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Glaucoma

A Pilot Study Assessing Retinal Blood Flow Dysregulation in Glaucoma Using Erythrocyte Mediated Velocimetry

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Purpose: The purpose of this study was to compare autoregulation of retinal arteriolar and venular blood flow in patients with glaucoma, glaucoma suspect participants, and control participants using erythrocyte mediated velocimetry.

Methods: This prospective cohort pilot study included 7 eyes of 5 participants with glaucoma, 15 eyes of 8 glaucoma suspect participants, and 11 eyes of 6 control participants. Mean erythrocyte velocity in retinal arterioles and venules was measured using erythrocyte mediated velocimetry at room air and after oxygen supplementation. Change in erythrocyte velocity was compared among all groups using generalized estimating equations.

Results: In total, 64 vessels (18 with glaucoma, 31 that were glaucoma suspect, and 15 controls) of 33 eyes of 19 participants were analyzed. There was no significant difference in baseline velocities in arterioles or venules among the three groups. With induction of hyperoxia, mean arterial erythrocyte velocity decreased in glaucoma ($-7.2 \pm 13.7\%$), which differed from controls and glaucoma suspects where erythrocyte velocity increased with hyperoxia by $4.6 \pm 13.3\%$ ($P = 0.002$) and $7.2 \pm 21.7\%$ ($P = 0.03$), respectively. A higher baseline arteriolar velocity ($\beta = -3.9\%$ per mm/s, $P = 0.002$), glaucoma diagnosis ($\beta = -21.1\%$, $P = 0.03$), and White race ($\beta = -20.0\%$, $P = 0.01$) were associated with decreased velocity in response to arterial hyperoxia.

Conclusions: Hyperoxia increased erythrocyte velocity in control and glaucoma suspect participants, but decreased erythrocyte velocity in glaucoma participants, possibly due to impaired autoregulation. Baseline velocity, glaucoma diagnosis, and White race were associated with a decrease in velocity with induction of hyperoxia.

Translational Relevance: Erythrocyte mediated angiography (EMA) permits precision measurements of blood flow which may aid in the development of biomarkers of glaucoma-related dysregulation of blood flow.

Introduction

Whereas current treatments of primary open-angle glaucoma target reduction of intraocular pressure (IOP), many patients experience worsening disease despite controlled IOP. Furthermore, a subset of patients with ocular hypertension has elevated IOP but no signs of disease, suggesting that other factors play a role in the pathogenesis and progression of glaucoma.[1] Low blood pressure (BP) and impaired ocular perfusion are independent risk factors for glaucoma, so measurement of ocular blood flow and its regulation may prove to be an alternative biomarker for glaucoma.[2,3]

Autoregulation of blood flow is defined as the "intrinsic ability of vascular beds to maintain constant blood flow despite fluctuating perfusion pressure and varying metabolic demand."[4] Patients with dysregulation of ocular blood flow have impaired ability to maintain perfusion with physiologic fluctuations in IOP and BP. This impairment may ultimately result in chronic, intermittent ischemic and reperfusion damage which contributes to glaucomatous optic neuropathy.[5] For this reason, the precise and accurate quantification of ocular autoregulation holds promise

as both an early biomarker for glaucoma as well as a novel therapeutic target. Retinal blood flow (RBF) autoregulation is of particular interest due to its ease of measurement and because RBF supplies the retinal ganglion cell soma. Like autoregulation of the cerebral circulation, RBF is dependent on local blood gas perturbations and the partial pressure of oxygen.[6–9] Hyperoxygenation induces a vasoconstrictive effect on the retinal microvasculature whereas hypercapnia induces vasodilatory effect.[10,11]

Microvascular retinal dynamic flow has been examined previously with various imaging modalities with promising results, although with certain limitations. Laser speckle flowgraphy offers reproducible measurements of relative RBF, measuring flow in arbitrary units.[12] Similarly, variants of optical coherence tomography angiography (OCTA) can assess relative RBF with limited detection in areas with low flow flux.[13,14] Adaptive optics scanning laser ophthalmoscopy (AOSLO) offers direct and dynamic quantification of RBF, but is limited spatially by a small field of view.[15] Consequently, our knowledge of the role of autoregulation of RBF in glaucoma remains incomplete.

Erythrocyte mediated velocimetry (EMAv) is a novel technique that permits direct visualization of indocyanine green (ICG)-labeled erythrocytes allowing quantification of erythrocyte velocity and dynamics. This allows for a highly precise and accurate assessment of autoregulation of retinal blood velocity.[16] We conducted a pilot study to characterize differences in the autoregulation of retinal blood velocity among control, glaucoma suspect, and glaucoma subjects. We tested autoregulation by measuring the change in retinal blood velocity to induced hyperoxia.

