Retinal Arteriolar Hemodynamic Response to a Combined Isocapnic Hyperoxia and Glucose Provocation in Early Sight-Threatening Diabetic Retinopathy

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Purpose. To quantify the magnitude of change of retinal arteriolar hemodynamics induced by a combined isocapnic hyperoxia and glucose provocation in diabetic patients with early sight-threatening diabetic retinopathy (DR) and in age-matched control subjects and to compare the response to that of an isocapnic hyperoxia provocation alone. The study hypothesis was that hyperglycemia reduces the retinal vascular reactivity response to a hyperoxic stimulus.

Methods. The sample comprised 17 control subjects (group 1), 15 patients with no clinically visible DR (group 2), 16 patients with mild-to-moderate nonproliferative DR (group 3), and 15 patients with diabetic macular edema (group 4). Retinal hemodynamic measurements were acquired in the subjects, at baseline and 1 hour after consuming a standardized oral glucose load drink while breathing oxygen isocapnic with baseline.

Results. Retinal blood velocity and flow significantly decreased in all groups ($P \leq 0.001$ and $P \leq 0.0002$, respectively) in response to a combined isocapnic hyperoxia and glucose provocation. The maximum-to-minimum velocity ratio significantly increased ($P \leq 0.005$), and wall shear rate (WSR) significantly decreased ($P \leq 0.0002$), in groups 1, 2, and 3, but not in group 4. The vascular reactivity response was not significantly different *across* the groups. The control group demonstrated a reduced change in flow ($P = 0.009$) and WSR ($P = 0.010$) to the combined isocapnic hyperoxia and glucose provocation compared with that of hyperoxia alone.

Conclusions. The vascular reactivity response to a combined isocapnic hyperoxia and glucose provocation produced a pronounced reduction in blood flow. Unlike the response to hyperoxia alone, the vascular reactivity response was not significantly different *across* the groups. Hyperglycemia reduced the retinal vascular reactivity response to hyperoxia in age-matched control subjects. (*Invest Ophthalmol Vis Sci.* 2008; 49:699–705) DOI:10.1167/iovs.07-0339

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Vascular reactivity represents the hemodynamic response of the vasculature to a given stimulus, such as hyperoxia[1,2] or hypercapnia.[3] In large retinal vessels, vascular reactivity in response to hyperoxia with simultaneous hyperglycemia is thought to be impaired in humans with diabetes.[4,5] By contrast, another study reported no effect of hyperglycemia on hyperoxia-induced retinal capillary vascular reactivity in humans.[6] Previous studies are limited, however, because many have not utilized simultaneous diameter and velocity measurements to calculate retinal blood flow and no previous studies have adequately controlled for systemic variation in arterial CO_2 during hyperoxic provocation. In addition, a unique aspect of this study is the focus on changes associated with the development of early sight-threatening diabetic retinopathy (DR) culminating in diabetic macular edema (DME). Impairment of vascular reactivity during acute hyperglycemia may yield important information about the pathophysiology associated with the development of early DR.

Previous work in our laboratory has shown that hyperoxia-induced change of retinal blood flow in patients with predominantly type 2 diabetes and early DR is impaired and that this impairment precedes change in homeostatic blood flow parameters. Vascular reactivity is a more sensitive marker of vascular dysfunction in early DR than is homeostatic blood flow.[2] Following on from this work, we used the same blood flow quantification techniques to investigate the impact of *acute* hyperglycemia on retinal blood flow in early DR. We were unable to reveal any impact of acute hyperglycemia on blood flow parameters and attributed this observation to an impaired magnitude of retinal vascular reactivity in patients with predominantly type 2 diabetes.[7] In the present study, we advanced this work by investigating the effect of a combined isocapnic hyperoxia and glucose provocation on retinal vascular reactivity. We hypothesized that hyperglycemia reduces the retinal vascular reactivity response to a hyperoxic stimulus.

The purpose of the study was to quantify the magnitude of change of retinal hemodynamics induced by a combined isocapnic hyperoxia and glucose provocation in groups of diabetic patients clinically stratified by retinopathy status and in age-matched control subjects without diabetes. The response to the combined isocapnic hyperoxia and glucose provocation was compared with that of an isocapnic hyperoxic provocation alone.[2] Volunteers also underwent noninvasive, objective assessment of DME (Macular Edema Module [MEM] of the Heidelberg Retina Tomograph II [HRT] Heidelberg Engineering, Heidelberg, Germany).[8] We correlated the retinal vascular reactivity response to a combined isocapnic hyperoxia and glucose provocation with the objective assessment of retinal edema.

