of the nature and possible consequences of the study, according to the tenets of the Declaration of Helsinki.

### Isocapnic Hyperoxia Delivery System

The isocapnic hyperoxia delivery system comprised a sequential rebreathing circuit made up of a fresh gas reservoir, an expiratory gas reservoir and a face-mask (Hi-Ox; Visys Healthcare, Yorba Linda, CA). The inspiratory and expiratory limbs were interconnected by a single positive end-expiratory pressure (PEEP) valve, allowing exhaled gas to be rebreathed when the gas in the inspiratory limb was depleted. Flow from gas tanks containing air (baseline) or oxygen (hyperoxia), respectively, was controlled using standard rotometers. This method has been described in detail in a previous publication.

### Quantification of Retinal Vessel Diameter, Blood Velocity, and Flow

The principal underlying the quantification of retinal hemodynamics is based on the Doppler effect. Laser light (frequency \(f\)) reflected from a moving particle is shifted in frequency by an amount \((\Delta f)\) that is proportional to the velocity of the moving red blood cells. A vessel that exhibits Poiseuille flow has a range of velocities and thus a range of frequency shifts up to a maximum frequency shift \((\Delta f_{\text{max}})\) that corresponds to the \(V_{\text{max}}\) of the blood moving at the center of the vessel. By using two photomultipliers separated by a known angle, it is possible to quantify the absolute center-line blood velocity.

The Canon Laser Blood Flowmeter (CLBF; Canon, Tokyo, Japan) utilizes a red diode laser to measure velocity and a green diode laser to measure vessel diameter and maintain centration of the laser at the measurement site. The vessel tracking system allows postacquisition rejection of velocity measurements affected by significant saccades. Two sequential measurements using different optical paths (paths 1 and 2) are taken to ensure consistency and averaged to give one reading. In combination with the average velocity \((V_{\text{mean}})\) over a pulse cycle and diameter \((D)\), flow through the vessel can be calculated using \(\frac{1}{2} \cdot \pi \cdot D^2 / 4 \cdot V_{\text{mean}} \cdot f_{\text{max}}\). Magnification effects associated with refractive and axial components of ametropia are corrected to provide absolute measurements of diameter (in micrometers), velocity (millimeters per second), and flow (microliters per minute). This device has been extensively evaluated in volunteers with and without retinal diseases.

### Quantitative Assessment of Retinal Edema

A confocal scanning laser tomograph that sequentially acquires two-dimensional section images along the optical axis was used. The distribution of reflected light intensity along the optical axis for a given pixel is described by the \(z\)-profile or confocal intensity profile. Studies have demonstrated a broadening of the \(z\)-profile signal width and a decrease in peak reflectance intensity in areas of retinal edema. Normalization of the reflectance values reduces the variation in intensity between successive scans. The MEM technique of the HRT II (Heidelberg Engineering) determines the \(z\)-profile signal width (at half peak height) and peak reflectance intensity. The edema index is derived as the quotient of signal width and peak reflectance intensity. MEM has been demonstrated to have high sensitivity and good specificity for the detection of DME.

### Procedures

One eye of each subject was randomly assigned to the study if both eyes met study criteria. Volunteers attended for two visits. Visit 1 was used to establish eligibility and baseline characteristics, determine group assignment, undertake objective assessment of DME, and famil-

<table>
<thead>
<tr>
<th>Group</th>
<th>Group Mean Age (y) (SD)</th>
<th>Group Mean Duration Diabetes (y) (SD)</th>
<th>Number Treated with Insulin</th>
<th>Male to Female Ratio</th>
<th>Group Mean A1c Value (SD)</th>
<th>Group Mean Random Glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49 (10)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7 M:14 F</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>52 (11)</td>
<td>10 (9)</td>
<td>7</td>
<td>8 M:11 F</td>
<td>0.072 (0.014)</td>
<td>10.5 (4.7)</td>
</tr>
<tr>
<td>3</td>
<td>51 (12)</td>
<td>14 (10)</td>
<td>12</td>
<td>9 M:8 F</td>
<td>0.083 (0.019)</td>
<td>7.8 (3.8)</td>
</tr>
<tr>
<td>4</td>
<td>55 (8)</td>
<td>13 (8)</td>
<td>7</td>
<td>13 M:4 F</td>
<td>0.084 (0.014)</td>
<td>10.0 (3.8)</td>
</tr>
</tbody>
</table>

A1c, glycosylated hemoglobin.
Gilmore et al.

