Impact of Pupil Dilation on Optical Coherence Tomography Angiography Retinal Microvasculature in Healthy Eyes

George Villatoro, MD,*† Christopher Bowl, PhD,* James A. Proundfoot, MS,*
Patricia I.C. Manalastas, MD,* Khoo D. Nguyen, MD,*‡
Huiyuan Hou, MD, PhD,* Rafaela C. Penteado, MD,* Andrew J. Li, BA,*§
Sasan Moghimi, MD,* Elham Ghabahi, MD,* Robert N. Weinreb, MD,*
and Linda M. Zangwill, PhD*

Précis: Small but significant decreases in optical coherence tomography angiography (OCTA)-measured circumpapillary capillary density (cpCD) were observed in healthy eyes dilated with 2.5% phenylephrine/0.5% tropicamide. Although likely clinically insignificant, ophthalmologists should consider these changes when interpreting OCTA results from dilated eyes.

Purpose: The purpose of this study was to investigate the effect of pupil dilation using 2.5% phenylephrine and 0.5% tropicamide on quantitative assessment of retinal microvasculature using OCTA.

Methods: OptoVue AngioVue high density (HD) and non-HD OCTA macula and optic nerve head (ONH) images were obtained at 15-minute intervals predilation and postdilation in 26 healthy participants (mean age: 40.0; 95% confidence interval = 33.9, 46.1). Superficial macular vessel density (VD) was measured in the whole image VD and the parafoveal region VD. ONH capillary density was measured in the whole image capillary density and the cpCD region. Differences between predilation and postdilation densities were assessed using linear mixed effects models to account for within-patient correlation.

Results: Instillation of dilating drops resulted in a small but statistically significant reduction in non-HD ONH whole image capillary density of 0.6%, from a mean of 45.2% (95% confidence interval = 41.9%, 48.4%) to 44.6% (41.4%, 47.8%) (P = 0.046). A similar reduction in non-HD ONH cpCD of 0.8% was also observed, from a mean of 49.3% (45.3%, 53.3%) to 48.5% (44.5%, 52.4%) (P = 0.025). No postdilation decreases in macular VD or HD ONH capillary density were observed.

Conclusions: Pupil dilation using topical 2.5% phenylephrine and 0.5% tropicamide results in a small but statistically significant reduction in non-HD ONH whole image and cpCD in healthy eyes.

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From the *The Viterbi Family Department of Ophthalmology and Shiley Eye Institute, Hamilton Glaucoma Center; †UCSD School of Medicine, University of California San Diego, La Jolla, CA; ‡Dartmouth College, Hanover, NH; and ††Northeast Ohio Medical University, Rootstown, OH.
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Reprints: Linda M. Zangwill, PhD, The Viterbi Family Department of Ophthalmology and Shiley Eye Institute, Hamilton Glaucoma Center, 9500 Gilman Drive, University of California San Diego, La Jolla, CA 92037; E-mail: l.zangwill@health.ucsd.edu.
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The observed reduction likely is not clinically significant because the observed reduction was within the previously reported range of measurement variability. Further studies should consider investigating these effects in nonhealthy eyes with glaucoma and media opacities, as well as older individuals.

Key Words: optical coherence tomography angiography, optical coherence tomography, vessel density, ganglion cell complex thickness, dilation

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Pupillary dilation is an essential component of noninvasive examination of the retina and retinal vasculature as well as many intraocular surgeries. Mydriatic agents such as the sympathomimetic agent phenylephrine and the antimuscarinic agent tropicamide are commonly used to achieve maximal dilation.1,2

The α1-adrenergic receptor family plays a critical role in the regulation of vascular tone and blood flow by mediating the vasoconstrictive effects of endogenous catecholamines and adrenergic agonists like phenylephrine. However, these effects are not limited to the peripheral vasculature. The functional role of α1-adrenoceptors has been shown in retinal arterioles,3–5 conjunctival vessels,6 and anterior ciliary arteries.7 Studies using laser Doppler flowmetry have demonstrated that topical phenylephrine decreases blood velocity in retinal vessels, including those supplying the optic nerve head (ONH) of rabbits, monkeys, and healthy humans.8,9