Methods

Study Design

We conducted a prospective cohort study assessing the differential effect of hyperoxia on erythrocyte velocity in a cohort of glaucoma, glaucoma suspect, and control participants. Our research adhered to the tenets of the Declaration of Helsinki and this protocol was approved by the Institutional Review Board of the University of Maryland School of Medicine.

Study Participants

We enrolled patients with glaucoma, patients who were glaucoma suspects, and control patients from the practice of the University of Maryland Department of Ophthalmology and Visual Sciences from February 2016 to April 2019. For this pilot study, sample size could not be ascertained a priori. Written informed consent was obtained from all participants after an explanation of the nature and possible consequences of the study. To be eligible for inclusion, participants had to be at least 18 years of age, had to be healthy control subjects or have clinical signs of open-angle glaucoma or were glaucoma suspects, had angles documented to be open on gonioscopy, and have best corrected visual acuity of at least 20/200 in the study eye. An experienced glaucoma specialist (author O.J.S.) determined the diagnosis of each enrolled participant based on the diagnostic criteria of the preferred practice patterns of the American Academy of Ophthalmology.[17,18] Participants were excluded if they had glaucoma secondary to any other cause, such as pigmentary dispersion, pseudoexfoliation, angle closure, or prior surgery, had significantly compromised visual acuity in the study eye due to concomitant ocular conditions, had retinal disease such as age-related macular degeneration or diabetic retinopathy, were participating in any other investigational drug study, had significant liver disease or uremia, had a known adverse reaction to ICG dye, iodine, or shellfish, or were pregnant or nursing. Blood pressure, pulse, oxygen saturation, intraocular pressure, and keratometry were recorded during the study visit. Participant demographics, medical history, ocular history, and medications were documented by review of the medical chart.

Erythrocyte Mediated Angiography

All participants underwent erythrocyte mediated angiography, which has been previously described.[16,19,20] Briefly, autologous erythrocytes are labeled with ICG and re-injected into the subject. Following pupillary dilation with tropicamide 0.5%, up to 1 mL of autologous ICG-loaded erythrocytes were injected intravenously. A Heidelberg Retinal Angiograph (HRA) 2 (Heidelberg Engineering GmbH, Heidelberg, Germany) was used to acquire 10 to 20 second angiograms of participants undergoing EMAv. Angiograms of the disc, macula, and peripapillary retina were obtained in both eyes of all participants except for one monocular participant. Angiograms were acquired at 24.6 frames per second with a 15-degree horizontal and 7.5-degree vertical field of view.

Erythrocyte Velocity Measurement

Erythrocytes were tracked manually frame by frame per a previously described protocol for erythrocyte velocity determination.[16] Graders were masked to

participant diagnosis and condition during analysis. To account for eye motion, images were registered using a custom MATLAB (MathWorks, version 2018a) script that performed spatial domain registration, as described previously.[16] Arterioles and venules were differentiated based on the direction of movement of labeled cells, either away from (arterioles) or toward (venules) the optic disc. Cells were eligible for tracking only if the same cell was visible on at least three consecutive frames within the vessel of interest. Erythrocytes visualized within the optic disc were excluded. All arterioles less than 80 microns in diameter and venules less than 100 microns in diameter which were captured within an individual angiogram were analyzed. Mean velocity for each vessel was calculated by averaging the individual erythrocyte velocity measurements with a minimum requirement of 30 measurements per angiogram. Angiograms were only eligible for inclusion if they included at least 85 frames. Angiograms and individual vessels were excluded from analysis if erythrocytes could not be visualized in the vessels for tracking or the inclusion criteria for tracking were not met. Lack of erythrocyte visualization was due to excessive eye movement or media opacity, such as dry eye or cataract, which precluded adequate image registration. In the case of the two participants with repeat imaging of eligible vessels in both room air and oxygen conditions, the first visit was included in the analysis.

To ensure uniformity in location for comparisons of average erythrocyte velocity between conditions and between groups, the same vessel segment was analyzed between conditions and vessel segments chosen for analysis were a maximum length of 1500 microns from the optic disc.