The volunteer with the technique used to quantify retinal hemodynamics. Three sets of MEM images centered on the fovea were acquired at visit 1 for each volunteer. Visit 2 was used to quantify retinal vascular reactivity to isocapnic hyperoxia (detailed in the next paragraph). Refraction, logMAR VA, resting blood pressure, and random blood glucose level were assessed before dilation of the study eye with 1% tropicamide (Alcon Canada, Mississauga, ON, Canada). At least five retinal hemodynamic measurements were attempted with the CLBF at baseline and also during isocapnic hyperoxia. Goldmann intraocular pressure assessment was undertaken after retinal blood flow measurements had been acquired. Axial length was measured by A-scan ultrasound (I3 Innovative Imaging, Inc., Sacramento, CA) to correct blood flow measurements for magnification effects due to ametropia. The median time interval from initial visit to hyperoxia visit was 8 days.

Volunteers initially breathed air for 10 minutes followed by oxygen (O2) for 10 minutes, using a sequential rebreathing circuit (Hi-Ox; Viasys Healthcare) to maintain isocapnia. This initial air breathing period was used to allow stabilization of baseline parameters (e.g., respiration rate, partial pressure of arterial oxygen [Pao2], and the partial pressure of arterial carbon dioxide [Paco2]) as indicated by measurements of end-tidal oxygen (PETO2) and carbon dioxide (PETCO2) and to establish baseline data. Retinal hemodynamic measurements were simultaneously acquired from an arteriole within 1 disc diameter from the optic nerve head using a straight vessel segment in one eye of each volunteer. Measurements were acquired during the second 5-minute period of each paradigm.

Gas Analysis and Systemic Vascular Responses

A rapid-response critical care gas analyzer (Cardiocap 5; Datex-Ohmeda, Madison, WI) was used to quantify the relative concentrations of O2 and CO2 in both the inspired and expired gases on a breath-by-breath basis. The relative concentrations of O2 and CO2 were sampled continuously by the gas analyzer and the inspired O2, inspired CO2, Pao2, and PETCO2 were downloaded to a personal computer every 5 seconds (S5 Collect software; Datex-Ohmeda). In addition, finger oxygen saturation, respiration rate and pulse rate were recorded continuously. Blood pressure was also measured noninvasively every minute over the course of the hyperoxic paradigm (Cardiocap 5; Datex-Ohmeda).

Analysis

A postacquisition analysis of the CLBF velocity waveforms was performed using a standardized protocol to remove aberrant waveforms affected by eye movement, tear film breakup, or improper tracking of the measurement laser. The maximum number of acceptable pulse cycles was used in the data analysis for each measurement (with a minimum of 1 complete velocity waveform required). In addition, maximum to minimum (max:min) velocity ratio was calculated during air breathing and compared to that during oxygen breathing for each individual. This ratio reflects vascular compliance, where an elevation of max:min ratio indicates increased vascular rigidity (the site of this change can be upstream of, downstream of, or at the CLBF measurement site). In the physiological situation, compliance is expected to reduce and rigidity increase during hyperoxia due to increased tonus of the vessel wall. In addition, wall shear rate (WSR = mean velocity · 8/diameter)29 was calculated because change in shear stress is believed to alter blood flow, and this mechanism is thought to be disturbed in diabetes and atherosclerosis.29,30

The normality of each hemodynamic parameter as a function of group and condition was confirmed before the use of parametric statistics. A normal distribution was confirmed for all parameters apart from max:min velocity ratio which was log transformed for statistical analysis. The change in each of the hemodynamic parameters in response to provocation within each group was determined using paired two-tailed t-tests. Repeated-measures ANOVA was used to determine any differences between the baseline hemodynamic parameters between groups and any difference in the response of the hemodynamic parameters between the groups. The magnitude of change of each of the hemodynamic parameters was correlated with systemic mean arterial blood pressure, duration of diabetes, A1c values and the edema indices within 500 and 1500 μm radii of the fovea. Two-tailed t-tests were used to determine differences between testing conditions where appropriate.