Muscarinic acetylcholine receptors are involved in various physiological actions in the eye, such as regulation of intraocular pressure (IOP), pupil size, and ocular growth.10 These receptors also have displayed vasoactive properties by mediating acetylcholine-induced vasodilation in retinal blood vessels, suggesting that interrupting muscarinic receptor communication at the level of the retina could have vascular consequences, potentially affecting vessel density (VD) measurements.11 A recent study supported this relationship by demonstrating reduced retinal capillary perfusion in healthy human individuals receiving topical 0.5% tropicamide.12

These studies have relied largely on laser Doppler flowmetry, a method that is limited by its inability to assess the entirety of the ocular vascular network, specifically the microvasculature. Instead, they have focused on the effect on large vessels of the ONH rather than the other vessels supplying the rest of the retina.13

Optical coherence tomography angiography (OCTA) is a noninvasive imaging modality that can be used to characterize ocular vasculature and microvasculature in various
Several OCTA instruments have recently received Food and Drug Administration approval. OCTA acquisition speed and diagnostic precision are similar to or better than other currently approved ophthalmic modalities.\textsuperscript{14,15} Furthermore, OCTA does not strictly require dilation, making it an ideal technique for assessing vascular changes in response to induced mydriasis. However, standard patient flow in ophthalmology clinics often will result in patients being imaged with OCTA after pupil dilation. In addition, pupillary dilation is commonly employed to enhance the image quality of this technique. Thus, recognizing the effects of common mydriatic agents on ocular blood vessels is important, especially as OCTA is increasingly used for investigating many ophthalmic conditions.

Understanding any influence mydriatic agents may have on ocular vascular measurements is imperative in interpreting clinical results and comparing conclusions from studies utilizing different agents to achieve pupillary dilation. Therefore, the objective in the present study is to evaluate the impact of topical 2.5% phenylephrine and 0.5% tropicamide, dilating agents commonly used during the clinical examination, on the vascular parameters measured by OCTA.

**METHODS**

**Study Subjects**

This study included 26 eyes from 26 healthy participants. Participants were recruited from the Hamilton Glaucoma Center and Shiley Eye Institute healthy subject pool, staff, and University of California San Diego School of Medicine students from July 2017 to October 2017. Informed consent was obtained from each participant and the UCSD Human Subjects Committee approved all methodology. All methods adhered to the tenets of the Declaration of Helsinki for research involving human subjects and to the Health Insurance Portability and Accountability Act. Screening involved the collection of demographic data including date of birth, ethnicity, sex, medical and surgical history, medications, height and weight, heart rate, and blood pressure. Study participants underwent a slit lamp and fundus examination to ensure patients had no pre-existing eye disease.

Participants were required to have clear ocular media bilaterally, best corrected visual acuity \(\geq 20/40\) bilaterally, predilation pupillary diameter \(\geq 3\) mm, IOP \(<21\) mm Hg by Goldmann applanation tonometry, and normal anterior and posterior segments on clinical examination by an ophthalmologist. Individuals were excluded from study if they were pregnant or intending to become pregnant, were lactating, had a history of prior intraocular surgery or eye disease, including glaucoma, had any medical condition that may affect ocular hemodynamics, including but not limited to diabetes mellitus, hypertension, arrhythmia, or vascular disease. Participants had not consumed caffeine, nicotine, or alcohol within the 12 hours before study testing.

**OCT Image Acquisition**

OCT scans were obtained using a commercially available spectral-domain-OCT system (Avanti; Optovue Inc., Fremont, CA) with AngioVue software (version 2017.1.0.144). This system uses an 840 nm super-luminescent diode with a bandwidth of 45 nm, operated at 70,000 A-scans per second. The AngioVue imaging system uses the Split-Spectrum Amplitude-Decorrelation Angiography (SSADA) algorithm, allowing for noninvasive measurement of VD using 2 subsequent aligned OCT images to detect between-image changes in relative voxel position that indicate the presence of flowing blood. OCTA with SSADA has been described in detail elsewhere.\textsuperscript{14,16,17}

One eye of each participant was randomly selected for imaging. Three dimensional OCTA macula [3×3 mm, 6×6 mm, and high density (HD) 6×6 mm] and optic disc (4.5×4.5 mm and HD 4.5×4.5 mm) images were obtained at 15-minute intervals with 2 baseline scans obtained before dilation and 2 scans obtained after dilation. Dilation was achieved using 1 drop of 2.5% phenylephrine and 1 drop of 0.5% tropicamide in both eyes, and study participants were required a pupil size \(\geq 6\) mm to continue with postdilation OCTA imaging. Lubricating eye drops were instilled before obtaining each image to decrease the possible effect of tear film disruption on image quality.

