

Acute Hyperglycemia Reverses Neurovascular Coupling During Dark to Light Adaptation in Healthy Subjects on Optical Coherence Tomography Angiography

Changyow C. Kwan,¹ Hee Eun Lee,¹ Gregory Schwartz,^{1,2} and Amani A. Fawzi¹

¹Department of Ophthalmology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States

²Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States

Correspondence: Amani A. Fawzi, Department of Ophthalmology, Feinberg School of Medicine, Northwestern University, 645 N. Michigan Avenue, Suite 440, Chicago, IL 60611, USA; afawzimd@gmail.com.

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PURPOSE. To test the hypothesis that hyperglycemia perturbs neurovascular coupling and compromises retinal vascular response during transition from dark to light in healthy subjects using optical coherence tomography angiography (OCTA).

METHODS. Ten eyes of 10 healthy subjects were tested, first during fasting and then after receiving a 75-g oral glucose solution. In both sessions, OCTA imaging was done in the dark-adapted state and at 50 seconds, 2 minutes, 5 minutes, and 15 minutes of ambient light. Parafoveal vessel density (VD) and adjusted flow index (AFI) were calculated for the superficial capillary plexus (SCP), middle capillary plexus (MCP), and deep capillary plexus (DCP), and vessel length density was calculated for the SCP. These measurements were compared among conditions after adjusting for age, refractive error, and OCTA scan quality.

RESULTS. Hyperglycemia leads to a complete reversal of dark/light adaptation trends in VD and AFI in all layers of the inner retina. In the dark, there is significantly decreased VD in the DCP in hyperglycemia. With a transition to light in hyperglycemia, we observed decreased VD in the SCP, increased vessel density in the MCP and DCP, and decreased AFI in all three layers.

CONCLUSIONS. Our results show that hyperglycemia significantly disrupts neurovascular coupling in healthy eyes, with potential metabolic deficits affecting photoreceptor oxygen demands during dark adaptation and the inner retina during light exposure. In pathological states, such as diabetic retinopathy, where the vasculature is already attenuated, retinal neurons may be exquisitely vulnerable to intermittent hyperglycemic challenge, which should be the focus of future studies.

Keywords: hyperglycemia, neurovascular coupling, dark adaptation, optical coherence tomography angiography, diabetic retinopathy

Neurovascular coupling describes the functional regulation of blood flow in response to neuronal activity in the brain and the eye.^{1,2} In the eye, neurovascular coupling anticipates the changing metabolic demands of the different retinal neurons under different lighting conditions.³ Photoreceptors have one of the highest metabolic rates of any cell in the body, and their greatest oxygen demand occurs in the dark.^{4,5} Laser Doppler studies have demonstrated increased retinal blood flow in the dark.^{6–8} Berkowitz et al.⁹ also demonstrated decreasing blood flow with light exposure in the inner retina of mice using monocrystalline iron oxide nanocolloid magnetic resonance imaging. Using optical coherence tomography angiography (OCTA), our group showed that the deep retinal capillary plexus has maximal density in the dark, which we hypothesized would be necessary to meet the metabolic demands of the photoreceptors. With transition to ambient light, we found that the density of the deep and middle retinal capillary plexuses decreased along with increased density of superficial reti-

nal capillaries, consistent with increased metabolic activity in the inner retina.¹⁰

Hyperglycemia has been shown to impact neurovascular coupling in human eyes. A previous study using a Zeiss retinal vessel analyzer (Carl Zeiss Meditec, Jena, Germany) found that acute hyperglycemia attenuates retinal vascular dilation in response to flicker stimulation in healthy subjects.¹¹ Another study using infrared photography in patients with type 2 diabetes mellitus and minimal or no diabetic retinopathy (DR) found decreased peripapillary vessel diameter during dark adaptation in hyperglycemia compared to fasting conditions.¹² Based on these studies, we hypothesized that hyperglycemia will perturb neurovascular coupling in healthy subjects and compromise retinal vascular response during dark adaptation.

Understanding the acute effects of hyperglycemia on retinal blood flow autoregulation is important because a loss of physiologic neurovascular coupling can be seen in many chorioretinal diseases, including DR.¹³ In pathological states,

the retinal vasculature is already compromised, with lower vascular density. In these situations, the attenuated remaining vasculature may be more vulnerable to the acute effects of hyperglycemia. Clarifying these effects in healthy subjects will inform future studies on neurovascular coupling in DR and other disease states.

In this novel study, we sought to clarify the acute effects of hyperglycemia on each of the three macular capillary plexuses during dark adaptation and transition to light in healthy subjects using OCTA, a non-invasive modality capable of high-resolution, depth-resolved imaging of the retinal capillary plexuses *in vivo*.¹⁴ The techniques used in these previous studies assess only changes in the relatively larger vessels of the superficial retina. To our knowledge, no previous studies have examined the changes in the individual retinal capillary plexuses in response to hyperglycemia during dark adaptation and transition to ambient light

METHODS

This was a prospective study that recruited 10 healthy subjects from the Department of Ophthalmology at Northwestern University in Chicago, Illinois, between February and April 2019. This study followed the tenets of the Declaration of Helsinki, was performed in accordance with the Health Insurance Portability and Accountability Act regulations, and was approved by the Institutional Review Board of Northwestern University. Healthy volunteers were recruited to participate, and written informed consent was obtained from all participants. Exclusion criteria included ocular disease, media or lens opacities, and refractive error greater than ± 6.0 diopters. We also excluded subjects with systemic conditions that could affect retinal circulation, such as diabetes mellitus, smoking, and hypertension.

