Caffeine Delays Retinal Neurovascular Coupling during Dark to Light Adaptation in Healthy Eyes Revealed by Optical Coherence Tomography Angiography

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PURPOSE. The purpose of this study was to investigate the acute effects of caffeine on retinal hemodynamics during dark to light adaptation using optical coherence tomography angiography (OCTA).

METHODS. Thirteen healthy individuals (13 eyes) underwent OCTA imaging after dark adaptation and at repeated intervals during the transition to ambient light in two imaging sessions: control and after ingesting 200 mg of caffeine. We analyzed the parafoveal vessel density (VD) and adjusted flow index (AFI) of the superficial capillary plexus (SCP), middle capillary plexus (MCP), and deep capillary plexus (DCP), as well as the vessel length density (VLD) of the SCP. After adjusting for age, refractive error, and scan quality, we compared parameters between control and caffeine conditions.

RESULTS. In the dark, MCP VD decreased significantly after caffeine ($-2.63 \pm 1.28\%$). During the transition to light, initially, DCP VD increased ($12.55 \pm 2.52\%$), whereas SCP VD decreased ($-2.09 \pm 0.91\%$) significantly with caffeine compared to control. By 15 minutes in light, DCP VD reversed and was significantly decreased ($-5.45 \pm 2.62\%$), whereas MCP VD increased ($4.65 \pm 1.74\%$). There were no differences in AFI or VLD.

CONCLUSIONS. We show that, overall, caffeine causes a trend of delayed vascular response in all three macular capillary plexuses in response to ambient light. Whereas the MCP is constricted in the dark, during the transition from dark to light, there is initially delay followed by prolonged constriction of the DCP and constriction followed by slow dilation of the SCP. We posit that these delayed vascular responses may present potential risk of capillary ischemia.

Keywords: caffeine, neurovascular coupling, dark adaptation, light adaptation, optical coherence tomography angiography

Caffeine, a purine alkaloid, is the most widely consumed psychostimulant in the Western world, with the average person consuming 200 to 300 mg per day.[1] At physiological levels, caffeine antagonizes the adenosine receptors,[2] leading to arousal[3] and alterations in systemic, cerebral, and ocular hemodynamics.[4,5] Systemically, caffeine increases systolic and diastolic blood pressure.[4] In the brain, caffeine induces cerebral vasoconstriction leading to decreased blood flow, as shown previously on transcranial Doppler ultrasonography and perfusion magnetic resonance imaging.[5–7] In the retina, Lotfi et al. first demonstrated decreased macular blood velocity measured by blue field entopic stimulation after caffeine intake.[8] Furthermore, vasoconstriction of the ocular circulation has been shown directly as constriction of the retinal arterioles on retinal vessel analyzer[9] and indirectly through increased resistive index of the ophthalmic, central retinal, and short posterior ciliary arteries on Doppler ultrasonography.[10] These effects of caffeine on the retinal vasculature are hypothesized to be due to inhibition of the adenosine A2a receptor that causes overall vasoconstrictive effects.[11]

Previous studies have found adenosine to be a mediator of neurovascular coupling in the brain and retina.[11,12] Neurovascular coupling is the local regulation of blood flow by neuronal signaling to meet their metabolic demand.[11,13] This response is seen in the brain[13] as well as the retina, where studies using blue field simulation and laser Doppler ultrasonography showed increased macular blood flow in response to flicker stimulus and to ambient light after dark adaptation.[14–16] During the transition to higher illuminance, retinal ganglion cells activity is increased, leading to the vasodilatory vascular response.[11,17]

Recent studies have indicated that each vascular plexus has its own vascular supply and drainage and independently regulates its neurovascular coupling.[18,19] Using optical coherence tomography angiography (OCTA), our group has demonstrated that the three vascular plexuses of the retinal circulation exhibit distinct neurovascular coupling

responses during dark and light adaptation.[20] In the dark, the middle capillary plexus (MCP) and deep capillary plexus (DCP) are maximally dilated while blood is shunted from the superficial capillary plexus (SCP) in order to perfuse the photoreceptors, which have the highest metabolic demand in the dark.[20,21] During light adaptation, the MCP and DCP constrict, whereas the large vessels in the SCP dilate[20] in order to meet the increased demand of retinal ganglion cells in light.[17]

Though caffeine as an antagonist of adenosine receptors seems to have overall vasoconstrictive effects in the retinal circulation, its effects on neurovascular coupling in the retina is not yet clear. A study of animal models showed attenuation of cerebral hemodynamic responses to increased neuronal stimulation after caffeine.[22] In the human brain, neuroimaging studies have shown uncoupling of vascular and metabolic responses after caffeine.[23–25] We hypothesize that the brain effects of caffeine extend to the retina, with dampened vascular responses to metabolic changes induced by different illumination conditions. In the current study, we aim to elucidate the effects of caffeine on retinal hemodynamics in healthy individuals during dark adaptation and transition to light in the three vascular plexuses using OCTA.