**Statistical Analysis**

The statistical significance of differences in OCT measures predilation and postdilation was assessed using linear mixed effects models and the Bonferroni-Holm method. Across each eye, all available measures were included as the dependent variables in these models, with fixed effects for time (predilation or postdilation) and image quality and a random intercept to account for within-eye correlation. Multivariable models including fixed effects for sex and age also were performed.

**RESULTS**

The mean age of participants was 40.0 years (95% confidence interval (CI) = 33.9, 46.1, range = 19.6 to 68.6) detailed in Table 1. Of the 26 healthy eyes investigated, 1 eye was excluded due to poor image quality. Predilation and postdilation measurements from 25 eyes with macula 3×3 and 6×6 mm imaging, 24 eyes with HD macula 6×6 mm and ONH 4.5×4.5 mm imaging, and 23 eyes with HD ONH 4.5×4.5 mm imaging were compared.

The mean non-HD ONH wiCD values predilation and postdilation were 45.2% (95% CI = 41.9%, 48.4%) and 44.6% (41.4%, 47.8%), respectively (Table 2). The mean (95% CI) reduction between predilation and postdilation was 0.6% (−1.2%, 0.0%), which was statistically significant \((P = 0.045)\). Mean (95% CI) non-HD ONH circumpapillary capillary density values predilation and postdilation were 49.3% (45.3%,
53.3% and 48.5% (44.5%, 52.4%), resulting in a statistically significant reduction of 0.8% (~1.5% to ~0.1%) (P = 0.025).

No significant decrease in postdilation VDs were observed in HD ONH measurements (all comparisons P ≤ 0.287). Similarly, no significant decrease in postdilation VDs were observed in either the non-HD macula 3×3 and 6×6 mm, or HD 6×6 mm scans (all comparisons P ≤ 0.251).

Average parafoveal GCC thickness increased significantly by 0.5 µm (0.0 µm, 1.0 µm; P = 0.040) in non-HD macula 3×3 mm scans. A similar significant increase of 0.4 µm (0.0 µm, 1.0 µm; P < 0.001) was observed in non-HD macula 6×6 mm scans. This effect was not observed in the HD macula 6×6 mm scans or in ONH circumpapillary retinal nerve fiber layer thickness.

Predilation and postdilation effects within scan type also were explored using the Bonferroni-Holm method of correcting for multiple comparisons. The capillary density reduction previously observed in both non-HD whole image circumpapillary ONH images and circumpapillary images no longer was statistically significant at the P-value < 0.05 level when using this approach (ONH whole images P = 0.090; circumpapillary images P = 0.075). Only the increase in parafoveal GCC thickness in non-HD macula 6×6 mm scans (significant in mixed effects models) was statistically significant (P = 0.006).

No relationships between subject age, sex, and quality index of image and differences in predilation and postdilation VD, capillary density, and GCC measures were observed. Similarly, there were no changes in systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate, or IOP after administration of the tropicamide and phenylephrine eye drops. See Table 2 for results from all OCT comparisons investigated.

Finally, there was a small decrease in postdilation SQI for all image types (Table 3). This decrease reached statistical significance for 6 mm high definition macular images only (P = 0.035, all other image types P ≥ 0.183).

**DISCUSSION**

The current study showed a small but statistically significant decrease in capillary density as measured by non-HD OCT.
OCTA in the ONH of healthy individuals after application of 2.5% phenylephrine and 0.5% tropicamide. This decrease was not observed in HD ONH scans. Furthermore, small but statistically significant postdilation increases in OCT-derived parafoveal GCC thickness of ~0.5 and 0.4 μm (an increase of ~0.4% of baseline thickness in both cases) also were observed in macula 3×3 and 6×6 mm non-HD images, respectively. These small differences possibly reflect the effect of a larger pupil increasing optical image blur in lower density images resulting in the inability of the instrument to accurately identify the position of the posterior GCC and the Imaging Data Evaluation and Analysis Center personnel to manually correct the segmentation. Finally, we observed a very small decrease in SQI postdilation, possibly related to study participant fatigue.