Optical Coherence Tomography Angiographic Imaging

We acquired 3×3 -mm OCTA scans centered on the fovea using the RTVue-XR Avanti OCT System (Optovue, Inc., Fremont, CA, USA), which incorporates the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm.¹⁵ The OCTA device uses a light source centered on 840 nm with a full width at half-maximum bandwidth of 45 nm and an A-scan rate of 70,000 scans per second. The system captures two consecutive B-scans (M-B frames) at each location on the retina, with each B-scan containing 304 A-scans, and uses an orthogonal registration algorithm to reduce motion artifacts and improve the signal-to-noise ratio.¹⁶ An M-scan is a repeated zero-dimensional scan at a single position, whereas a B-scan is a one-dimensional scan along a single axis. An M-B frame is comprised of multiple A-scans taken at one lateral position before switching to the next position.¹⁷ The SSADA algorithm extracts angiographic flow information by quantifying the OCT reflectance decorrelation between two consecutive B-scans. Only images with a quality score (Q-score) of 7 or greater (manufacturer's recommendation), with signal strength index (SSI) of 50 or greater, and without large movement or shadow artifacts were considered eligible for further analysis.

Imaging Protocol

In the control experiment, 10 healthy subjects underwent dark adaptation in one eye by wearing a thick eye patch over the left eye for 45 minutes in a completely dark room. The room was completely dark except for the OCTA computer monitor, which was adjusted to display only red light. Red light does not disrupt dark adaptation, as rods (the primary cells involved in low-light vision) are insensitive to the long wavelengths of red light.¹⁸ We then removed the patch and scanned the macula while the eye was still dark adapted. We maintained OCTA focus on the retina following the dark-adapted eye scan to ensure rapid imaging in light. The lights in the room were turned on to ambient levels (800 candela/m²), and we then obtained single OCTA images at four time points (50 seconds and 2, 5, and 15 minutes).

In the glucose experiment, baseline blood glucose was measured. Each subject was given 5 minutes to consume a standard 75-g oral glucose tolerance test solution. Subjects then underwent dark adaptation and OCTA imaging as described above.

The same 10 subjects participated in both experiments. Participants were instructed not to consume any food or drinks other than water for 8 hours prior to the experiment. All images were obtained before noon in order to minimize diurnal variation.

Image Analysis

Using the built-in AngioVue Analytics software (version 2017.1.0.151) with projection artifact removal, we first segmented the superficial capillary plexus (SCP), middle capillary plexus (MCP), and deep capillary plexus (DCP). The SCP was segmented from the internal limiting membrane (ILM) to 10 μm above the inner plexiform layer (IPL) to encompass the nerve fiber and ganglion cell layers. The MCP was segmented from 10 μm above to 30 μm below the IPL to encompass the IPL. The DCP was segmented from 30 μm below the IPL to 10 μm below the outer plexiform layer (OPL) to encompass the OPL. For thresholding, we segmented the full retinal thickness OCTA from the ILM to 10 μm below the OPL.

We used the AngioVue Analytics software to obtain parafoveal vessel density (VD) for each of the three capillary plexuses. The parafovea was defined as an annulus centered on the fovea with inner and outer ring diameters of 1 mm and 3 mm, respectively. VD was calculated as the percent of total retinal blood vessels comprising the parafoveal area so that vasodilation presents as an increase in VD and vasoconstriction presents as a decrease in VD.

We then exported the SCP, MCP, and DCP angiograms into ImageJ software (National Institutes of Health, Bethesda, MD, USA)¹⁹ to calculate the parafoveal adjusted flow index (AFI). The AFI is an indirect and relative measure of flow velocity based on pixel intensity, which is related to flow velocity within a limited range in OCTA.²⁰ Two independent graders (CCK, HEL) obtained these measurements through a global threshold as previously described.²¹ We also calculated the vessel length density (VLD) for the SCP to eliminate the influence of larger arterioles and venules on the density measurement. We binarized and skeletonized the parafoveal SCP angiogram and used the following equation to obtain the VLD (in units of mm⁻¹): skeletonized vessel length (mm)/parafoveal area (mm²). **Figure 1** is a schematic of how the AFI and VLD measurements were obtained.