Materials and Methods

Sample

Using previously published data from our group,[1] we found the vascular reactivity response of healthy young subjects in terms of

TABLE 1. Group Characteristics

Group	Group Mean Age (y) (SD)	Group Mean Known Duration Diabetes (y) (SD)	Number Treated with Insulin	Male to Female Ratio	Group Mean A1c Value (SD)
1	49 (10)	—	—	6 M:11 F	—
2	54 (11)	6 (5)	5	5 M:10 F	0.070 (0.010)
3	51 (12)	15 (10)	12	8 M:8 F	0.084 (0.019)
4	55 (8)	13 (9)	6	11 M:4 F	0.087 (0.014)

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. Assuming an approximate 50% reduction in vascular reactivity response when comparing healthy subjects to our most advanced DR group,[2,9] the difference between groups to reach statistical significance would have to be 0.72 μL/min (i.e., [50% of 4.3 μL/min]/3). Therefore, the standardized effect size (difference between the mean/SD) was calculated to be 0.85, and the resultant sample size using an α of 0.05 and a power of 0.9 was estimated to be 16 per group. The sample comprised 17 nondiabetic control subjects (group 1; mean age, 49 ± 10 [SD] years), 15 patients with no clinically visible DR (group 2; mean age, 54 ± 11 years), 16 patients with mild-to-moderate nonproliferative DR[10] (group 3; mean age, 51 ± 12 years) and 15 patients with DME (group 4; mean age, 55 ± 8 years; Table 1). The number of patients classified as having type 1 diabetes as a function of group was 2, 2, and 1 for groups 2, 3, and 4, respectively.

Volunteers were stratified into groups according to their retinal status using dilated stereo fundus biomicroscopy. All volunteers were aged between 30 and 70 years and had a logMAR visual acuity 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 D sphere or ±2.00 D cylinder and glaucoma in a first-degree relative. None of the volunteers was a regular smoker or had undergone retinal laser treatment. Nondiabetic control subjects were asked to refrain from caffeine-containing drinks or snacks for at least 8 hours before the study visit. Lens clarity was graded with the Lens Opacity Classification System III[11] (LOCS III). The study was approved by the University of Waterloo Office Research Ethics and the University Health Network Research Ethics Board, Toronto. Informed consent was obtained form each volunteer after explanation of the nature and possible consequences of the study according to the tenets of the Declaration of Helsinki.

Oral Glucose Tolerance Test

An oral glucose load drink (75 g glucose suspended in 300 mL water) was given to all participants. Diabetic patients were asked to fast for a minimum of 8 hours before the study and to omit their usual doses of insulin or oral hypoglycemic agents during this fasting period.

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; ViasysHealthcare, Yorba Linda, CA). Adhesive dressing (Tegaderm; 3M Health Care) was used to ensure an air-tight seal of the mask to the face. 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 oxygen and air, respectively, was controlled with standard rotometers as flowmeters. This method has been described in detail in a previous publication.[12]

Quantification of Retinal Vessel Diameter and Blood Velocity and Flow

The methodology used to quantify retinal hemodynamics has been detailed.[1–3,7,8] The technological principles of the Canon Laser Blood Flowmeter (CLBF; Canon, Tokyo, Japan) have been described in detail elsewhere.[13–18] The CLBF has been extensively evaluated in volunteers, with[2,7,18–20] and without[1,21–23] retinal diseases and after therapeutic intervention.[24]

Quantitative Assessment of Retinal Edema

The methodology used to quantify retinal edema has been detailed previously.[8,25] The Macular Edema Module (MEM) technique of the Heidelberg Retina Tomograph (HRT; Heidelberg Engineering, Heidelberg, Germany) is an objective measure of retinal edema and has been demonstrated to have high sensitivity and good specificity for the detection of DME.[8]

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 familiarize 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 the retinal vascular response to a combined isocapnic hyperoxia and glucose provocation. Refraction, logMAR visual acuity, resting blood pressure, and random blood glucose level were assessed before dilation of the study eye with 1% tropicamide (Alcon, Mississauga, ON, Canada). Retinal hemodynamic measurements were simultaneously acquired from an arteriole approximately 1 to 2 disc diameters from the optic nerve head using a straight vessel segment. At least five retinal arteriolar hemodynamic measurements were acquired at baseline and 1 hour after glucose ingestion while simultaneously breathing oxygen under isocapnic conditions. Intraocular pressure was measured by Goldmann applanation tonometry after retinal blood flow measurements had been acquired. Axial length was measured by A-scan ultrasound (I^3 Innovative Imaging Inc, Sacramento, CA) to correct blood flow measurements for magnification effects due to ametropia.