METHODS

This prospective study took place in the Department of Ophthalmology at Northwestern University in Chicago, Illinois, between February 2019 and March 2019 with approval from the Institutional Review Board of Northwestern University. Our study was conducted in accordance with the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act regulations. Healthy individuals, determined per patient report and review of electronic health records, were recruited from the clinic. Written informed consent was obtained from all participants. Exclusion criteria included ocular disease, media or lens opacities, and refractive error >6.0 diopters. We also excluded subjects with systemic diseases that could affect retinal circulation, such as diabetes mellitus and hypertension. All participants with a daily caffeine intake of more than two cups were also excluded from the study.

Optical Coherence Tomography Angiographic Image Acquisition

We used the RTVue-XR Avanti system (Optovue Inc., Fremont, CA) with split-spectrum amplitude-decorrelation angiography (SSADA) algorithm[26] to obtain 3×3 mm^2 scans centered on the fovea of eyes without pharmacological pupillary dilation. In brief, the system captured two consecutive B-scans (M-B frames), each containing 304 A-scans at a sampling location on the retina. An A-scan rate of 70,000 scans per second was applied by the device using a light source with a bandwidth of 45 nm centered on 840 nm. The SSADA algorithm extracted angiographic flow information by quantifying the OCT reflectance decorrelation between two consecutive B-scans. Three-dimensional projection artifact removal (3D-PAR) technology by Optovue was applied to the images before extraction for further analysis. The RTvue software provided the signal strength index (SSI), which represents the scan's reflectance signal strength, and a quality index (Q-score), which represents the overall quality of the image, taking factors like SSI and motion artifacts into account.[27,28] In our study, we only included images with a Q-score of 7 or above, a SSI above 50, and without motion or shadow artifacts.

Imaging Protocol

Imaging experiments were performed on the same 14 eyes of 14 subjects under 2 conditions: control (without caffeine) and after ingestion of caffeine. Before both experiments, patients were asked to fast for 9 hours and abstain from caffeine-containing beverages and chocolate for 12 hours prior to imaging. All images were obtained before noon in order to minimize diurnal variation.

In the dark adaptation imaging protocol, subjects wore a thick eye patch over the left eye for 45 minutes in a completely dark room. Prior to removing the eye patch, the OCTA computer monitor was adjusted to display only red light. After removing the eye patch, the dark-adapted left eye underwent a dark-adapted OCTA scan. Following the dark-adapted scan, we refocused the OCTA on the retina to ensure rapid imaging in light. One OCTA image was taken at each of the four consecutive timepoints (50 seconds, 2 minutes, 5 minutes, and 15 minutes) after the lights in the room were turned on to ambient levels (800 cd/m^2).

In the control experiment, participants underwent the dark adaption imaging protocol without ingestion of food or caffeine. The caffeine experiment was performed on a separate day from the control experiment. Each subject was given a 200 mg caffeine capsule at the beginning of the experiment. Two hundred milligrams of caffeine is equivalent to around two cups of standard brewed coffee[29] and represents the average amount of daily caffeine intake per person in Western cultures.[1] After caffeine ingestion, subjects underwent dark adaptation and OCTA imaging, as described above. Blood pressure was measured for each participant prior to caffeine ingestion and after completing the imaging experiment.

Image Analysis

We used the built-in AngioVue Analytics software (version 2017.1.0.151) to automatically segment the SCP and full retina angiograms. The SCP was segmented from the internal limiting membrane (ILM) to 10 μm above the inner plexiform layer (IPL). Full retinal thickness OCTA was segmented from the ILM to 10 μm below the outer plexiform layer (OPL) and was used to define the threshold value in subsequent manual image analysis. Manual segmentation was performed on the machine to obtain the MCP (10 μm above to 30 μm below the IPL) and DCP (30 μm below the IPL to 10 μm below the OPL), as described previously.[30]

We obtained OCTA parameters in the parafoveal region, defined as the annulus centered on the fovea with inner and outer ring diameters of 1 mm and 3 mm, respectively (Fig. 1B). Vessel density (VD) was obtained from the built-in machine software while adjusted flow index (AFI) and vessel length density (VLD) were obtained through manual image analysis. VD for the SCP, MCP, and DCP were measured by the machine, which calculated VD as the percentage of pixels in the parafoveal region occupied by blood vessels.