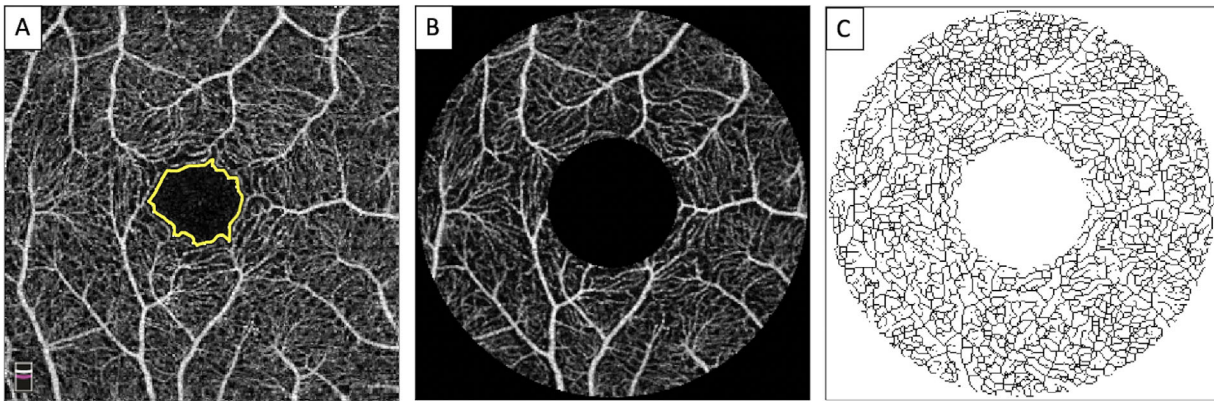


FIGURE 1. Schematic of OCTA parameters. (A) Full retinal thickness OCTA with yellow outline delineating the foveal avascular zone used to establish the noise threshold. (B) OCTA image of parafoveal zone of the SCP with elimination of pixels below threshold. Average pixel intensity above the threshold was used to calculate adjusted flow index. (C) Binarized and skeletonized image of parafoveal SCP vessels used to calculate vessel length density.

TABLE 1. Subject Characteristics

Characteristic	Value (mean \pm SD)
Number of males, n (%)	5 (50)
Age, y	28.4 \pm 5.72
Refractive error, diopters	-1.10 \pm 1.81
Blood glucose, mg/dL	
Pre-oral glucose tolerance test	91.3 \pm 11.01
Post-oral glucose tolerance test	134.0 \pm 21.78
Change	42.7 \pm 21.03

Additionally, we used bUnwarpJ,²² an algorithm for elastic image registration, to align images and create pseudo-colored composites (see Fig. 3).

Statistics

We performed statistical tests with SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA). A two-way random intraclass correlation coefficient (ICC) was used to assess intergrader reliability for AFI and VLD measurements. Shapiro-Wilk tests were used to determine if data were normally distributed. Paired *t*-tests were used to determine if there was a significant difference in blood sugar before and after administration of the glucose solution. Pearson correlations were used to determine whether Q-score or SSI had a stronger correlation with vessel density in each of the three layers. We then used a mixed linear model adjusted for age, Q-score (based on stronger Pearson correlation than SSI), and refractive error to assess for differences in the AFI, VD, and VLD at different time points between conditions. Bonferroni correction was used to account for multiple comparisons. Values at each time point in ambient light were normalized to the value in dark within each condition. $P < 0.05$ was considered statistically significant.

RESULTS

Ten eyes of 10 healthy subjects (age, 28.4 \pm 5.7 years; 5 females) were included in the study. Blood sugar increased significantly after consumption of the glucose solution ($P < 0.01$). Subject characteristics are reported in Table 1. Intergrader reliability for AFI and VLD was excellent, with an

ICC of 0.996 (95% confidence interval [CI], 0.992–0.998) for AFI and 0.994 (95% CI, 0.977–0.999) for VLD. Shapiro-Wilk tests indicated that the data were normally distributed. Pearson correlation demonstrated moderate correlation between Q-score and VD in the SCP (0.484), MCP (0.388), and DCP (0.487) ($P < 0.05$ for all three layers). Pearson correlation between SSI and VD was significant in the SCP (0.421; $P < 0.05$) and DCP (0.476; $P < 0.05$) but not the MCP (0.024; $P = 0.867$).

After adjusting for age, refractive error, and Q-score, we compared the OCTA parameters in hyperglycemia to those in control conditions. In the dark, there was an increase in VD in the SCP and a decrease in the MCP and DCP in hyperglycemia (Fig. 4A); however, only DCP VD reached significance ($P < 0.001$). Figures 2 and 3 shows representative OCTA images of the SCP and DCP, respectively, at each time point in each condition. There was a trend of increased AFI in all three layers, but none reached significance (Fig. 5A). There were no significant differences in VLD at any time point (Table 2).

With transition to ambient light, hyperglycemia caused a distinct reversal of VD and AFI trends in all three layers of the retina. In the control condition, with light adaptation, VD increased in the SCP and decreased in the MCP and DCP. In hyperglycemia, VD decreased in the SCP and increased in the MCP and DCP (Figs. 4B–4D). The difference between hyperglycemia and control was significant at all time points of ambient light in all three layers (Table 2). The AFI followed a similar trend in all of the layers and increased with ambient light in the control condition. In hyperglycemia, the reverse trend of decreased AFI was seen in all layers (Figs. 5B–5D). The difference in the AFI between hyperglycemia and control was significant at 2 minutes, 5 minutes, and 15 minutes in the SCP and MCP and at 5 minutes in the DCP (Table 2).