The response to the combined isocapnic hyperoxia and glucose provocation was compared with the response of an isocapnic hyperoxia provocation alone, determined at a separate visit.[2] The median time between the two provocations was 7 days.

Gas Analysis and Systemic Vascular Responses

A rapid response critical care gas analyzer (Datex-Ohmeda Cardiocap 5; GE Healthcare, Mississauga, ON, Canada) 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 concentrations of O_2 and CO_2 were sampled continuously by the gas analyzer and the levels of inspired O_2, inspired CO_2, $P_{ET}O_2$, and $P_{ET}CO_2$ were downloaded to a personal computer every 5 seconds (Datex-Ohmeda S5 Collect software; GE Healthcare). In addition, finger-oxygen saturation, respiration rate, and pulse rate were re-

TABLE 2. Group Mean Percentage Change Retinal Hemodynamics after a Combined Isocapnic Hyperoxic and Glucose Provocation as Function of Group

Group Mean Change Relative to Baseline	Group 1	Group 2	Group 3	Group 4
Diameter	−4.0 (6.0)	−3.6 (4.4)	−3.2 (6.7)	−4.1 (4.7)
Velocity	−30.5 (12.0)	−30.6 (11.4)	−23.1 (13.8)	−24.2 (10.6)
Flow	−36.0 (11.8)	−35.5 (11.1)	−27.8 (14.6)	−31.5 (12.6)

Data are the mean percentage ± SD. A negative value indicates reduction from baseline.

corded continuously. $P_{ET}CO_2$ was analyzed by calculating the upper 10th and lower 90th percentiles. Data points lying outside the upper 10th or lower 90th percentiles were excluded from the analysis since all these values were found to be erroneous—that is, these points resulted from inappropriate interpretation of tidal waveforms by the gas monitor. Blood pressure was also measured noninvasively once every minute over the course of the hyperoxic paradigm (Datex-Ohmeda Cardiocap 5; GE Healthcare).

Analysis

A postacquisition analysis of the CLBF velocity waveforms was performed by 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 one complete velocity waveform required). In addition, the 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, were an elevation of the max-min ratio indicates increased vascular rigidity. (The site of this change can be upstream of, downstream of, or at the measurement site.) In the physiological situation, compliance is expected to reduce and rigidity increase during hyperoxia due to increased tonus of the vessel wall. Furthermore, wall shear rate (WSR = mean velocity × 8/diameter)[26] was calculated, assuming a parabolic velocity profile, because change in shear stress is believed to alter blood flow, and this mechanism is thought to be disturbed in diabetes and atherosclerosis.[26,27]

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 the max-min velocity ratio, which was log transformed to meet normality before 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, any difference in the response of the hemodynamic parameters between the groups and any difference in the response between combined isocapnic hyperoxia and glucose versus hyperoxia alone within each group. The dependent variables were diameter, velocity, blood flow, max-min velocity ratio, and WSR. The within-subject factor was glucose and isocapnic hyperoxia, and the between-subject factor was group. 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 index values within the 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 the retinal hemodynamic outcome measures at baseline. Group mean change of retinal arteriolar diameter, blood velocity, and flow for each group are shown in Table 2.

Within Groups Comparison

Retinal blood velocity and flow significantly decreased in all groups ($P \leq 0.0010$ and $P \leq 0.0002$, respectively) in response to a combined isocapnic hyperoxia and glucose provocation (Fig. 1). Retinal arteriolar diameter manifested a nonsignificant trend to reduce in all groups (Bonferroni corrected $P < 0.0100$). The max-min velocity ratio significantly increased ($P \leq 0.0050$), and WSR significantly decreased ($P \leq 0.0002$) in groups 1, 2, and 3, but not in group 4.