Vessel Diameter Measurement

The diameter of each vessel at baseline was determined by conventional ICG or fluorescein angiography conducted for all subjects concurrently with EMAv imaging. Diameter measurements were obtained from ICG or fluorescein angiograms using the Automated Retinal Image Analyzer (ARIA) MATLAB script published by Bankhead et al.[21] To account for possible variations in vessel diameter with the cardiac cycle, diameter was measured for the same vessel segment across 5 separate frames, each approximately 200 milliseconds apart, then the measurements were averaged.[22] Arterioles less than 80 microns in diameter and venules less than 100 microns in diameter were analyzed as the higher velocity of erythrocytes in larger vessels could not be accurately captured at our temporal resolution and larger vessels may have higher lateral erythrocyte movement, artificially increasing velocity measurements. Branching vessels were excluded to limit the analysis to a single vessel segment and eliminate the variation in velocity attributable to branching.

Autoregulation Testing With Supplemental Oxygen

Image acquisition was conducted under two conditions – room air and mild hyperoxia. In the mild hyperoxia condition, 3 liters per minute of supplemental oxygen (100% oxygen concentration) was administered by nasal cannula, resulting in approximately 32% fraction of inspired oxygen. Five minutes were allotted for equilibration of oxygen saturation prior to imaging when switching from the room air condition to supplemental oxygen or vice versa. Participants were instructed not to breathe through their mouths while the nasal cannula was in place. Absolute change and percent change in velocity from baseline to hyperoxia conditions was calculated.

Statistical Analysis

We compared glaucoma, glaucoma suspect, and control participants in all demographic and clinical variables using analysis of variance (ANOVA) with post hoc Tukey analysis. To account for repeated measures in participants, such as multiple vessels or multiple eyes measured, generalized estimating equations (GEEs) were used for bivariate and multivariable analysis. To determine the predictors of autoregulation, we conducted a bivariate analysis using percent change in velocity from room air to oxygen as the dependent variable and all demographic and clinical variables as independent variables. Variables that were significant on bivariate analysis were then considered for inclusion in the multivariable model with percent change in velocity as the outcome variable. All statistical analysis was completed on SPSS version 25.0 (IBM, Armonk, NY, USA).

Results

Thirty-seven subjects were enrolled for this study. The enrollment flowchart is shown in Figure 1.

A total of 64 vessels (27 arterioles and 37 venules) of 33 eyes of 19 participants (5 with glaucoma, 8 who were glaucoma suspect, and 6 controls) were analyzed in this study. The demographic characteristics of the participants are summarized in Table 1. Table 2 shows the baseline comparison of arterioles and Supple-