DISCUSSION

Using OCTA, we assessed inner retinal hemodynamics and neurovascular coupling in response to glucose challenge while modulating light adaptation and adjusting for age, refractive error, and scan quality in healthy subjects. We hypothesized that hyperglycemia would attenuate neurovascular coupling during the transition from dark to light;

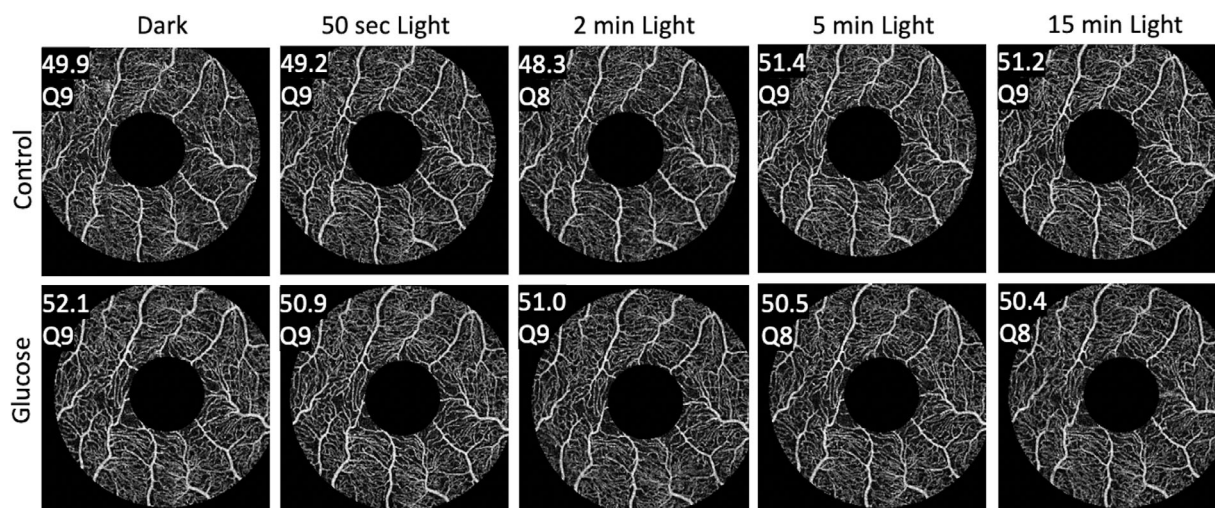


FIGURE 2. Representative OCTA images of the SCP during light modulation in the same subject. Top row shows representative OCTA images of the parafoveal zone of the SCP at each measured time point from the control condition. Bottom row shows representative images from the same subject during the glucose condition. Each image is labeled with corresponding parafoveal VD measurements and Q-scores at the top left corner.