Between-Groups Comparison and Correlations

The magnitude of change of the retinal hemodynamic outcome measures in response to combined isocapnic hyperoxia and glucose provocation was not significantly different between the groups (Fig. 1; Table 2).

There was a significant correlation between the magnitude of change in blood glucose and age ($r = 0.339$; $P = 0.007$). There were no other correlations evident between change in the hemodynamic parameters and age, systemic mean arterial blood pressure, duration of diabetes, blood glucose, and A1c values.

Comparison with Hyperoxia Alone

The magnitude of change of flow ($P = 0.009$) and WSR ($P = 0.010$) was significantly less for the age-matched control group to the combined isocapnic hyperoxia and glucose provocation than that of hyperoxia alone (Bonferroni-corrected $P = 0.01$). In the nondiabetic group, flow decreased by 43.8% in response to isocapnic hyperoxia but only by 36.0% in response to the combined isocapnic hyperoxia and glucose provocation, whereas WSR declined by 36.8% in response to hyperoxia and by 27.3% in response to the combined isocapnic hyperoxia and glucose provocation. There were no significant differences in any of the outcome parameters between the combined isocapnic hyperoxia and glucose provocation and hyperoxia alone for any of the *diabetic* groups.

Isocapnic Hyperoxia

Group mean baseline and effect values for relevant respiratory and systemic parameters as a function of group are shown in Table 3. Fractional inspired oxygen (FiO_2) changed significantly in each group with combined isocapnic hyperoxia and glucose provocation ($P < 0.0001$; paired two-tailed *t*-test). Expired carbon dioxide ($P_{ET}CO_2$) did not change in any group. The group mean arterial blood pressure (MAP) was not significantly different between baseline and combined isocapnic hyperoxia and glucose provocation for any of the groups. Pulse rate did not change significantly in any group with combined isocapnic hyperoxia and glucose provocation.

Oral Glucose Tolerance Test

Table 4 details blood glucose before and 1 hour after ingestion of glucose. At baseline, group 1 blood glucose was significantly