Manual analyses of the AFI and VLD were performed by two separate graders (C.C.K. and H.E.L.) on ImageJ[31] using previously described methods.[32,33] First, we determined the threshold of each scan based on the foveal avascular zone signal on the full thickness angiogram as previ-
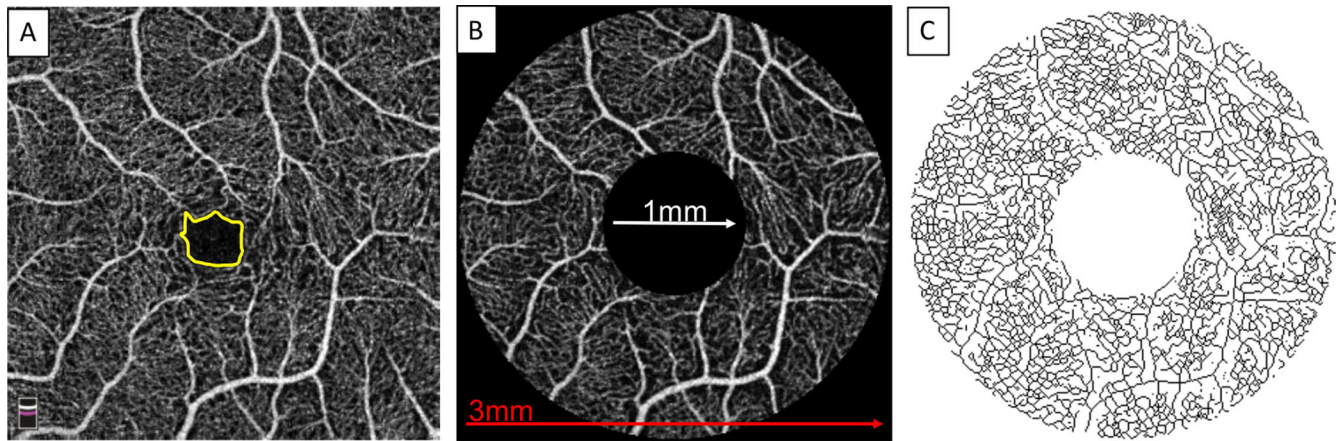
FIGURE 1. **Schematic of optical coherence tomography angiography (OCTA) parameters.** (**A**) Full retinal thickness OCTA image with the foveal avascular zone outlined in yellow and used to establish the noise threshold. Average pixel intensity above the threshold was used to calculate the adjusted flow index. (**B**) OCTA image of the parafoveal region of the SCP delineated between rings of 1.0 mm and 3.0 mm. All parameters were obtained in the parafoveal region. (**C**) Vessel length density was calculated from this image after binarizing and skeletonizing the parafoveal SCP vessels.

ously described (Fig. 1A).[32,33] This threshold subsequently distinguished vessel from background noise and was used to binarize images in subsequent analysis.

We calculated AFI, defined as the average decorrelation value, in the three capillary plexuses as the average pixel intensity of all vessels in the parafoveal region above the background threshold.[34] The decorrelation value in the SSADA algorithm is a reflection of blood velocity based on motion contrast and has been shown to have a linear relationship with true blood flow within a limited range of flow velocity.[34,35] Therefore, AFI is an indirect and relative measure of flow velocity. VLD was defined as the ratio of the total length occupied by all blood vessels in the parafovea. Because all vessels are skeletonized to be one pixel wide, as shown in Figure 1C, VLD eliminates the influence of larger superficial vessels on the density measurement. To calculate VLD, we binarized the image and used skeletonize 2D/3D an open source plugin created for ImageJ (developed by Ignacio Arganda-Carreras; available at http://imagej.net/Skeletonize3D) to skeletonize all vessels into one pixel wide.[36] The density of the skeletonized vessels was calculated as percentage of pixels covered by skeletonized vessels in the parafoveal area.

In the analysis of OCTA parameters in ambient light time points, we converted absolute VD, AFI, and VLD values to measurements that were normalized to the dark, with measurements in the dark set to a value of zero. For example, a normalized SCP VD at 50 seconds in ambient light would be calculated as SCP $VD_{50\ seconds}$ − SCP VD_{dark}.

Statistical Analysis

We performed statistical tests using SPSS version 21 (IBM SPSS Statistics; IBM Corporation, Chicago, IL). A two-way random intraclass correlation coefficient (ICC) was used to assess intergrader reliability for AFI and VLD measurements. We used the Student's *t*-test to compare blood pressures before and after caffeine ingestion. Pearson correlation was used to evaluate associations between the OCTA parameters and potential confounding variables, including age, refractive error, and Q-score.

TABLE 1. Demographic Information

Characteristics	Baseline	Caffeine	*P* value
Age, y[†]	27.7 ± 1.42		
Sex, F/M	7/6		
SBP, mm Hg[†]	112.4 ± 2.33	120.9 ± 2.81	0.029[*]
DBP, mm Hg[†]	70.5 ± 1.57	77.5 ± 1.86	<0.01[*]
MAP, mm Hg[†]	84.4 ± 1.67	92.0 ± 2.01	<0.01[*]
Refractive error, D[†]	−1.43 ± 0.53		

[*] Statistical significance (*P* value < 0.05).
[†] Values reported as mean ± standard error.
SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; mm Hg, millimeters of mercury; F, female; M, male; D, diopters.

To account for the fact that we were taking repeated measurements of the same subjects under different conditions, we chose a mixed effects model that adjusted for age, refractive error, and Q-score. We used the linear mixed effects model to assess for differences in VD, AFI, and VLD in the dark and light time points between the caffeine and control groups. All analysis of measurements in ambient light were normalized to the dark, with the measurements in the dark set to zero. A Bonferroni correction for multiple comparisons was applied in our linear mixed effects model and a *P* value of <0.05 was considered statistically significant.

RESULTS

We recruited 14 participants (14 eyes) with 1 participant (1 eye) excluded for poor image quality due to poor fixation. Ultimately, 13 eyes of 13 healthy subjects (aged 27.7 ± 1.42 years) were included in the study. Systolic (*P* = 0.029), diastolic (*P* < 0.01), and mean arterial (*P* < 0.01) blood pressure increased in all participants 1 hour after consumption of the 200 mg caffeine capsule (Table 1). Subject characteristics are summarized in Table 1.