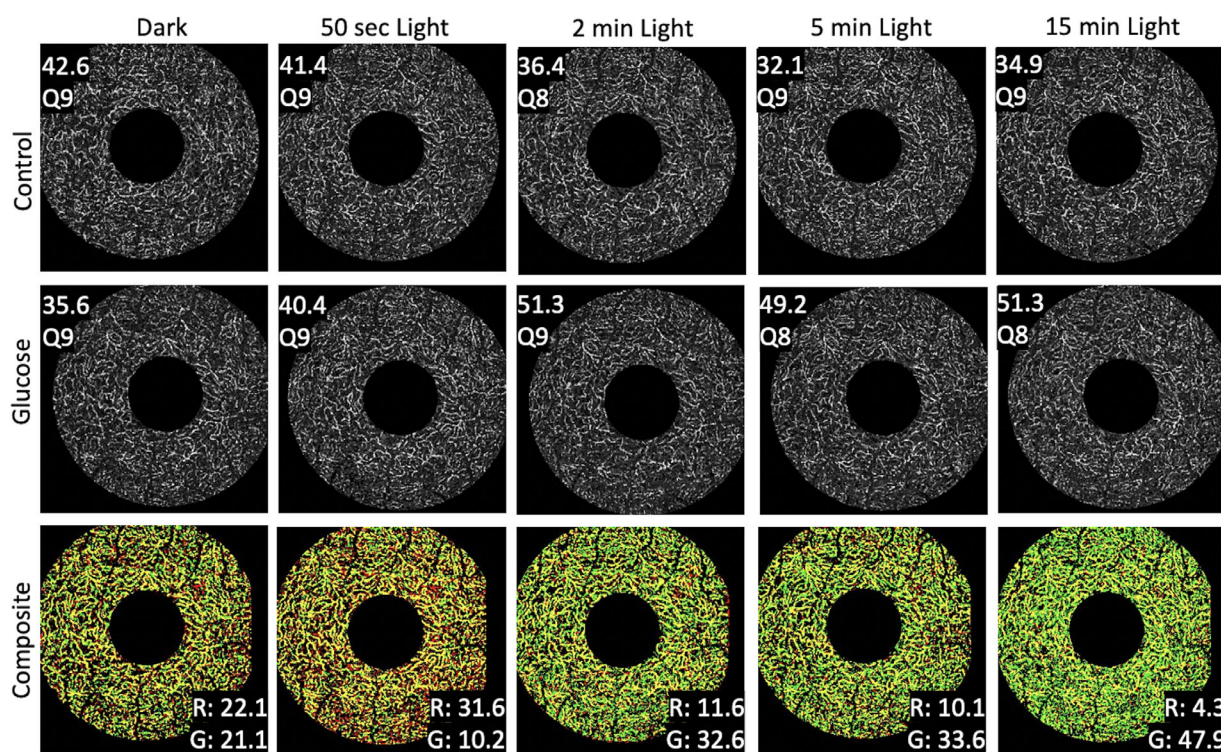


FIGURE 3. Representative OCTA images of the DCP during light modulation in the same subject. Top row shows representative OCTA images of the parafoveal zone of the DCP at each measured time point from the control condition. Middle row shows representative images from the same subject during the glucose condition. Each image in the top two rows is labeled with corresponding parafoveal VD measurements and Q-scores at the top left corner. Bottom row shows pseudo-colored composite images at each time point. Red pixels represent vessels present only in the control image. Green pixels represent vessels present only in the glucose image. Yellow pixels represent vessels present in both conditions. Each image in the bottom row is labeled with corresponding percentage of red (R) and green (G) pixels in the lower right corner.

however, the overall findings show complete reversal of the normal response at each of the layers. Under hyperglycemia in the dark, flow was increased in all three layers, and VD was increased in the SCP and decreased in the MCP and DCP

compared to control, although only DCP VD reached significance. With transition to ambient light in hyperglycemia, we saw significantly decreased flow in all three layers, along with decreased VD in the SCP and increased VD in the MCP

TABLE 2. Mean Differences in Parafoveal OCTA Vessel Parameters Between Glucose and Control Conditions

OCTA Parameter [†]	Dark	Values for Light Normalized to Dark			
		50 s	2 min	5 min	15 min
SCP AFI	0.014 ± 0.008	-0.14 ± 0.11	-0.023 ± 0.011	-0.051 ± 0.012	-0.032 ± 0.011
<i>P</i> value	0.98	0.237	0.042*	0.000*	0.006*
MCP AFI	0.017 ± 0.10	-0.022 ± 0.014	-0.035 ± 0.014	-0.67 ± 0.14	-0.034 ± 0.14
<i>P</i> value	0.103	0.128	0.015*	0.000*	0.017*
DCP AFI	0.008 ± 0.14	-0.010 ± 0.18	-0.024 ± 0.18	-0.060 ± 0.19	-0.027 ± 0.018
<i>P</i> value	0.576	0.594	0.188	0.002*	0.141
SCP VD, %	1.099 ± 0.671	-2.342 ± 0.807	-1.228 ± 0.807	-3.066 ± 0.826	-1.583 ± 0.802
<i>P</i> value	0.105	0.005*	0.132*	0.000*	0.052*
MCP VD, %	-2.725 ± 1.529	5.147 ± 2.124	6.573 ± 2.124	6.076 ± 2.175	4.544 ± 2.109
<i>P</i> value	0.078	0.018*	0.003*	0.006*	0.034*
DCP VD, %	-15.809 ± 2.592	22.245 ± 3.058	30.215 ± 3.058	33.179 ± 3.130	22.868 ± 3.036
<i>P</i> value	0.000*	0.000*	0.000*	0.000*	0.000*
SCP VLD, mm ⁻¹	-0.248 ± 0.431	-0.826 ± 0.464	0.184 ± 0.464	0.868 ± 0.475	-0.239 ± 0.461
<i>P</i> value	0.566	0.079	0.693	0.071	0.606

* *P* < 0.05.

[†] Mean difference (glucose - control) ± SE.