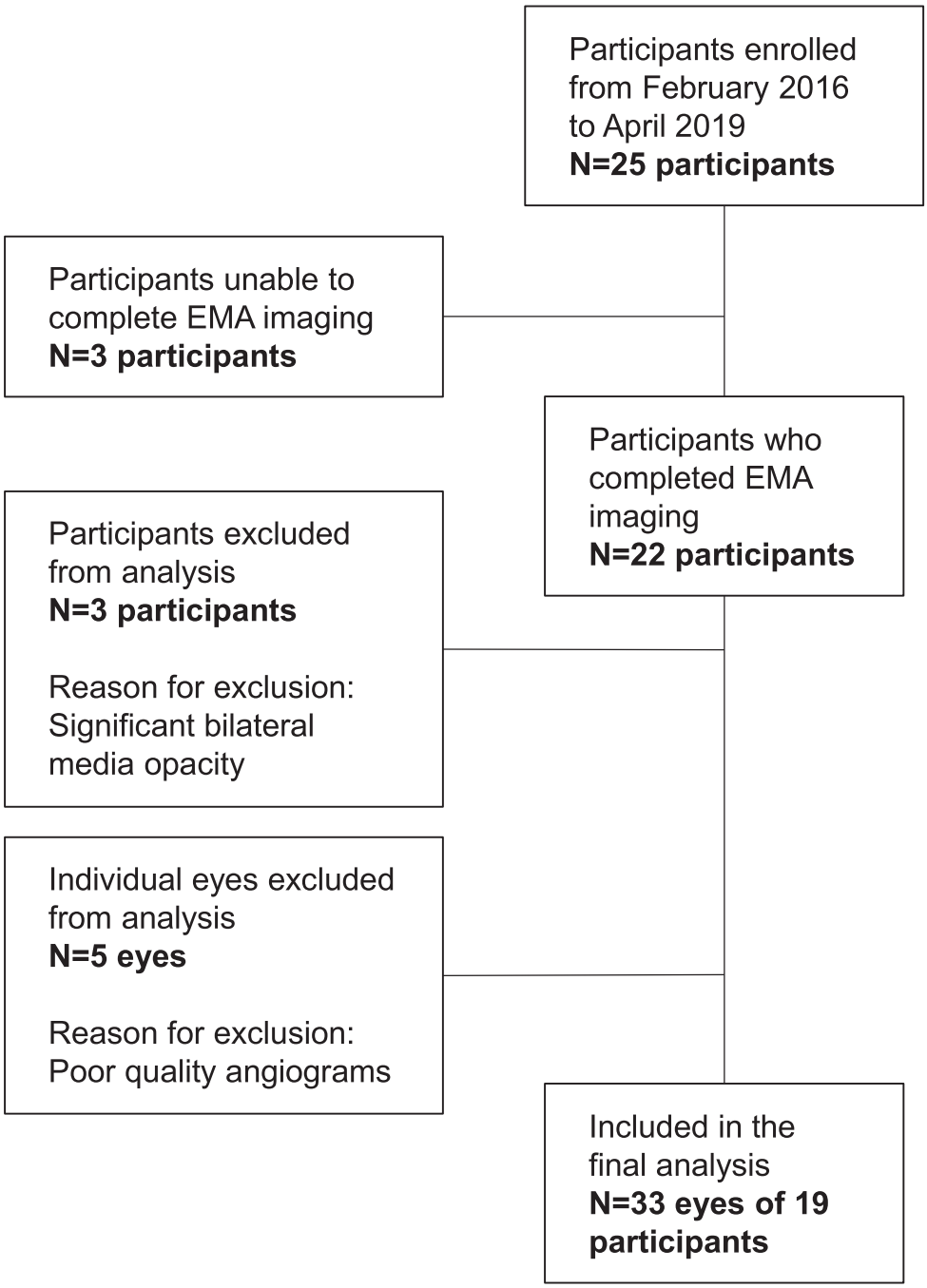

Figure 1. Study enrollment flowchart. Incomplete EMA imaging was due to equipment malfunction, elevated blood pressure, and vasovagal reaction. Angiograms were graded as poor quality if the minimum requirement of 30 measurements per angiogram could not be met. EMA, erythrocyte mediated angiography.

mental Table S1 shows the baseline comparison of venules.

Baseline arteriole diameter was smallest in participants with glaucoma (34.2 ± 9.0 microns), as compared to those who were glaucoma suspect (42.9 ± 11.1 microns) and control (68.8 ± 27.9 microns) participants ($P < 0.001$), consistent with arteriolar narrowing in glaucoma.[23] When comparing arteriole measurements, the cup-to-disc ratio and OCT retinal nerve fiber layer (RNFL) thickness was significantly different among groups, and glaucoma suspects and participants with glaucoma were more likely to be on glaucoma medications as expected. The glaucoma suspect group had more women as compared to the

Table 1. Participant Demographics

Characteristic	Control	Glaucoma Suspect	Glaucoma
Number of participants	6	8	5
Age, y	53.2 ± 14.0	55.6 ± 10.6	59.0 ± 6.0
Sex, *n* (%)[a]			
Male	3 (50)	0	2 (40)
Female	3 (50)	8 (100)	3 (60)
Race/ethnicity, *n* (%)[a]			
White	4 (80)	4 (50)	1 (20)
Black	2 (20)	3 (38)	4 (80)
Asian	0	1 (12)	0
Diabetes mellitus, *n* (%)	1 (17)	1 (12)	3 (60)
Hypertension, *n* (%)	3 (50)	6 (75)	1 (20)
Use of any IOP-lowering medication, *n* (%)[a]	0	3 (38)	4 (80)
Topical beta blocker[a]	0	1 (13)	3 (60)
Systemic and topical beta blocker[a]	0	2 (25)	4 (80)
Prostaglandin analog	0	3 (38)	3 (60)

Data are presented as mean ± standard deviation for continuous variables or as number (%) for categorical variables. IOP, intraocular pressure.

[a]Statistical significance at the $P < 0.05$ level by one-way analysis of variance for the comparison of continuous variables and chi-square tests for the comparison of categorical variables.

control and glaucoma groups ($P = 0.01$), and the glaucoma group had more African Americans as compared to the control and glaucoma suspect groups ($P = 0.01$). There was no significant difference in IOP, mean ocular perfusion pressure (MOPP), or mean deviation (MD) between groups, but there was a trend towards higher IOP, lower MOPP, and lower MD in participants with glaucoma as expected.

In the comparison of clinical and demographic variables for eyes in which venular velocity was measured (see Supplemental Table S1), participants with glaucoma had thinner RNFL, larger cup-to-disc ratios, and were more likely to be on glaucoma medications. Participants within this subset with glaucoma also had a higher incidence of diabetes mellitus ($P = 0.01$). Venules had an average baseline diameter of 43.0 ± 13.1 microns in the healthy control group, 49.0 ± 11.0 microns in the glaucoma suspect group, and 38.5 ± 6.7 microns in the glaucoma group ($P > 0.05$).