AFI and VLD measurements by the two separate graders showed an ICC of 0.996 for AFI (95% confidence interval of 0.992–0.998) and 0.994 (95% confidence interval of 0.992–

TABLE 2. Mean Difference in Parafoveal Optical Coherence Tomography Angiographic Parameters Between the Caffeine and Control Conditions

OCTA Parameter (Caffeine–Control)	Dark	Values in Light are Normalized to Dark			
		50 Seconds	2 Minutes	5 Minutes	15 Minutes
SCP AFI	-0.001 ± 0.007 (0.844)	-0.011 ± 0.009 (0.207)	-0.002 ± 0.009 (0.836)	-0.007 ± 0.009 (0.463)	0.006 ± 0.009 (0.509)
MCP AFI	-0.008 ± 0.009 (0.369)	-0.006 ± 0.011 (0.566)	-0.001 ± 0.011 (0.913)	-0.006 ± 0.011 (0.567)	0.018 ± 0.011 (0.106)
DCP AFI	-0.013 ± 0.012 (0.288)	-0.05 ± 0.013 (0.678)	0.008 ± 0.013 (0.512)	-0.006 ± 0.013 (0.615)	0.024 ± 0.013 (0.070)
SCP VD (%)	0.36 ± 0.71 (0.615)	-2.09 ± 0.91 (0.024)[*]	-1.36 ± 0.91 (0.138)	-1.29 ± 0.91 (0.158)	-0.86 ± 0.94 (0.365)
MCP VD (%)	-2.63 ± 1.28 (0.042)[*]	2.60 ± 1.67 (0.123)	2.61 ± 1.68 (0.123)	3.80 ± 1.67 (0.025)[*]	4.65 ± 1.74 (0.009)*
DCP VD (%)	0.16 ± 2.39 (0.947)	12.55 ± 2.52 (<0.001)[*]	8.71 ± 2.52 (<0.01)[*]	1.33 ± 2.52 (0.597)	-5.45 ± 2.62 (0.039)[*]
SCP VLD (%)	-0.10 ± 0.30 (0.734)	-0.42 ± 0.37 (0.258)	-0.34 ± 0.37 (0.354)	0.088 ± 0.37 (0.812)	-0.62 ± 0.38 (0.103)

Optical coherence tomography angiography parameters reported as mean difference (caffeine –control) $\pm$ standard error. *P* values reported in brackets.
[*] Statistical significance (*P* value < 0.05).

0.998) for VLD. Pearson correlation demonstrated that Q-score was significantly correlated with both VD and AFI in the SCP, MCP, and DCP ($P < 0.05$ for all three layers).

In the dark, we found a decrease in MCP VD ($P = 0.042$) in the caffeine group after adjusting for confounding variables. Results of VD, AFI, and VLD at ambient light time intervals are reported as values normalized relative to dark, with the measurement in the dark set to zero (Table 2). After adjusting for age, refractive error, and Q-score, we found a significantly decreased SCP VD ($P = 0.024$) and increased DCP VD ($P < 0.001$) during the initial 50 seconds after ambient light transition in the caffeine condition compared with controls. After caffeine, the significantly increased DCP VD at 50 seconds ($P < 0.001$) and 2 minutes ($P < 0.01$) was followed by a significantly decreased DCP VD at 15 minutes ($P = 0.039$) in ambient light compared with controls. After 5 minutes of light adaptation, the MCP of the caffeine condition showed a significantly increased VD ($P = 0.025$) lasting until 15 minutes ($P = 0.009$). Overall, there is a trend of delay in VD changes in all three layers after caffeine compared with controls (Fig. 2).

There were no statistically significant differences in the AFI of any layer or in the SCP VLD between the two conditions at any time point (Table 2). However, after caffeine, all 3 layers of the retina showed a trend of delayed rise in AFI during light adaptation, with the AFI peaking in the control condition at 5 minutes and in the caffeine condition at 15 minutes (Fig. 3).

DISCUSSION

In this study, we investigated the effects of caffeine on the three macular vascular plexuses on OCTA during dark adaptation and transition to ambient light in healthy subjects while adjusting for age, refractive error, and Q-score. In the dark, the MCP was significantly constricted after caffeine, congruent with caffeine's vasoconstrictive effects.[2,5] During light adaptation, we found that across all three layers, VD and AFI changes were delayed after caffeine ingestion compared to the control condition. In our current and previ-

ously published control data,[20] we found that during the transition from dark to light, there is a rise in AFI in all layers, an increase in SCP VD, and a decrease in MCP and DCP VD. However, Figure 2 and Figure 3 show that overall, caffeine induces a trend of delayed VD and AFI response to ambient light, with early significantly decreased SCP VD and prolonged decrease in DCP VD. Taken together, this delay suggests that caffeine hinders physiologic neurovascular coupling during the transition from dark to ambient light, as detected on OCTA.