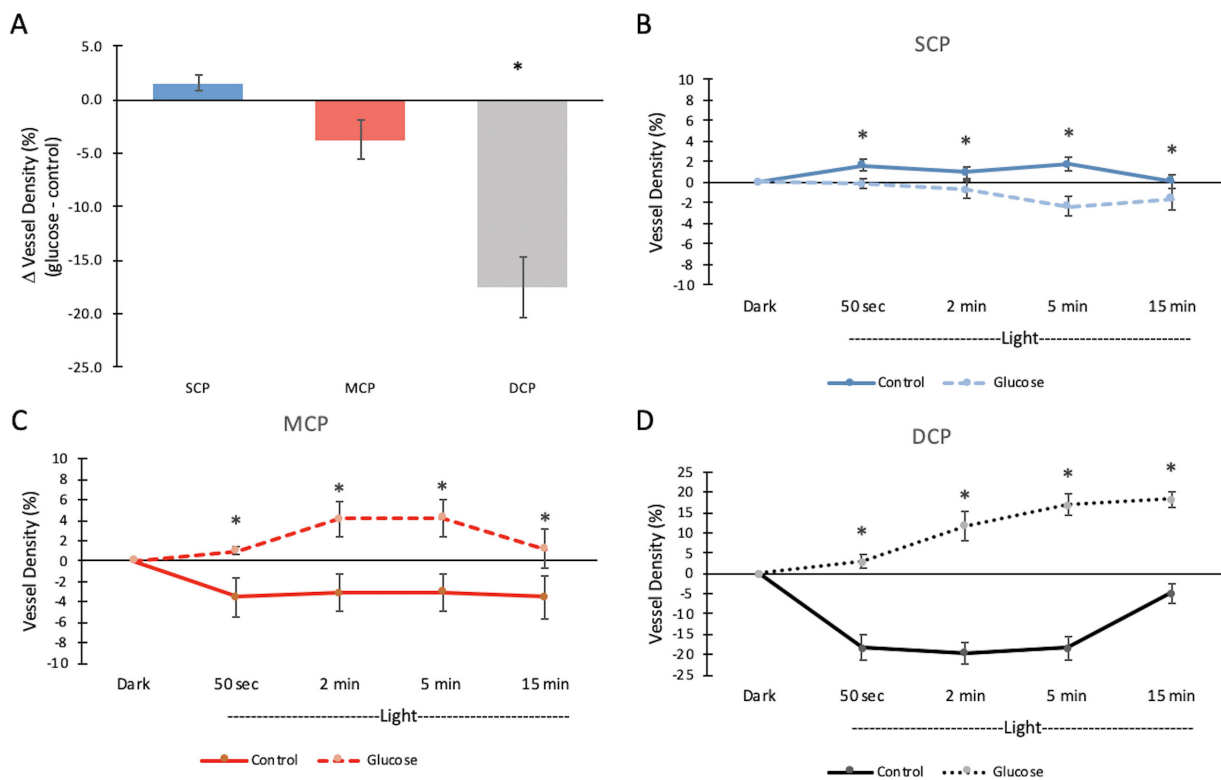


FIGURE 4. Change in parafoveal VD using OCTA during transition from dark adaptation to ambient light after glucose challenge. (A) Difference in VD between the glucose condition and control during darkness in each capillary plexus. VD is significantly decreased in the DCP (*P* < 0.001). Values at each time point in light were normalized to dark, which was set to zero. (B) In the SCP, VD was significantly decreased at all time points with hyperglycemia as compared with control during transition from darkness to ambient light. (C, D) In the MCP and DCP, VD was significantly increased with hyperglycemia as compared with control at all time points during transition from darkness to ambient light. Error bars represent standard errors. Asterisks represent a significant difference between hyperglycemia and control (*P* < 0.05).

and DCP compared to control. Overall, these results show that hyperglycemia causes complete and significant reversal of the vascular responses of the retina during the transition from dark to light adaptation in all three capillary layers of the macula.

These results suggest that acute hyperglycemia compromises neurovascular coupling in healthy retinas. During

hyperglycemia in the dark, we found a trend of increased AFI in all three layers, increased VD and VLD in the SCP, and decreased VD in the MCP and DCP. The only parameter that reached significance in the dark was DCP VD, which decreased. Normally, the DCP is maximally dilated in the dark, which we believe allows it to meet the increased metabolic demands of the photoreceptors in the dark.¹⁰