The mean baseline erythrocyte velocity across all included vessels was 6.41 ± 2.11 mm/s. Consistent with physiologic differences, arterioles had a higher mean baseline velocity of 7.09 ± 2.41 mm/s compared to venules at 5.90 ± 1.71 mm/s ($P = 0.02$). For arterioles, the mean erythrocyte velocities at baseline were 6.73 ± 2.30 mm/s for control participants, 6.73 ± 2.16 mm/s for glaucoma suspect participants, and 7.94 ± 2.94 mm/s for participants with glaucoma ($P > 0.05$ across all groups). For venules, the mean erythrocyte velocities at baseline were 5.01 ± 1.79 mm/s for control participants, 6.32 ± 1.51 mm/s for glaucoma suspect participants, and 6.12 ± 1.76 mm/s for participants with glaucoma ($P > 0.05$ across all groups).

The change in velocity between conditions is demonstrated in the EMAv superimposed images in Figure 2, which show the frame-by-frame tracking of an ICG-labeled erythrocyte at baseline and with supplemental oxygen in a glaucomatous arteriole.

We identified a total of 46 vessels from the larger data set of 64 vessels that were imaged and analyzed in both room air and oxygen conditions to compare their paired velocities. Among this subset of paired vessels, the mean absolute change in arterial velocity in glaucoma eyes declined by an average of 0.79 ± 1.28 mm/s from room air to hyperoxia, which differed significantly from control eyes (increased by 0.27 ± 0.75 mm/s, $P = 0.001$) but not glaucoma suspect eyes (increased by 0.22 ± 1.59 mm/s, $P = 0.06$; Fig 3a). To account for baseline velocity, percent change from baseline in the arterioles was calculated to be -7.2 ± 13.7% in glaucoma vessels, which differed significantly from control (4.6 ± 13.3%, $P = 0.002$) and glaucoma suspect vessels (7.2 ± 21.7%, $P = 0.03$; Fig 3b). There were no significant differences in venule velocity among groups.

On bivariate analysis, we found that diagnosis, baseline velocity, and RNFL thickness in corre-

Table 2. Baseline Comparison of Demographics and Clinical Variables for Arterioles by Diagnosis in a Bivariate GEE Model

Parameter	Control	Glaucoma Suspect	Glaucoma
Number of participants	3	7	5
Number of eyes	4	9	6
Number of vessels	5	14	8
Age, y	60.4 ± 4.7	56.8 ± 9.2	58.1 ± 6.2
Race[a]			
Black	1 (20)	2 (14)	6 (75)
White	4 (80)	11 (78)	2 (25)
Asian	0 (0)	1 (7)	0 (0)
Gender[a]			
Male	3 (60)	0 (0)	2 (25)
Female	2 (40)	14 (100)	6 (75)
Hypertension	3 (60)	11 (78)	3 (38)
Diabetes mellitus	1 (20)	2 (14)	4 (50)
Systolic blood pressure, mm Hg	131.8 ± 16.2	125.8 ± 19.7	127.4 ± 21.5
Diastolic blood pressure, mm Hg	81.6 ± 10.7	77.4 ± 9.7	73.2 ± 9.0
Mean arterial pressure, mm Hg	98.3 ± 12.0	93.5 ± 12.4	91.3 ± 12.8
IOP, mm Hg	16.3 ± 2.3	15.7 ± 4.8	18.0 ± 8.6
Mean ocular perfusion pressure, mm Hg	54.7 ± 6.3	46.9 ± 8.1	42.9 ± 13.6
Spherical equivalent refractive error, D	0.9 ± 2.2	−1.2 ± 2.8	−0.2 ± 0.4
Mean deviation, dB	Not available	0.5 ± 1.5	−10.6 ± 14.8
RNFL thickness (total), µm[a]	94.8 ± 4.6	90.9 ± 11.8	61.25 ± 27.4
RNFL thickness (quadrant), µm[a]	102.4 ± 30.8	87.0 ± 34.1	42.1 ± 24.5
Cup-to-disc ratio[a]	0.34 ± 0.02	0.65 ± 0.18	0.84 ± 0.13
Use of any IOP-lowering medications, *n* (%)[a]	0 (0)	11 (79)	8 (100)
Topical beta blockers[a]	0 (0)	0 (0)	4 (50)
Topical and/or systemic beta blockers[a]	0 (0)	7 (50)	6 (75)
Prostaglandin analogs[a]	0 (0)	4 (28)	4 (50)

Data are presented as mean ± standard deviation for continuous variables or as number (%) for categorical variables. D, diopters; IOP, intraocular pressure; mm Hg, millimeters of mercury.