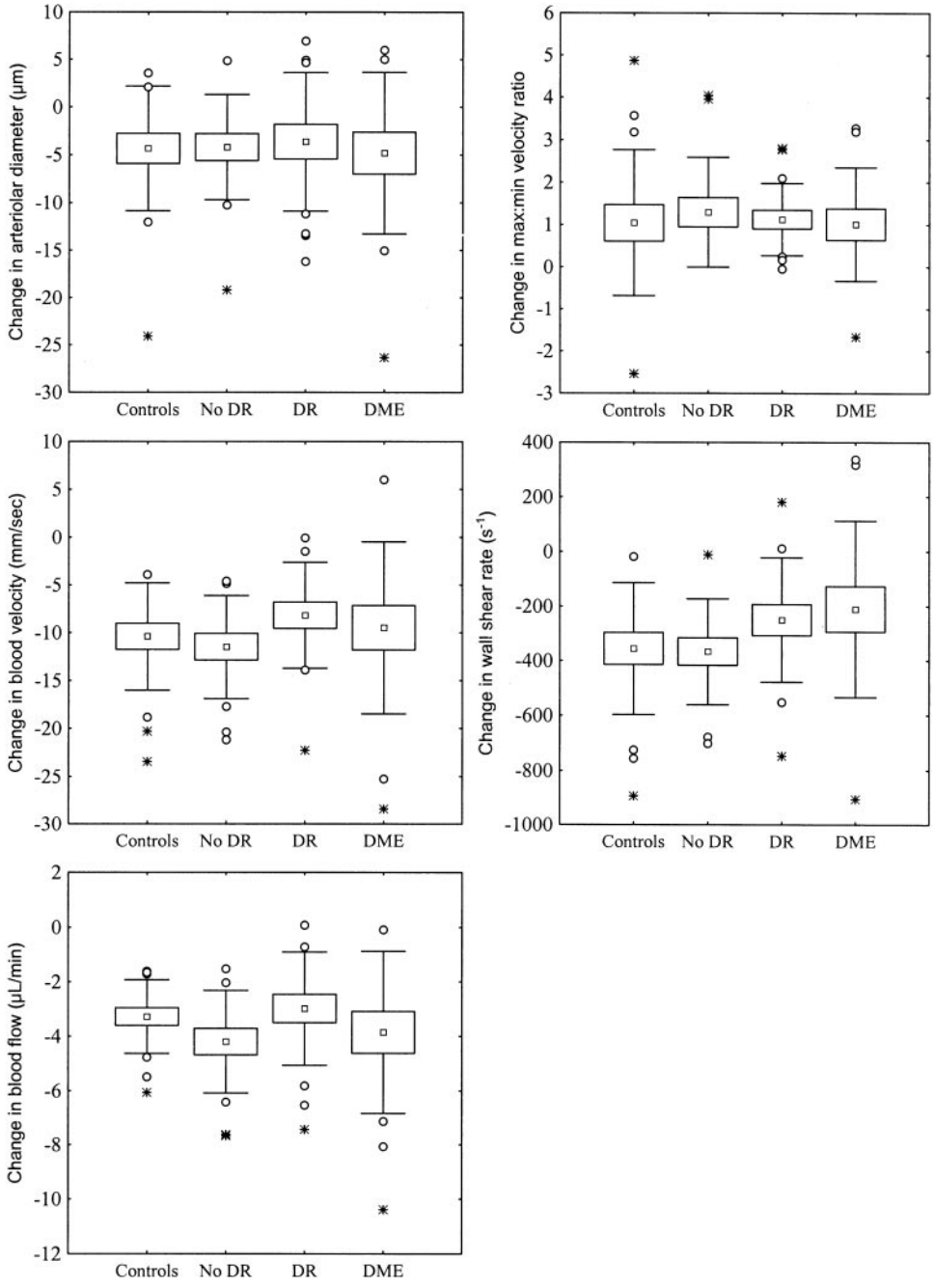

FIGURE 1. Change relative to baseline in retinal arteriolar diameter (*top left*), blood velocity (*middle left*), blood flow (*bottom left*), max-min velocity ratio (*top right*), and wall shear rate (*middle right*) after combined isocapnic hyperoxia and glucose provocation, as a function of group. For each graph, the center of the box represents the group mean response, the limits 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 value of the box. Extremes and outliers were not included in the derivation of box plot means. Control, nondiabetic, age-matched subjects (group 1); No DR, patients with no clinically visible diabetic retinopathy (group 2); DR, patients with mild-to-moderate nonproliferative diabetic retinopathy in the absence of clinically evident diabetic macular edema (group 3); DME, patients with diabetic macular edema (group 4). The changes in vascular reactivity as a function of hyperoxia alone are described elsewhere.[2]

lower than in groups 3 and 4 ($P = 0.001$). Blood glucose increased significantly in all groups ($P \leq 0.0005$). The increase in blood glucose was significantly less in group 1 than that of the other three groups ($P < 0.0001$).

Quantitative Assessment of Retinal Edema

Group mean edema indices within the 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 groups 1 and 2 for both the 500- and 1500-μm radii ($P \leq 0.0005$; 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). There was no correlation between baseline edema indices within the 500- or 1500-μm radius circle and the magnitude of change in diameter, velocity, or flow in response to combined isocapnic hyperoxia and glucose provocation.

DISCUSSION

The present study investigated change in retinal arteriolar diameter, blood velocity, flow, max-min velocity ratio, and WSR induced by a combined isocapnic hyperoxia and glucose provocation in a group predominantly comprising patients with type 2 diabetes, stratified by severity of retinopathy and compared with age-matched subjects without diabetes. The vascular reactivity response in terms of the reduction of blood flow relative to baseline was significant in all groups, but the magnitude of the change in flow was not significantly different *across* the groups. The magnitude of change of flow and WSR

TABLE 3. Respiratory and Systemic Parameters before and after Glucose Challenge

	Group 1	Group 2	Group 3	Group 4
FiO_2 air/before glucose (%)	20.5 (1.7)	20.1 (0.2)	20.0 (0.5)	20.2 (0.6)
FiO_2 O_2/after glucose (%)	92.5 (4.5)*	92.8 (2.5)*	92.8 (2.8)*	93.1 (2.5)*
$P_{ET}CO_2$ air/before glucose (%)	4.8 (0.4)	5.0 (0.4)	5.1 (0.3)	5.1 (0.4)
$P_{ET}CO_2$ O_2/after glucose (%)	4.8 (0.4)	5.0 (0.5)	5.1 (0.4)	5.0 (0.4)
MAP air/before glucose (mmHg)	89.4 (7.5)	94.9 (8.3)	93.1 (8.8)	99.2 (9.8)
MAP O_2/after glucose (mmHg)	90.7 (7.2)	94.4 (8.1)	94.7 (8.4)	102.7 (8.8)
PR air/before glucose (bpm)	66.3 (7.0)	72.9 (8.3)	74.3 (12.9)	78.8 (11.0)
PR O_2/after glucose (bpm)	63.0 (6.7)	70.1 (9.2)	71.9 (13.0)	75.8 (11.8)