In an animal model, caffeine has been shown to attenuate neurovascular coupling by diminishing vasodilatory responses and reducing the expected increase in cerebral blood flow during somatosensory stimulation.[22] Studies of the human brain using blood oxygen level dependent functional magnetic resonance imaging (BOLD-fMRI) and electroencephalogram (EEG) showed that caffeine uncoupled the relationship between cerebral neuronal activity or metabolic rate of oxygen consumption ($CMRO_2$) and cerebral blood flow (CBF).[24,25] In normal conditions, $CMRO_2$ and CBF increase together, because increased neuronal metabolic demand leads to increased blood flow through neurovascular coupling.[24,25] After consumption of caffeine, $CMRO_2$ on fMRI and neuronal activity on EEG increase due to the neurostimulatory effect of adenosine A1 receptor inhibition.[23–25] However, instead of an expected compensatory increase in blood flow, caffeine causes decreased CBF due to the vasoconstrictive effects of adenosine A2a receptor inhibition, leading to potential relative ischemia that is postulated to be salvaged by increased oxygen extraction in the brain.[23–25,37] The localization and relative proportion of these adenosine receptors could reflect the topographical distribution of vascular response to caffeine in the central nervous system.[23] In the human retina, both adenosine A1 and A2a receptors are present,[38] which could mediate similar caffeine effects in the eye as in the brain, namely a state of heightened neuronal activity but relative vasoconstriction. Because inhibition of A1 produces neurostimulatory effects in the brain,[23–25] the delay in retinal vascular responses during dark to light transition is better explained
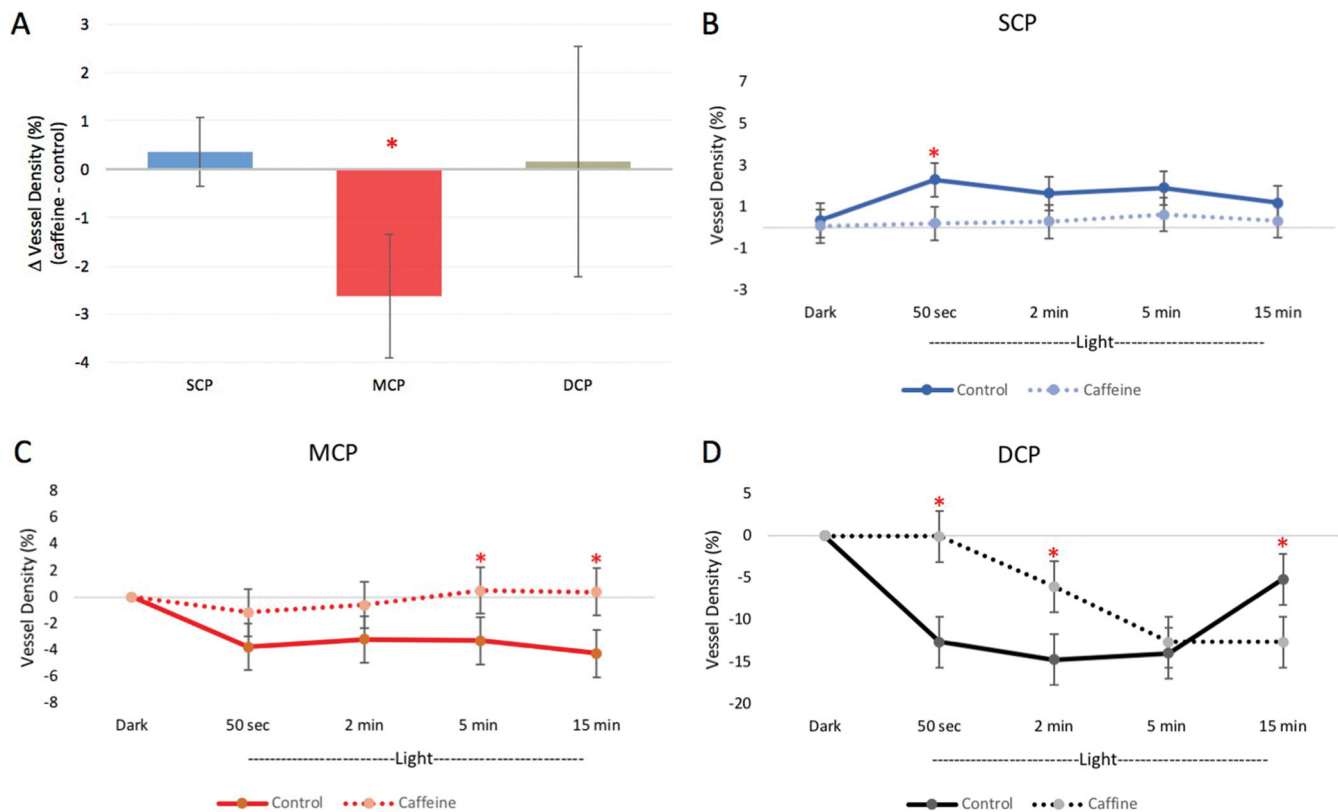
FIGURE 2. Parafoveal vessel density on optical coherence tomography angiography during dark and light adaptation. (A) Absolute change in VD in the dark comparing the caffeine and control groups shows significantly decreased MCP VD ($-2.63 \pm 1.28\%$; $P = 0.042$) after caffeine. VD in ambient light at each timepoint in (B), (C), and (D) are shown as values normalized to dark, which was set to zero. (B) Shows significantly decreased VD in the superficial capillary plexus ($-2.09 \pm 0.91\%$; $P = 0.024$) at 50 seconds of ambient light in the caffeine compared to control group. (C) After caffeine, MCP VD was significantly increased at 5 minutes ($3.80 \pm 1.67\%$; $P = 0.025$) and 15 minutes ($4.65 \pm 1.74\%$; $P = 0.009$) in ambient light compared to the control condition. (D) In the deep capillary plexus, the caffeine group first showed a significantly increased VD at 50 seconds ($12.55 \pm 2.52\%$; $P < 0.001$) and 5 minutes ($8.71 \pm 2.52\%$; $P < 0.01$) in ambient light then a significantly decreased VD at 15 minutes ($-5.45 \pm 2.62\%$; $P = 0.039$) compared to controls. All time points with statistically significant differences ($P < 0.05$) between the caffeine and control conditions are marked with an asterisk (*). Error bars represent standard errors.