[a]Statistical significance at the $P < 0.05$ level in the comparison of control, glaucoma suspect, and glaucoma groups as determined by generalized estimating equation (GEE) to account for multiple vessels per individual.

sponding quadrants to the measured vessels were significantly associated with percent change in arteriolar velocity (Table 3). Given the covariance between RNFL thickness and glaucoma, we did not include RNFL thickness in the final multivariable model.

Multivariable analysis controlling for glaucoma diagnosis, baseline velocity, sex, and race found that higher baseline arteriolar velocity was associated with a decrease in percent change in arteriolar velocity of 3.9% per mm/s ($P = 0.002$). Glaucoma diagnosis was associated with a decrease of 21.1% as compared to control participants ($P = 0.03$), and White race was associated with a decrease of 20.0% as compared to non-White participants ($P = 0.01$).

Discussion

We present the first study to directly show impaired retinal microvascular autoregulation in human subjects with glaucoma as compared to controls using EMAv. This is also the first study to quantify differences in autoregulation of blood velocities in vessels as small as 30 microns and to find that the difference in vasoreactivity between participants with glaucoma and healthy controls is most prominent in arterioles. Conversely, there was no statistically significant difference in vasoreactivity in the venules. Although we did not see any differences in baseline velocity between participants with glaucoma and control participants,

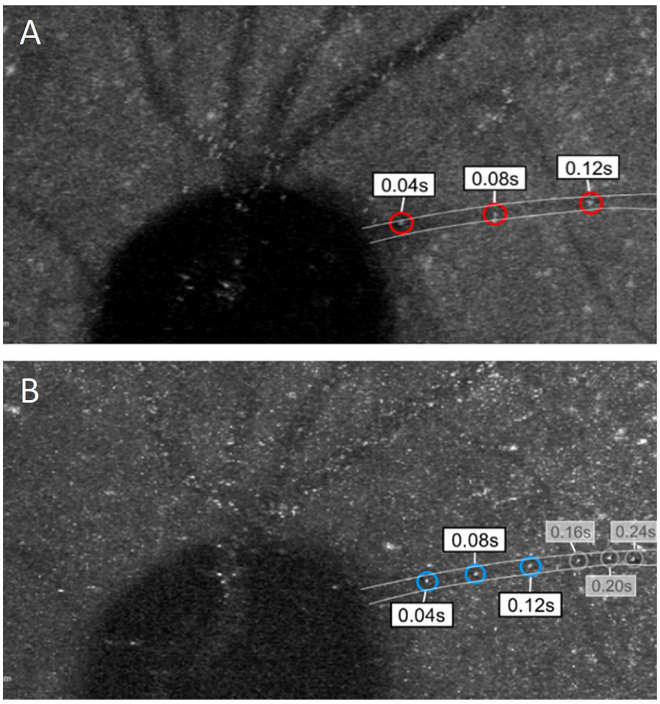

Figure 2. Superimposed images demonstrating tracked erythrocytes within the same retinal arteriole in (**A**) room air and (**B**) hyperoxia conditions using erythrocyte mediated velocimetry. Consecutive frames at 0.04 seconds, 0.08 seconds, 0.12 seconds, and additional 0.4 second intervals were superimposed to demonstrate the movement of one cell over time.

Table 3. Bivariate GEE Analysis of Demographics and Clinical Variables With Percent Change in Arteriolar Velocity With Hyperoxia

Parameter	Difference in Percent Change in Arteriolar Velocity	*P* Value
Age	0.23	0.65
Race		
White	REF	REF
Black/Asian	1.15	0.85
Sex		
Male	REF	REF
Female	6.17	0.21
Hypertension	2.53[b]	0.69
Diabetes mellitus	2.08[b]	0.75
Systolic blood pressure	0.15	0.17
Diastolic blood pressure	0.45	0.09
Mean arterial pressure	0.31	0.09
Intraocular pressure	0.51	0.27
Mean ocular perfusion pressure	0.17	0.52
Spherical equivalent error	1.04	0.48
Mean deviation	0.27	0.10
Diagnosis		
Control	REF	
Suspect	2.67	0.72
Glaucoma[a]	−11.8	0.002
RNFL (total)	0.12	0.25
RNFL (quadrant)[a]	0.11	0.008
Cup-to-disc ratio	−14.9	0.21
Baseline velocity[a]	−3.89	0.002
Baseline vessel diameter	0.21	0.18
Velocity after oxygen supplementation	0.12	0.95

IOP, intraocular pressure; RNFL, retinal nerve fiber layer.
[a]Statistical significance at the $P < 0.05$ level.
[b]Reference group was participants without the listed condition.

our work shows that there are profound differences in the microvascular arterial response to mild hyperoxia between the two groups, hence providing new information on RBF dysregulation in glaucoma.