Data are the mean ± SD.
* Indicates significantly different from baseline.

was significantly less for the age-matched nondiabetic group to the combined isocapnic hyperoxia and glucose provocation than to that of hyperoxia alone (i.e., *within*-group comparison). There were no significant differences in any of the hemodynamic outcome parameters between the combined isocapnic hyperoxia and glucose provocation and hyperoxia alone *within* any of the *diabetic* groups.

An intriguing finding of this study was that the age-matched control group demonstrated a reduced response of change in flow and WSR to the combined isocapnic hyperoxia and glucose provocation compared with that of hyperoxia alone. Careful examination of the characteristics of the age-matched control group showed that three of the subjects had possible impaired glucose tolerance (IGT). When these subjects were excluded from the analysis, the difference in flow and WSR response between the combined isocapnic hyperoxia and glucose provocation and that of hyperoxia alone was not significant (note that this finding was based on a sample size of 14). This result suggests that hyperglycemia may influence the retinal vascular reactivity response to hyperoxia, especially in patients with IGT (i.e., before the development of clinically defined type 2 diabetes mellitus and the resulting loss of retinal vascular reactivity). Unlike the response to hyperoxia alone,[2] which showed a stepped profile of reduced reactivity with increasing severity of retinopathy, the vascular reactivity response to a combined isocapnic hyperoxia and glucose provocation was not significantly different *across* the groups.

Previous work in our laboratory has demonstrated that retinal blood flow significantly decreases in patients with diabetes and in those without diabetes in response to isocapnic hyperoxia alone; however, the magnitude of the change in flow is significantly less in patients with mild-to-moderate nonproliferative DR,[2] indicating a loss of vascular reactivity with increasing severity of retinopathy. Magnetic resonance imaging techniques have been used to measure change in preretinal vitreous oxygen tension in patients with diabetes and in healthy control subjects.[28,29] These studies show a supernormal vitreous oxygenation response in patients with diabetes compared with control subjects, suggesting that diabetic retinal disease results in an inability to regulate oxygen supply and demand.[30] Also, we have shown that retinal blood flow is not affected by acute increases of blood glucose in patients with type 2 diabetes and subjects without diabetes.[7] In agreement with our previous work, this study showed that WSR decreased significantly in response to a combined isocapnic hyperoxia and glucose provocation in each group, with the exception of the DME group. WSR is a component that determines shear stress (i.e., shear stress = WSR × viscosity). This finding suggests that patients with DME have impaired ability to regulate shear stress in response to provocation. However, we cannot rule out the possibility that the observed change in WSR may be secondary to hyperglycemia-induced change in blood viscosity. The max-min velocity ratio significantly increased in all groups of the present study, except that of the DME group. This finding is the same as that of the response to isocapnic hyperoxia alone[2] and indicates reduced capacity to regulate vascular tonus characteristics of the retinal arterioles in patients with DME.

Vascular reactivity changes in response to hyperoxic provocation typically occur independent of change in blood pressure. Increased PaO_2 is thought to result in the release of endothelia-derived constricting factors, especially endothelin-1, by the vascular endothelium, which in turn results in a vasoconstrictive response.[31] Although the change in diameter did not reach significance in this study for any of the groups (Bonferroni-corrected $P < 0.010$), the trend toward vasoconstriction can be the only logical explanation for the subsequent change in velocity and blood flow (in the absence of any significant change in blood pressure).