by delayed neurovascular responses rather than an appropriate vascular response to decreased metabolic demand after caffeine intake. Ultimately, the disruption of neurovascular coupling could potentially lead to inadequate distribution of blood flow to retinal neurons.

Our study suggests that caffeine delays the vascular response in the SCP during the initial transition to ambient light. Multiple studies have shown that during flicker stimulation and ambient light adaptation, vessel diameter and flow velocity should increase in the large retinal vessels that reside in the SCP,[16,20,39–42] a response that is postulated to meet the higher metabolic demand of retinal ganglion cell activity in response to irradiance.[17] On OCTA, we have previously shown that healthy individuals respond to ambient light with an increase in SCP VD and decrease in MCP and DCP VD.[20] This was replicated in the control condition of the current study, where the trend of increased SCP VD in the absence of remarkable change in SCP VLD also suggest that the large vessels are responsible for the SCP response to light. However, after caffeine ingestion, at 50 seconds in ambient light, we find significantly decreased SCP VD along with significantly increased DCP VD compared to controls (Table 2; Figs. 2B, 2D). Effectively, the ingestion of caffeine prevented the full dilation of the SCP and the constriction of

the DCP, which combined would reverse the flow of blood away from superficial layers, contrary to what happens in healthy controls in the early phase of the transition to light.[20]

Later, at 15 minutes in ambient light, caffeine causes a relatively curtailed distribution of blood to the DCP. In the control condition, with light stimulation, the DCP VD initially decreases but returns to baseline vasodilation by 15 minutes in ambient light, whereas the MCP VD remains decreased for the entirety of the adaptation period (Figs. 2C, 2D). After caffeine, the MCP VD increased significantly at 5 and 15 minutes in light while the DCP VD decreased significantly by 15 minutes compared with controls. In fact, caffeine causes the DCP to remain constricted for a prolonged period from 5 to 15 minutes in light. This suggests that, in the later stages of light adaptation while the dilated MCP may be adequately perfused after caffeine, blood may not be distributed appropriately to the DCP, which remains constricted. The profound constriction of the DCP is consistent with histological evidence of the highest density of A2a receptors in the outer retina,[43] because inhibition of those receptors would lead to vasoconstriction.[11,44]