Prior work supports the concept that differences in the response to hyperoxia reflect underlying changes in the ocular microvasculature in subjects with glaucoma. Prior work has focused on differences in the response of hyperoxia in the optic nerve head[4] or on differences in capillary density.[24] Color Doppler ultrasound has been used to show that hyperoxia affects larger retrobulbar arteries and that this response is blunted in subjects with glaucoma.[25] Kiyota et al.[4] showed that systemic hyperoxia resulted in a weaker response in optic nerve head microcirculation as measured with laser speckle flowgraphy. They hypothesized that preexisting vasoconstriction in subjects with glaucoma may reduce the capacity of a vasoconstrictive response to hyperoxia, and our study suggests that this may be true on the level of the individual vessel. Additionally, there is controversy over whether neurovascular coupling specifically occurs at the level of the arteriole with smooth muscle playing a role or at the level of the capillary due to pericytes.[26–29] In our study, we demonstrate differences in vasoreactivity in arterioles, but not in venules. Like cerebral vasculature, we hypothesize that retinal arterioles, which are resistance vessels, play a key role in the vasoreactive response to oxygen.[26,27] Further studies focused on capillary flow are needed.

Neurovascular coupling in glaucoma has been previously studied using the retinal vessel analyzer (RVA) which measures vessel dilation in response to flicker light. Within larger arteries and veins, Gugleta et al. found that general vessel response to flicker light was decreased in subjects with glaucoma compared to controls.[30] Our study further demonstrates impaired vascular responses in glaucoma at the level of the