A recent OCTA study by Cheng et al18 observed a 4.63% reduction in non-HD peripapillary VD (with no reduction in macula VD) in individuals who received a 0.5% tropicamide/0.5% phenylephrine mixture. Hohberger et al,19 however, did not observe any decrease in retinal vasculature (macula or ONH VD) after introduction of 5% phenylephrine and 0.5% tropicamide using high resolution OCTA imaging (Heidelberg OCT Spectralis). A prior study using Canon Laser Blood Flowmetry also did not observe changes in vascular reactivity of the major retinal arterioles following the application of 1% tropicamide, combination of 0.8% tropicamide and 5% phenylephrine, or 1% cyclopentolate.20 The current results do not directly support results from these previous studies, but instead fall somewhere in between, reporting a small reduction in macula and nasal ONH capillary blood VD observed surrounding the ONH could confound the effect of a larger pupil postdilation effects between our results and the OCTA measurements in conditions involving the optic nerve head.

The observed lack of significant vascular reactivity in the macula and minimal effect on the ONH vasculature could be explained by anatomic and physiological barriers that prevent topical applied drugs from reaching the posterior segment of the eye at therapeutic levels. Typically, ~3% of the topically instilled dose reaches the aqueous humor, and an even smaller fraction reaches the posterior segment of the eye in sub-therapeutic levels, although the percentage reaching the posterior segment may be larger in pseudophakic (or aphakic) eyes. The challenge of drug delivery to the posterior segment of the eye is well understood and documented.26–28

Although our study suggests that miodriatic agents have a small impact on non-HD ONH blood flow in healthy individuals that is within reported measures of test repeatability, this may not be the case in people with compromised ocular blood flow, such as patients with diabetes.29 As OCTA becomes more widely used in assessing diabetic and other diseased eyes, it will be important to consider if unhealthy eyes exhibit a different response to miodriatic agents compared with healthy eyes.30,31 It is possible that a slight but statistically significant reduction in blood VD observed surrounding the ONH could confound OCTA measurements in conditions involving the optic nerve such as glaucoma, potentially resulting in diagnostic misclassification or unwarranted conclusions of disease-related change. Many studies have shown decreases in VD in glaucoma eyes and any potential effects that miodriatic agents may have on VD measurements may be of significance.14,32,33

By only including healthy eyes, we were able to explore the normal physiological response of retinal vasculature to miodriatic agents but were limited in observing any potential effect that miodriatic agents may have on diseased and pseudophakic or aphakic eyes. Another limitation of this study included testing only a combination of phenylephrine and tropicamide, the magnitude of the effects observed likely is clinically insignificant.

### TABLE 3. Mean (95% CI) SQI Predilation and Postdilation

|           | Predilation [Mean (95% CI)] | Postdilation [Mean (95% CI)] | Difference [Mean (95% CI)] | P   |
|-----------|-----------------------------|-------------------------------|-----------------------------|-----|
| Macula 3 mm | 7.7 (7.5, 8.0)              | 7.5 (7.3, 7.8)                | −0.2 (−0.5 to 0.1)          | 0.183 |
| Macula 6 mm | 7.4 (7.2, 7.6)              | 7.2 (7.0, 7.5)                | −0.2 (−0.4 to 0.1)          | 0.239 |
| Macula 6 mm HD | 7.6 (7.3, 7.8)             | 7.3 (7.1, 7.5)                | −0.3 (−0.5 to −0.0)         | 0.035 |
| ONH        | 7.7 (7.4, 8.0)              | 7.4 (7.3, 7.8)                | −0.3 (−0.4 to 0.2)          | 0.489 |
| ONH HD     | 7.8 (7.5, 8.0)              | 7.7 (7.4, 8.0)                | −0.1 (−0.4 to −0.3)         | 0.724 |

Statistically significant difference P < 0.05 values are in bold. CI indicates confidence interval; HD, high density; ONH, optic nerve head; SQI, Scan Quality Index.
was smaller in the HD scans, dilatation is not likely to affect measurements of microvasculature used for patient management. Future studies are needed to investigate the use of HD imaging in both healthy and nonhealthy eyes.

In conclusion, this OCTA study demonstrated that a combination of topical 2.5% phenylephrine and 0.5% tropicamide in healthy eyes causes a small, but likely clinically negligible, reduction in VD of the ONH in the healthy population. Although no changes in macular VD were observed postdilatation, small but significant increases in macular GCC thickness in non-HD images were observed.

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