Results

There were no significant differences between the groups for all primary outcome measures at baseline.

Group mean baseline and effect magnitudes of retinal arteriolar diameter, blood velocity, flow, max:min velocity ratio and WSR for each group are shown in Table 2. The magnitudes of change of each of these parameters in response to isocapnic hyperoxia are shown in Figure 1. Retinal arteriolar diameter significantly decreased in response to isocapnic hyperoxia in groups 1, 3, and 4 (P < 0.005); group 2 exhibited a nonsignificant trend toward vasoconstriction (P = 0.090). Retinal blood velocity, flow, and WSR significantly decreased in response to isocapnic hyperoxia in all groups (P ≤ 0.0002, P < 0.0001, and P ≤ 0.005, respectively). Max:min velocity ratio significantly increased in groups 1, 2, and 3 (P ≤ 0.007).

Group mean reduction in diameter, velocity, and flow shown as percentage change in response to isocapnic hyperoxia as function of group are shown in Table 3. The magnitude of the reduction of blood velocity in response to isocapnic hyperoxia was significantly reduced with increasing severity of retinopathy (P < 0.0001). The responses of groups 3 and 4 were significantly less than that of groups 1 and 2. The magnitude of the reduction of blood flow was significantly reduced with increasing severity of retinopathy (P < 0.004). The responses of groups 3 and 4 were significantly less than that of group 1 and the response of group 3 was significantly less than that of group 2.

The magnitude of the increase of max:min velocity ratio in response to isocapnic hyperoxia was significantly reduced with increasing severity of retinopathy (P < 0.001). The response of group 1 was significantly greater than that of group 2. Group 2 exhibited a nonsignificant trend toward vasoconstriction (P = 0.090). Retinal blood velocity, flow, and WSR significantly decreased in response to isocapnic hyperoxia in all groups (P ≤ 0.0002, P < 0.0001, and P ≤ 0.005, respectively). Max:min velocity ratio significantly increased in groups 1, 2, and 3 (P ≤ 0.007).

Table 2. Group Mean Diameter, Velocity, Flow, Max:Min Velocity Ratio and WSR during Air and Oxygen Breathing as a Function of Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter air (μm)</th>
<th>Diameter O2 (μm)</th>
<th>Velocity air (mm/s)</th>
<th>Velocity O2 (mm/s)</th>
<th>Flow air (μL/min)</th>
<th>Flow O2 (μL/min)</th>
<th>Max:min air</th>
<th>Max:min O2</th>
<th>WSR air (s^-1)</th>
<th>WSR O2 (s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>110.7</td>
<td>106.6</td>
<td>34.8</td>
<td>21.0</td>
<td>10.2</td>
<td>5.8</td>
<td>3.1</td>
<td>5.1</td>
<td>1.280</td>
<td>807</td>
</tr>
<tr>
<td>Group 2</td>
<td>114.8</td>
<td>111.9</td>
<td>36.4</td>
<td>23.9</td>
<td>11.4</td>
<td>7.3</td>
<td>3.9</td>
<td>4.6</td>
<td>1.262</td>
<td>838</td>
</tr>
<tr>
<td>Group 3</td>
<td>113.5</td>
<td>109.3</td>
<td>31.9</td>
<td>24.7</td>
<td>11.9</td>
<td>7.5</td>
<td>4.1</td>
<td>5.3</td>
<td>1142</td>
<td>911</td>
</tr>
<tr>
<td>Group 4</td>
<td>115.9</td>
<td>109.5</td>
<td>32.4</td>
<td>26.4</td>
<td>10.4</td>
<td>7.5</td>
<td>4.1</td>
<td>5.5</td>
<td>1159</td>
<td>970</td>
</tr>
</tbody>
</table>

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Figure 1. Change in retinal arteriolar diameter (A), blood velocity (B), blood flow (C), max:min velocity ratio (D), and WSR (E) with isocapnic hyperoxia provocation as a function of group. In each graph, the center of the box represents the group mean response, the boundaries of the box represent ±1 SE and the whiskers represent ±1 SD. (○) Outlier values; (♦) extreme values. Extreme values are outside the three-box-length range from the upper and lower values of the box. Group 1: nondiabetic, age-matched control subjects; group 2: patients with no clinically visible DR; group 3: patients with mild-to-moderate nonproliferative DR in the absence of clinically evident DME; and group 4: patients with diabetic macular edema.
ing severity of retinopathy ($P = 0.0002$). The response of group 1 was significantly greater than that of groups 3 and 4 and the response of group 2 was significantly greater than that of group 4.