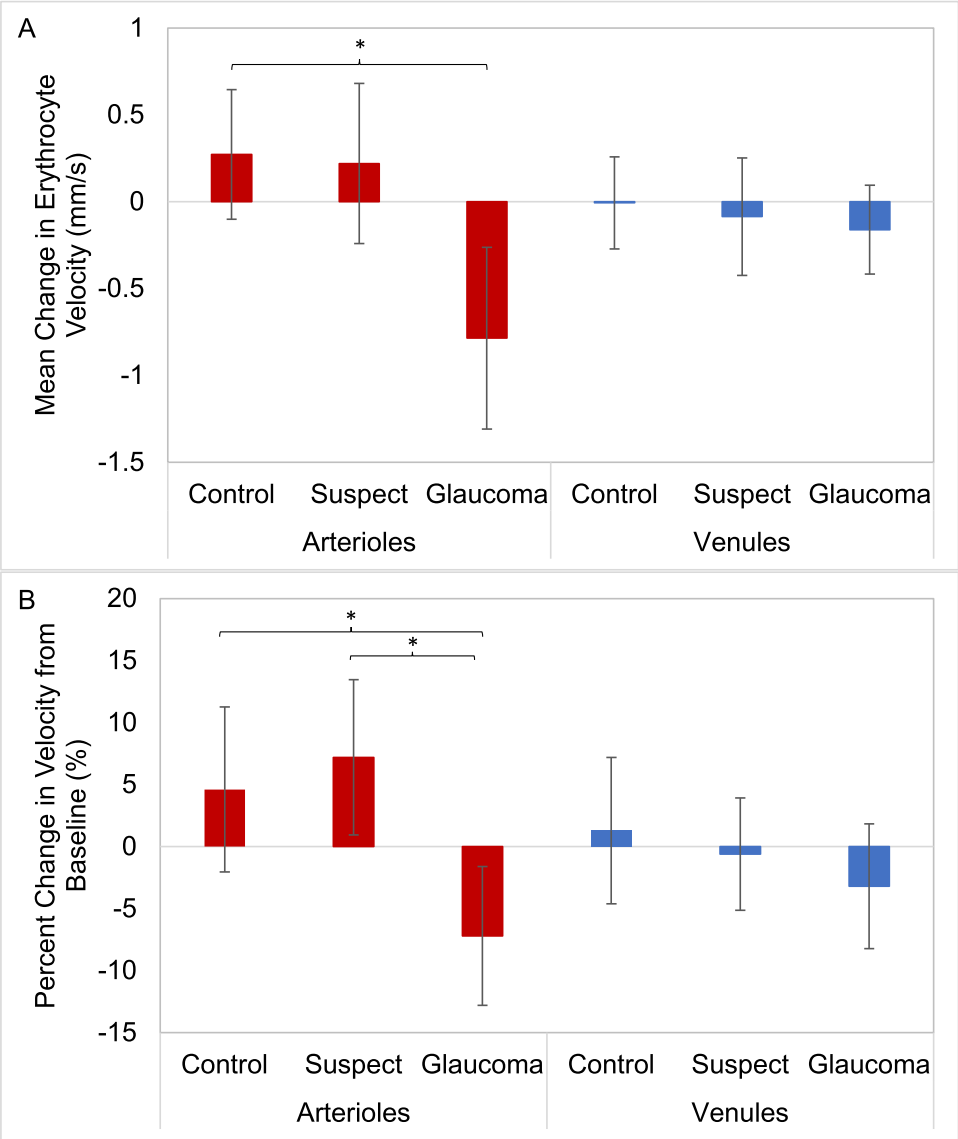

Figure 3. Bar graphs illustrating (**A**) mean absolute change in erythrocyte velocity and (**B**) percent change from baseline velocity with hyperoxia in arterioles and venules from the subset of paired vessels ($n = 46$) among the three study groups. Means are shown with standard error of the mean. *Statistical significance at the $P < 0.05$ level as determined by generalized estimating equation (GEE) to account for multiple vessels per individual.

arterioles. Notably, RVA measures diameter changes whereas this study assessed blood velocity. One limitation of RVA is that it is unable to assess vessels of diameter less than 90 micrometers, limiting its applicability in determining microvascular RBF.[31]

Although no significant differences were noted in velocities in baseline conditions, erythrocyte velocity declined in glaucomatous retinal arterioles but increased in controls when mild hyperoxia was induced. These changes in velocity may be secondary to changes in blood flow, arteriolar diameter secondary to abnormal relative vasodilation, or diameter of upstream or downstream vessels from the measured region. Vasoconstriction in larger upstream arteries and lack of vasoconstriction in downstream arterioles and capillaries may explain the findings in glaucomatous eyes. Further work looking at the changes in vessel diameter and flowrates before and after induction of hyperoxia in large and small retinal vessels is needed.

EMAv allows for the assessment of local autoregulation, assessing the change in velocity within an individual vessel, which can then be compared to the structural changes in the tissue supplied by that vessel. Our data show an association of the thickness of the RNFL quadrant associated with a given vessel and the change in velocity with hyperoxia, indicating that areas more affected by glaucoma may have greater dysregulation of blood flow. This is further illustrated in

Supplemental Figure S2, which shows superotemporal RNFL thinning in a patient who is a glaucoma suspect, and potentially impaired autoregulation in a vessel adjacent to the area of thinning. In this adjacent area, the RNFL is at the lower limit of normal, but the vessel identified within it feeds an area of relative ganglion cell layer thinning as evidenced by asymmetry across the midline. The impaired autoregulation may represent a potential earlier vascular change that precedes detection of structural loss.

Whereas this pilot study presents promising data regarding vascular dysregulation in glaucoma and will aid in sample size calculations for future studies, we note that the relatively small sample size may limit the conclusions that may be made. Although diabetes may affect the retinal vascular response to hyperoxia,[32] there was no significant difference in diabetes prevalence in all three groups within the arteriole subset analysis and none had overt diabetic retinopathy. ICG angiography was not consistently repeated in both baseline and hyperoxia conditions, so changes in diameter or flowrate could not be analyzed. The use of nasal cannula allowed for the assessment of the effect of mild hyperoxia on blood flow using a modest dose of supplemental oxygen, which increased oxygen saturation from an average of 98.5% to 99.3%; but a nonrebreathing system may have allowed for greater control of oxygen and carbon dioxide levels, both of which affect flow rates.[33] Due to limitations in temporal resolution (frame rate), we excluded arterioles larger than 80 microns and venules larger than 100 microns. Whereas this allowed for an assessment of autoregulation in the microvasculature, future studies will utilize higher frame rates to measure erythrocytes moving at higher velocities in larger vessels. A multimodal approach to the study of autoregulation using different technologies for assessing blood flow may be required for a comprehensive assessment of autoregulation in glaucoma.

Ultimately, dysregulation of RBF may be a candidate biomarker for early glaucoma and, thus, its characterization may aid in the identification of patients at high risk for glaucoma progression and the development of novel therapeutics aimed at the ocular vasculature. Further work will be required to achieve this goal.

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