Previously published studies have investigated retinal vascular reactivity using a nonisocapnic oxygen stimulus in patients with diabetes during homeostatic conditions and hyperglycemia.[4–6] These studies have reported either no difference in the magnitude of vascular reactivity response to hyperoxia during normoglycemic and hyperglycemic conditions,[6] or an impaired vascular reactivity response during hyperglycemia.[4,5] However, these studies are limited because of small sample sizes, many have not used simultaneous diameter and velocity measurements, and all did not control for systemic variation in arterial CO_2 during hyperoxic provocation. In contrast to our

TABLE 4. Blood Glucose Levels before and after Glucose Challenge, by Group

Group Blood Glucose	Group 1	Group 2	Group 3	Group 4
Baseline (mmol/L)	5.5 (0.7)	7.7 (2.3)	8.1 (3.7)	9.4 (2.6)
Effect (mmol/L)	8.9 (3.0)*†	14.8 (4.7)*	16.0 (4.1)*	18.2 (3.8)*
Absolute change (mmol/L)	+3.4 (3.0)	+7.1 (4.5)	+7.9 (2.4)	+8.7 (2.6)
Relative change (%)	+62	+92	+98	+94

Data are the mean ± SD.
* Significantly different from baseline.
† Significantly different from the other three groups.

TABLE 5. Edema Indices by Group

	Group 1	Group 2	Group 3	Group 4
500 μm radius (arbitrary units)	1.09 (0.29)	1.14 (0.40)	1.32 (0.24)	1.56 (0.39)*†
1500 μm radius (arbitrary units)	1.17 (0.21)	1.16 (0.23)	1.39 (0.20)*	1.48 (0.24)*†

Data are the mean ± SD.
* Significantly different from group 1.
† Significantly different from group 2.

study, no other work has been focused on early sight-threatening DR, culminating in DME. In most of the previous studies, investigators have measured vascular reactivity in venules; however, we studied the retinal arteriolar response, since the arterioles are responsible, at least in part, for the regulation of retinal vascular reactivity.

Patel et al.[4] assessed the vascular reactivity response to a nonisocapnic hyperoxic stimulus in patients with diabetes under conditions of normoglycemia and hyperglycemia. They concluded that patients with diabetes have impaired vascular reactivity that is exacerbated by hyperglycemia (>15 mmol/L). A possible explanation for the differences in results between that of Patel et al.[4] and our study is that a 60% FiO_2 stimulus (as opposed to >90% FiO_2) may reveal smaller alterations in retinal vascular reactivity between groups. Grunwald et al.[5] induced a reduction in blood glucose level in poorly controlled patients using exogenous insulin. Because of the vasoactive properties of insulin,[32] their findings are not directly comparable to ours. In agreement with our results, Davies et al.[6] reported that vascular reactivity was not affected by acute changes of blood glucose. In support of our observation is that glucose has a low basal retinal influx[33] and glucose transport operates near saturation levels at normal physiological glucose concentrations.[34] Glut-1 is one of the most important glucose transporters and is responsible for movement of glucose across the inner and outer blood retinal barriers.[35] Glut-1 expression is unchanged during short-term elevations of glucose, preventing increased glucose uptake across bovine retinal endothelial cells.[33]

Vascular reactivity has also been assessed in other vascular beds in animals[36] and humans.[37,38] Hamaty et al.,[36] reported that short-term hyperglycemia per se did not result in abnormal vascular responses in rat tail artery. Houben et al.[37] reported that endothelium-dependent or independent vasoreactivity was not affected by moderate-to-severe hyperglycemia in humans assessed by measuring skin and forearm blood flow. In addition, Capaldo et al.[38] were unable to detect a difference in change of velocity to a vasodilatory agent in the coronary circulation in normal subjects under baseline and hyperglycemic conditions. Previously published studies in animals have investigated preretinal oxygen tension using hyperoxia during hyperglycemia. Ernest et al.[39] reported increased oxygen tension, whereas others have reported no change in preretinal tension.[40,41] In addition, retinal oxygen consumption has been reported to increase[42] or remain unchanged[43] during hyperglycemia.

Previous work from our laboratory has shown that the retinal vascular reactivity response to isocapnic hyperoxia is a more sensitive marker of vascular dysfunction in patients with type 2 diabetes with early DR than homeostatic blood flow assessment.[2] When isocapnic hyperoxia was combined with concomitant hyperglycemia, however, the response was reduced in age-matched control subjects, whereas it was unaffected in a sample of patients with predominantly type 2 diabetes, so that the magnitude of vascular reactivity was equivalent between control subjects and patients with type 2 diabetes with early DR and DME. Our findings suggest that hyperglycemia may influence the retinal vascular reactivity response to hyperoxia in nondiabetic subjects. Future work will focus on the impact of hyperglycemia on vascular reactivity in patients with IGT.

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