There was no correlation between the magnitude of change of each of the hemodynamic parameters and age, systemic mean arterial blood pressure, duration of diabetes and A1c values.

Group mean baseline and effect values for relevant respiratory and systemic parameters as a function of group are shown in Table 4. Fractional inspired oxygen ($\text{FiO}_2$) changed significantly in each group with isocapnic hypoxic provocation ($P < 0.0001$; paired two-tailed t-test). Expired carbon dioxide ($P_{\text{ET}}$CO$_2$) did not change in any group. The group mean arterial blood pressure [MAP; $(1/3 \cdot$ diastolic BP) + $(2/3 \cdot$ systolic BP)] was not significantly different between baseline and isocapnic hyperoxia in any of the groups. Pulse rate did not change significantly in any group with isocapnic hyperoxic provocation.

Group mean edema indices within 500- and 1500-μm radii of the fovea as a function of group are shown in Table 5. Edema indices were significantly greater in group 4 than in group 1 for both the 500- and 1500-μm radii circles ($P \leq 0.0005$; paired two-tailed t-test). Edema indices were significantly greater in group 3 than in group 1 for the 1500-μm circle only ($P = 0.0005$; two-tailed t-test). Group 4 was significantly greater than group 2 for both the 500- and 1500-μm radius circles ($P \leq 0.0005$; two-tailed t-test). There was a significant correlation between baseline edema indices within the 500-μm radius circle and the magnitude of change in velocity in response to isocapnic hyperoxia ($r = 0.3; P = 0.03$). Baseline edema indices within the 1500-μm radius circle also correlated with the magnitude of change in velocity ($r = 0.3; P = 0.03$) and flow ($r = 0.3; P = 0.04$), in response to isocapnic hyperoxia.

### Table 3. Group Mean Reduction in Diameter, Velocity and Flow in Percentage Change Due to Isocapnic Hyperoxia as a Function of Group

<table>
<thead>
<tr>
<th>Group Mean Reduction (%)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>3.7% (4.8%)</td>
<td>2.5% (5.4%)</td>
<td>3.7% (4.8%)</td>
<td>5.5% (6.4%)</td>
</tr>
<tr>
<td>Velocity</td>
<td>40% (8%)</td>
<td>35% (15%)</td>
<td>22% (13%)</td>
<td>17% (13%)</td>
</tr>
<tr>
<td>Flow</td>
<td>44% (9%)</td>
<td>36% (15%)</td>
<td>28% (14%)</td>
<td>26% (16%)</td>
</tr>
</tbody>
</table>

### Table 4. Group Mean Baseline and Effect Values for $P_{\text{ET}}$CO$_2$, $\text{FiO}_2$, MAP, and Mean Pulse Rate during Air and Isocapnic Hyperoxia as a Function of Group