In the inner retina, the SCP supplies the nerve fiber layer, the ganglion cell layer, and the IPL, the MCP supplies the IPL and inner nuclear layer (INL), whereas the DCP supplies the
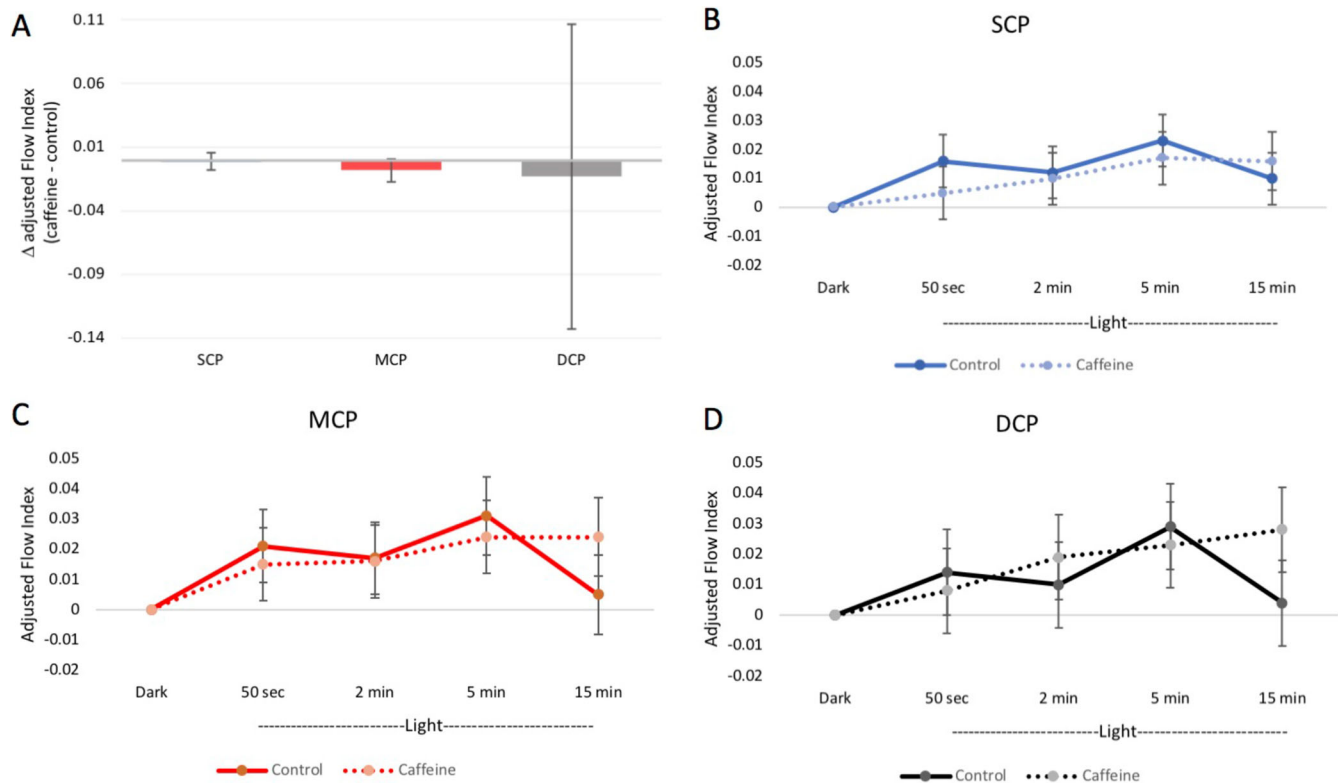
FIGURE 3. Parafoveal adjusted flow index on optical coherence tomography angiography during dark and light adaptation. (**A**) Absolute change in AFI in the dark between the caffeine and control groups. AFI in ambient light at each timepoint in (**B**), (**C**), and (**D**) are shown as values normalized to dark, which was set to zero. (**B**), (**C**), and (**D**) show a rise in AFI in ambient light that peaks at 5 minutes in the control but at 15 minutes in caffeine condition.

outer plexiform, outer nuclear, and partly the photoreceptors of the outer retina.[42] Clinically, it is unclear whether MCP constriction in the dark, SCP constriction at 50 seconds, or delayed DCP constriction at 15 minutes in ambient light after caffeine consumption can precipitate relative ischemia in these respective layers. In the brain, in the face of decreased cerebral blood flow, the increased oxygen demand caused by caffeine is met by acutely increasing the oxygen extraction[37] and chronically by compensatory upregulation of adenosine receptors in caffeine consumers.[45] It is plausible that the healthy retina, like the brain, could have a similar compensatory mechanisms, a question to be explored in future studies using different technology to study retinal oxygen extraction.[37] Interestingly, animal models of diabetic retinopathy also show upregulation of adenosine A1 and A2a receptors[46] and knock-out models of the A2a receptor show retinal cell apoptosis, most prominent in the ganglion cell layer.[47] This suggests that diabetic individuals, who already have impaired neurovascular coupling,[48] could potentially be more susceptible to the disruption of retinal hemodynamic responses caused by caffeine.

The effects of caffeine on neurovascular coupling may also play a potential role in clinical entities like acute macular neuroretinopathy (AMN) and paracentral acute middle maculopathy (PAMM), which have been associated with heavy caffeine intake.[49–51] Risk factors for AMN and PAMM include vasoconstrictive substances and hypoperfusion, suggesting vascular etiologies.[51,52] AMN mainly affects the outer retinal layers with disruption of the ellipsoid zone on OCT[52] and the cone mosaic on adaptive optics scan-

ning laser ophthalmoscopy (AOSLO).[53] The proposed vascular etiology of AMN involves the DCP, with evidence of DCP nonperfusion on OCTA in zones of AMN lesions.[50,52,54] On the other hand, PAMM lesions affect the IPL and INL with sparing of the outer retina.[55] On OCTA, PAMM presents with significant flow reductions in the MCP with secondary DCP reduction.[50]

Each of the three macular vascular plexuses has its own vascular supply and drainage with independent regulation of neurovascular coupling.[18–20] The differential involvement of the DCP and MCP in AMN and PAMM, respectively, illustrates the significance of independent regulation of the three vascular networks in the retina. Our current study further demonstrates that differential dysregulation of the individual plexuses by caffeine, evident by the constriction of the MCP in the dark, and the constriction of the SCP and DCP in light, can place distinct levels of the retina at risk for ischemia. Ultimately, this raises the important question whether caffeine would exacerbate the risk of ischemia in subjects with diabetes who already have impaired neurovascular coupling[48] and decreased capillary density.[32] This will be an important avenue of investigation for future studies, especially given the widespread prevalence of caffeine consumption.[29]