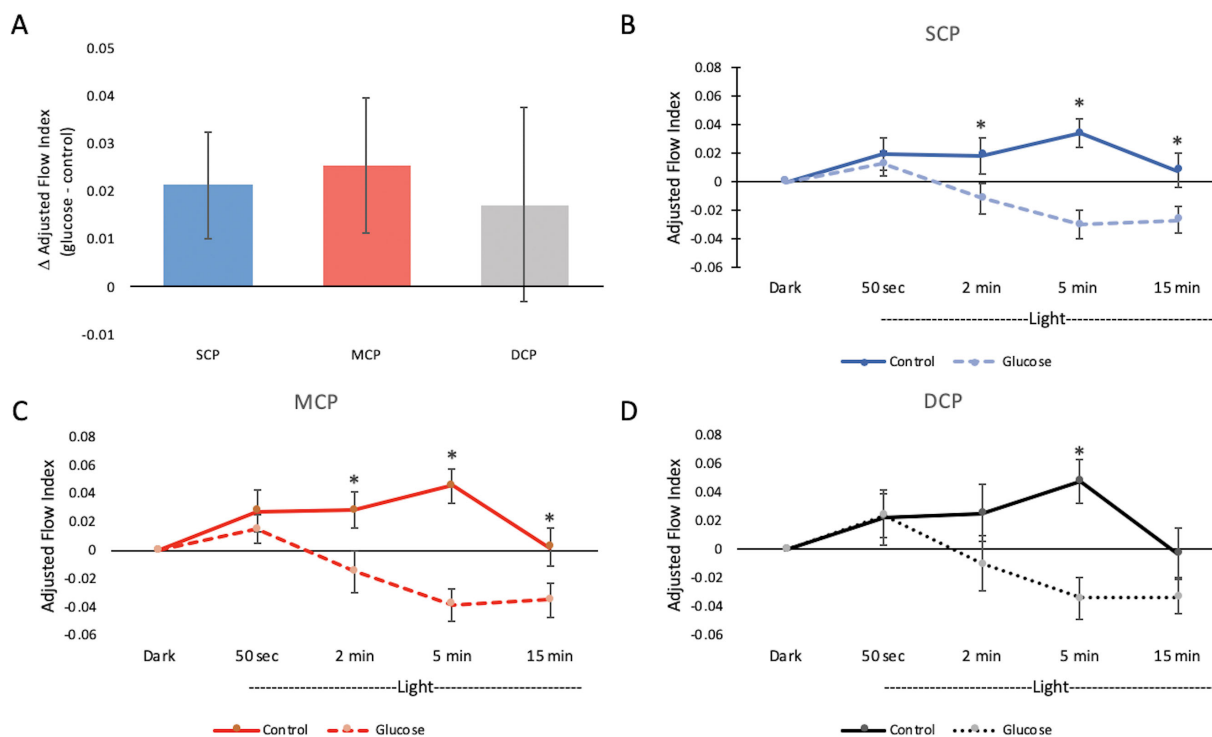


FIGURE 5. Change in the parafoveal AFI using OCTA during transition from dark adaptation to ambient light after glucose challenge. (A) Difference in the AFI between the glucose condition and control during darkness in each capillary plexus. Values at each time point in light were normalized to dark, which was set to zero. With transition from darkness to ambient light in hyperglycemia, the AFI was decreased in the superficial (B), middle (C), and deep (D) capillary plexuses compared to control. Error bars represent standard errors. Asterisks represent a significant difference between hyperglycemia and control ($P < 0.05$).

Thus, the finding of significantly decreased DCP VD with hyperglycemia in the dark may lead to relative photoreceptor hypoxia in the dark, when their metabolic needs are at their peak.

With transition to ambient light, SCP VD and the AFI were significantly decreased at almost all time points in hyperglycemia compared to fasting. There were no significant differences in SCP VLD at any time points. VLD preferentially measures changes in small capillaries, whereas VD measurement is more heavily weighted by larger vessels. This suggests that, in the SCP, hyperglycemia causes constriction of larger vessels leading to overall decreased blood flow during the transition to ambient light. The vascular beds in the retina are interconnected, with the large arterioles of the SCP supplying the MCP and DCP.²³ Therefore, constriction of the larger vessels in the SCP may limit the overall flow to the deeper layers. Consistent with this concept, the AFI in the MCP and DCP was significantly decreased with ambient light (at 2 minutes, 5 minutes, and 15 minutes in the MCP and 5 minutes in the DCP), even though VD was significantly increased at all time points in the MCP and DCP. This suggests that, during hyperglycemia, VD in the deeper layers of the retina increases in the face of overall decreased flow, which could suggest a reactive vasodilator response at the deep layers in response to the overall decreased flow.^{24,25}

To our knowledge, this is the first study to explore the effects of acute hyperglycemia on the capillary layers of the retina while modulating retinal light adaptation and glucose. Current evidence suggests that there are distinct vascular control mechanisms for each of the three plexuses of the retina in healthy and diseased eyes.²⁶ Previous studies by

our group demonstrated that OCTA parameters in the SCP change in different directions than in the MCP and DCP during dark/light adaptation, as well as with the advancing severity of DR.^{10,21,27} The results of the current study further extend our previous findings to show that VD in the SCP changes in a direction opposite that of the MCP and DCP in response to hyperglycemia (Fig. 4). Additionally, these capillary effects occur in the opposite direction of the response under normal glycaemia. Although the mechanisms underlying physiologic neurovascular coupling in response to light stimulus are still under investigation, there is evidence that neuronal stimulation of glial cells leads to the release of vasoactive metabolites.¹³ A recent study has also implicated a particular type of amacrine cell, which releases nitric oxide in response to light, as a potential key player in functional autoregulation.²⁸ Furthermore, there are multiple proposed mechanisms for how glucose could alter blood vessel diameter, such as inhibition of nitric oxide, which may cause vasoconstriction, or via increasing NADH/NAD⁺ ratios, which may cause vasodilation.²⁹⁻³¹ It is plausible that different mechanisms could operate in the different neurovascular compartments of the retina. These mechanisms are likely further confounded by the complex interactions between glucose and insulin, which has also been shown to have vasodilator effects in the eye.³² Further studies are needed to elucidate the specific mechanisms at work in each capillary plexus of the retina and the relative contribution of insulin and glucose to these mechanisms.