<table>
<thead>
<tr>
<th>Group Mean Values</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{ET}}$CO$_2$ air (%)</td>
<td>4.9</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>$P_{\text{ET}}$CO$_2$ O$_2$ (%)</td>
<td>4.8</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>$\text{FiO}_2$ air (%)</td>
<td>20.0</td>
<td>(0.5)</td>
<td>(0.2)</td>
<td>(0.3)</td>
</tr>
<tr>
<td>$\text{FiO}_2$ O$_2$ (%)</td>
<td>92.9</td>
<td>(2.9)</td>
<td>(2.8)</td>
<td>(4.2)</td>
</tr>
<tr>
<td>MAP mm Hg</td>
<td>90.7</td>
<td>(10.3)</td>
<td>(6.8)</td>
<td>(9.8)</td>
</tr>
<tr>
<td>MAP O$_2$ mm Hg</td>
<td>92.3</td>
<td>(10.4)</td>
<td>(7.2)</td>
<td>(9.9)</td>
</tr>
<tr>
<td>HR bpm</td>
<td>65.3</td>
<td>(9.2)</td>
<td>(11.4)</td>
<td>(12.0)</td>
</tr>
<tr>
<td>HR O$_2$ bpm</td>
<td>60.8</td>
<td>(8.4)</td>
<td>(11.1)</td>
<td>(13.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Edema Index Values</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 μm radius</td>
<td>1.12</td>
<td>(0.28)</td>
<td>(0.38)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>1500 μm radius</td>
<td>1.19</td>
<td>(0.21)</td>
<td>(0.24)</td>
<td>(0.20)</td>
</tr>
</tbody>
</table>

DISCUSSION

The vascular reactivity response to isocapnic hyperoxia was significant in all groups but the magnitude of the change in flow was significantly reduced with increasing severity of early retinopathy and sight-threatening DME. DME patients and patients with mild-to-moderate DR without clinically evident DME demonstrated significantly reduced vascular reactivity compared to age-matched subjects without diabetes and patients with no clinically visible DR. Few studies have examined retinal vascular reactivity at such an early stage in the development of DR. Max:min velocity ratio increased significantly as a result of isocapnic hyperoxia in each group except the DME group. The absence of a difference in change in max:min velocity ratio between groups 3 and 1 is probably attributable to the relatively small sample size of the study. The magnitude of change of max:min and WSR was significantly less in the DME group than in the age-matched subjects without diabetes. The correlation between the objective assessment of retinal edema and retinal vascular reactivity has not been previously reported. Taken as a whole, these results indicate a loss of retinal vascular reactivity in patients with moderate DR without clinically evident DME and in patients with DME. Furthermore, the results indicate an inability to increase vessel tonus and reduce shear stress (as indicated by the absence of change in max:min velocity ratio and WSR, respectively), in response to isocapnic hyperoxic provocation in patients with DME. Of note, random glucose levels were not significantly different pre- and post-isocapnic hyperoxia for any of the groups, whereas MAP was significantly higher for group 4 than any of the other three groups. This suggests that hypertension that typically occurs concomitantly in diabetes may also contribute to the findings of the study. However, group 3 diabetic patients demonstrated a loss of retinal vascular reactivity but not difference in MAP compared with groups 1 and 2.

Retinal blood flow varies inversely with the partial pressure of arterial oxygen (PO$_2$) to maintain retinal oxygenation at a relatively constant level and also varies directly with the partial pressure of arterial carbon dioxide (PCO$_2$). The change of end-tidal CO$_2$ concentration ($P_{\text{ET}}$CO$_2$), the maximum concentration of CO$_2$ during each expiration) reflects the change in arterial PCO$_2$ in healthy subjects. We are unaware of any evidence to suggest that this relationship is different in patients with diabetes. Oxygen supply to the retina during...
Vascular Reactivity in Diabetic Retinopathy

Hyperoxia is controlled by either a direct reduction of vessel diameter, or by change in WSR via an upstream flow-induced mechanism that initiates a secondary retinal diameter response; however, the exact mechanism by which retinal vessels respond to changes in PO₂ has yet to be fully elucidated. Hyperoxia stimulates ET-1 release from retinal vascular endothelial cells in vitro and is the primary factor modulating retinal vascular reactivity induced by hyperoxia, in animals and humans. Previously published studies investigated retinal vascular reactivity in diabetic patients and a nonisocapnic hyperoxic stimulus. A reduced magnitude of vascular reactivity to hyperoxia relative to subjects without diabetes has been demonstrated in patients with a spectrum of diabetic retinopathy severity up to that of proliferative retinopathy. These studies have been limited because many have not used simultaneous diameter and velocity measurements, all did not control for systemic variation in arterial CO₂ during hyperoxia, and none has focused on changes associated with the development of early sight-threatening diabetic retinopathy culminating in DME. Most of these studies have measured vascular reactivity in venules. We studied the retinal arteriolar response because the arterioles are known to be primarily responsible for the regulation of vascular reactivity and to obey more closely the Poiseuille flow principles (given their more circular cross section). The complexity of the experimental paradigm did not permit the comparison of arteriolar and venular response in this study. Using MRI based techniques, Trick et al. have shown a supernormal retinal oxygenation response in patients with type 1 diabetes prior to clinically visible DR.