Retinal studies have shown that caffeine decreased baseline blood velocity on blue field stimulation[8] and vessel diameter on retinal vessel analyzer.[9] On OCTA, Karti et al. showed decreased flow and VD in the SCP and DCP after caffeine, but did not investigate the MCP or discuss the level of illumination during imaging.[56] However, in our data, we

found significantly decreased VD in the MCP only during dark adaption, which illustrates the differential effects of caffeine on various retinal layers. In addition, despite an overall trend of delay in rise of AFI in ambient light, we did not find significant differences in AFI, a surrogate of flow, between the caffeine and control groups in the dark. The discrepancies between the current study and previous reports could be explained by the fact that our OCTA images were taken after 45 minutes of dark adaptation, whereas previous study did not report this. We also segmented the retinal vasculature into three vascular plexuses in our analysis and corrected for age, refractive error, and Q-score of the OCTA scans as well as for multiple comparisons, which was not performed in any of the prior studies. Correcting for confounding variables is crucial as our analysis showed that Q-score significantly correlated with OCTA parameters.

Previously, caffeine has been shown to increase baseline systolic and diastolic blood pressure,[4] consistent with our findings. Elevated mean arterial blood pressure in itself may cause reactive vasoconstriction in the retinal circulation according to the Bayliss effect.[57,58] Caffeine has also been shown to cause pupillary dilation and enhance accommodation,[59] although this effect is not consistently observed and poorly understood.[5] Previous studies suggest the repeatability of OCTA without mydriasis and similar vascular parameter results with and without mydriasis.[60,61] In addition, although the pupillary response between the baseline and caffeine conditions may be different due to the effects of caffeine, we adjusted for the quality scores of the OCTA scans, which accounts for some of the discrepancies in light intensity or fixation that may be caused by different pupillary responses. Therefore, our results may reflect a combination of caffeine's effects on systemic blood pressure, pupillary responses, as well as its direct pharmacologic effects in the retina.

The rapid detection of the microcirculation by OCTA and our manual segmentation of the MCP allowed us to obtain vascular data from all three capillary plexuses and, thus, to detect the changes in individual plexuses. The use of OCTA further allowed noninvasive and simultaneous visualization of structure and blood flow information.[62] In addition, the repeatability (coefficient of variability = 1.2%–6.8%) of OCTA has been shown in the healthy and diseased retina, with and without mydriasis.[60,61,63–65] On the other hand, OCTA measures flow-induced changes indirectly through the detection of motion contrast; and although OCTA-based flow-index has been shown in phantoms to have a linear relationship with true flow, this relationship is only valid within a limited range.[35] On OCTA, potential background motion or shadow artifacts may also interfere with angiographic signals.[66] We have limited the contribution of these artifacts by review of images for motion artifacts, excluding one participant for poor image quality, adjusting for the quality of the image in our analysis, and including individuals without media opacities like cataracts or hemorrhage that may cause shadow artifacts. Direct measurements of retinal blood flow can be achieved with fluorescent labeled blood cells,[67] Doppler imaging,[68] and adaptive optics scanning laser ophthalmoscopy,[69] but none of these allow the rapid, high-resolution, three dimensional, and noninvasive microvascular imaging that is possible with OCTA.[70]

The strengths of our study include the rigor of the analyses, using two separate graders with excellent ICC for all manual analyses. We statistically also adjusted for various confounding variables as well as for multiple comparisons.

We also excluded high-frequency caffeine drinkers to better understand the acute effects of caffeine on retinal hemodynamics. In addition, our study included vascular data on all three capillary plexuses, which allowed us to detect the changes in individual plexuses. Limitations of our study include the small sample size. In addition, we did not obtain intraocular pressure (IOP) measurements, which has been reported to be elevated by caffeine.[5] Last, our final imaging timepoint at 15 minutes in ambient light may not have been sufficiently late to capture whether the DCP VD returned to baseline.

Because an estimated 85% of the population in the United States consumes a caffeinated beverage daily,[29] it will be critical to elucidate whether the impaired neurovascular coupling caused by caffeine will ultimately affect perfusion in healthy eyes, or in those predisposed or diseased. Future studies using more functional tools are needed to define the potential clinical implications of impaired neurovascular coupling caused by acute caffeine consumption. In addition, future studies with larger sample sizes, comparing between habitual versus caffeine-naïve individuals, with complete physiologic measurements, including IOP, and with an extended imaging duration after caffeine consumption are needed to better understand the effects of caffeine on retinal hemodynamics. Studies with a placebo-control design should also be considered in the future, to further eliminate the potential nonpharmacological effects of caffeine ingestion.

In conclusion, our study demonstrates that caffeine impairs neurovascular coupling in the three retinal vascular plexuses during dark and light adaptation. We found that in dark adaptation, caffeine caused significant constriction of the MCP. In the initial transition to light, caffeine significantly dampened the dilation response in the SCP, likely due to failure of constriction in the DCP layer that remain significantly vasodilated. This initial vasodilation in the DCP was followed by a delayed but prolonged constriction in the caffeine compared to control condition. Future studies are needed to elucidate whether impaired neovascular coupling after caffeine could lead to retinal ischemia in susceptible individuals.

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