The finding that acute hyperglycemia affects neurovascular coupling during dark/light adaptation could have important implications for DR. Attenuation of neurovascu-

lar coupling is one of the earliest signs of DR and has been demonstrated in human and animal studies.^{13,33–35} Decreased retinal vasodilation in response to flickering light as assessed using a dynamic vessel analyzer is correlated with severity of DR and is associated with increased risk of DR progression at 1 year in adults with diabetes.^{36,37} Dark adaptometry studies have demonstrated that patients with DR have slower dark adaptation and elevated sensitivity thresholds.^{38,39} A recent study found that increasing severity of DR is associated with a significant decrease in OCTA VD ($P < 0.001$), as well as prolonged rod intercept ($P < 0.001$), a measure of rod function during dark adaptation.⁴⁰ These authors also demonstrated a more substantial association between DCP VD and rod intercept ($R^2 = 0.28$) than between SCP VD and rod intercept ($R^2 = 0.14$). This is consistent with a previous study from our group suggesting that the maximally dilated DCP in dark adaptation reflects its functional relevance to photoreceptor metabolic needs during dark adaptation.¹⁰ Thus, with hyperglycemia, decreased VD in the MCP and DCP in the dark, when photoreceptors experience their highest metabolic demands, may leave these cells exquisitely vulnerable to ischemia in disease states such as DR, where the DCP VD is already pathologically compromised.^{21,27} Our group has previously shown photoreceptor abnormalities associated with nonperfusion in the DCP in eyes with DR.^{42,43} Our current results suggest that hyperglycemia, especially at night, may exacerbate these metabolic deficits, although this concept needs to be verified by future studies exploring the effects of acute hyperglycemia on dark adaptometry functional measurements in patients with DR.

In contrast to our findings, a previous study using infrared photography in patients with diabetes and no or mild DR found that the fasting peripapillary vessel diameter during dark adaptation was greater than after consumption of the oral glucose tolerance solution.¹² This finding is in direct contradiction to our SCP VD findings, which may be due to a difference in the study imaging technique (infrared photography vs. OCTA), the vessels measured (peripapillary vs. parafoveal), or the subject population studied (diabetic vs. healthy). Therefore, additional studies are necessary in subjects with diabetes to further explore the effects of hyperglycemia on the neurovascular coupling in the macular capillary plexuses in greater detail.

This study is unique in analyzing the effects of hyperglycemia on the three capillary plexuses during dark adaptation and transition to light in healthy individuals, using rigorous clinical and statistical approaches. In general, autoregulation changes are most visible in the larger vessels as dilation and constriction, measured in real time within seconds.^{2,6,44} In contrast, our approach has been modified to fit with our limited imaging timeline. Due to hardware limitations, we are not able to stimulate (by flicker, for example) and image simultaneously. Moreover, each acquisition of the OCTA requires a few seconds to obtain two scans registered in time in order to calculate the decorrelation. To overcome these limitations and obtain dynamic metrics, we used OCTA to obtain a series of images, beginning with dark adaptation and then subsequently over 15 minutes of light, which allowed us to construct an accurate timeline of the dynamic changes in the entire macular capillary vasculature, which has not been possible with previous technologies. However, we acknowledge several limitations in this study. We had a small sample size, did not mask the readers during AFI and VLD analyses, and did not measure blood pressure, intraocu-

lar pressure, axial length, or other physiologic variables that could affect retinal hemodynamics.

There are also limitations associated with OCTA technology and using indirect hemodynamic measurements. Although the reliability of OCTA measurements has been established in various conditions and disease states, we did not take multiple images at each time point to confirm the repeatability of OCTA measurements in hyperglycemia.^{45–47} Additionally, the AFI is a measure of flow that is relative to a threshold value for each image set. The overall relationship between the AFI and absolute blood flow has been shown to be linear within a limited range of flow that is in line with physiologic measurements in the macular capillaries.^{20,48} Bulk flow artifacts in OCTA imaging due to eye movements may overwhelm capillary flow signals and explain why we found no significant differences in VLD. Novel algorithms to correct for these artifacts may improve the reliability of OCTA measurements.⁴⁹ Finally, we used the standard oral glucose tolerance solutions to induce hyperglycemia without control or continuous monitoring of blood sugar and insulin levels. Future studies using glucose and insulin clamps will be critical to control the potential confounding effect of insulin secretion, as well as allow sustained higher blood sugar levels, which could potentially affect neurovascular coupling.⁵⁰

In summary, we found that acute hyperglycemia significantly decreases DCP vessel density in the dark, which may leave the photoreceptors more susceptible to ischemia, especially in disease states where DCP capillary density is compromised. During the transition to ambient light, glucose challenge resulted in complete reversal of light adaptation trends in all three layers of the retina. There was a significant decrease in SCP VD, an increase in MCP and DCP VD, and a transient decrease in the AFI in all three layers, effects that would limit the vascular supply of the inner retinal layers during their light-adapted metabolic activity. These findings suggest that glucose interferes with neurovascular coupling and support the concept of differential regulation at the different macular capillary plexuses. Additional studies are necessary to explore how these findings translate to subjects with diabetes, especially as these individuals are likely to experience a wide range of glucose fluctuation during their lifetime. Attenuation of neurovascular coupling is one of the earliest signs of DR,^{13,33} and characterizing changes in neurovascular coupling with hyperglycemia in DR may have potential as an early diagnostic screening tool for DR. These studies promise to help us identify approaches to optimize retinal vascular supply in addition to confirming another, previously unrecognized, potential benefit for sustained, tight glucose control. It is plausible that avoiding wide fluctuations in glucose could be important in maintaining neurovascular coupling and retinal neuronal health and potentially mediating the long-term beneficial effects of tight glucose control on DR.

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