Functional hemodynamic indices such as max:min velocity ratio, resistivity index, pulsatility index, and WSR have been investigated previously using the retinal vasculature. Increased peripheral arterial stiffness (measured in the arm and ankle) has been positively correlated with severity of DR. Resistivity and pulsatility indices were not used in this study because of the extraneous influence of downstream impedance. WSR is a measure of shear stress (i.e., shear stress = WSR * viscosity). To the best of our knowledge, this study is the first to detail WSR in groups of diabetic patients and age-matched subjects without diabetes, and the change in WSR in response to isocapnic hyperoxia. A short-term increase in WSR using a hyperoxic stimulus in cats has been demonstrated. Our work agrees with that of Nagoaka et al., by demonstrating a decrease in WSR during a hyperoxic stimulus in all groups except those with DME. Our results show that patients with DME are unable to regulate WSR in response to isocapnic hyperoxic provocation. It has previously been shown that increased shear stress results in increased hydraulic conductivity across the vessel wall and the propensity for edema formation. MEM allows objective assessment of retinal edema and has previously been shown to have a high sensitivity and good specificity for detection of DME.

No previous publications investigating change in vascular reactivity in DR have used objective techniques for the assessment of retinal edema. The correlation between the magnitude of the vascular reactivity response and the edema index suggests a continuum of edema formation and impairment of vascular reactivity with increasing severity of DR. Diabetes is universally regarded as a systemic disease that impacts the vasculature as a whole. Consequently, it is not surprising to find correlation between parameters which reflect blood-retinal barrier function and retinal hemodynamics, irrespective of specific retinal measurement sites.

The reason for impairment of vascular reactivity in patients with diabetes is uncertain but may include structural alterations to the smooth muscle cells (SMCs) or pericytes and functional alterations of the endothelial cells. Some studies have suggested that ET-1 is upregulated in diabetes, resulting in increased media-to-lumen ratio, matrix accumulation, and vascular remodeling. However, other groups would question the role of ET-1 in diabetes. Nitric oxide (NO) bioactivity is reduced in diabetes due to decreased production or inactivation, resulting in increased arterial stiffness due to alteration of the collagen/elastic ratio of the vessel wall. Taken as a whole, these vascular remodeling changes will result in reduced compliance and vascular reactivity.

In diabetes, SMCs undergo abnormal growth, proliferation, and migration thereby preventing normal function. In addition, pericytes are capable of responding to changes in oxygen concentration and can regulate endothelin-1 and inducible (i)NOS release from endothelial cells. As pericytes are progressively reduced in number during DR, impairment of vascular reactivity may be due, in part, to loss of pericytes.

We hypothesize that increasing arterial stiffness and a reduced vascular reactivity response are important in the development of retinal edema. Retinal arterioles are the resistance vessels of the retina and control downstream hydrostatic pressure. An absence of an effective vascular reactivity control mechanism will result in increased hydrostatic pressure at the level of the capillary bed. According to Starling’s law, increased hydrostatic pressure will result in the net movement of fluid out of the vascular compartment into the extracellular space and the formation of edema.

In summary, an isocapnic hyperoxic stimulus was used in this study to assess retinal vascular reactivity in diabetic patients and in subjects without diabetes. The magnitude of change in blood velocity and flow in response to isocapnic hyperoxia was reduced in those individuals with clinically evident diabetic retinopathy relative to subjects without diabetes. A reduced change in max:min velocity ratio and WSR in response to isocapnic hyperoxia in patients with DME was also demonstrated. Vascular reactivity is impaired in early sight-threatening DR, and this impairment is related to the magnitude of edema. Altered production or sensitivity to various biochemical factors and/or structural/functional changes of the endothelial cells, smooth muscle cells, or pericytes may be involved.

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