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Chronic Effects of e-Cigarette Aerosol Inhalation on Macular Perfusion Assessed Using OCT Angiography

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Abstract

Purpose: To determine whether there are significant differences in the microvasculature and central retinal thickness (CRT) between e-cigarette users (user group) and age-matched nonusers (control group) using optical coherence tomography angiography (OCTA). **Methods:** In this prospective cross-sectional observational study, OCTA images were acquired of 52 eyes of 26 users and 25 eyes of 25 age-matched nonusers. Daily e-cigarette users with no ocular history were identified from provider information in the electronic medical record. A custom algorithm was used to calculate the foveal avascular zone (FAZ), vessel area density (VAD), and vessel length density (VLD). OCT software was used to calculate the foveal, superior, inferior, nasal, and temporal CRT. Generalized estimating equations using the Z-statistic were used to determine how the FAZ, VAD, VLD, and CRT parameters varied between groups and to assess the differential contribution of descriptive data in the user group. **Results:** No statistically significant difference was found between the user group and control group in the FAZ, superficial vascular complex (SVC) VAD, SVC VLD, or deep vascular complex (DVC) VAD. A statistically significant difference was found for DVC VLD ($P = .002$), with the user group having a slightly higher VLD on average. Superior, temporal, and inferior inner macular thicknesses were significantly thinner in the user group ($P = .038$, $P = .012$, and $P = .035$, respectively). **Conclusions:** Significant negative differences were found in CRT measures but not in retinal microvasculature parameters between e-cigarette users and nonusers. Decreased inferior, temporal, and superior inner macular thickness in e-cigarette users may show an early chronic structural effect that warrants further assessment of retinal effects as this population ages and continues to use e-cigarettes.

Keywords

retina, optical coherence tomography (OCT), optical coherence tomography angiography (OCTA), retinal vascular disease, retinal manifestations of systemic disease

Introduction

The popularity of electronic cigarettes (e-cigarettes) has increased substantially in recent years, and rampant use by teenagers and young adults[1] is a pressing public health issue as effects on long-term health are largely unknown. In e-cigarettes, an electric heating element aerosolizes the components of e-liquids that contain flavor additives, nicotine, base ingredients of propylene glycol and glycerol, and components used to dissolve flavorants.[2] The ultrafine metal particles generated by the heating element and highly reactive free radicals created through the heating of the e-liquid translocate into the systemic circulation after use.[2–4]

The use of e-cigarettes has been shown to be associated with acutely impaired microvascular function and endothelial dysfunction, with immediate increases in arterial stiffness and decreases in flow in large vessels after immediate use.[5–8] Chronic microvascular effects of e-cigarette use remain largely underexplored, especially in tissues dependent on the health of small vessels, where the effects of compromised vascular function would likely present first.

The retina is a highly metabolically active tissue that is densely vascularized and very sensitive to changes in vessel integrity and function. Impairment of retinal vessel health and flow leads to marked alterations and degradation in vision in affected patients.[9] Although the acute and chronic effects of traditional cigarette use on retinal microvasculature have been explored,[10–13] the effects of e-cigarette use are not known.

Traditional methods of imaging the retinal vasculature have largely relied on direct visualization with direct ophthalmoscopy and invasive fluorescein angiography. Optical coherence tomography angiography (OCTA) allows for noninvasive, high-resolution imaging of the microvasculature of the retina

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and choroid without the need for intravenous dye administration.[14,15] OCTA also allows for depth-encoded visualization of different vascular complexes through the retina and separate analysis of microvascular parameters from these separate complexes.[15] The use of this imaging modality permits quick and thorough collection of information on the retinal and choroidal microvasculature of e-cigarette users to determine whether long-term use leads to acute and chronic macular perfusion changes at baseline and immediately after the use of e-cigarettes.

The use of e-cigarettes in younger populations is increasing, and dramatic endothelial dysfunction in large vessels of the body immediately after use has been reported.[5–8] Thus, it is imperative to determine whether e-cigarette use leads to changes in microvasculature that could progress to compromise function in sensitive tissues, such as the retina and choroid, in years to come.

In this cross-sectional clinical observational study, we assessed whether there are chronic differences between e-cigarette users and age-matched nonsmoker controls in the retinal microvasculature parameters of the foveal avascular zone (FAZ), retinal vessel area density (VAD), and vessel length density (VLD) as well as in the central retinal thickness (CRT).

Methods

Study Population

In this cross-sectional clinical observational study, eyes of daily e-cigarette users (user group) and eyes of healthy young adult age-matched nonusers (control group) were imaged between October 2020 and December 2020. The study protocol was approved by the Duke University Health System Institutional Review Board and adhered to the US Health Insurance Portability and Accountability Act and all tenets of the Declaration of Helsinki. Informed written consent was obtained from all volunteers.

The participants were recruited from adult patients 18 years or older visiting the Duke Eye Center for general eye examinations or correction of mild refractive errors, students from the Duke campus, visitors of Duke Hospital, and Duke University Health System patients whose providers had indicated use of e-cigarettes in the electronic medical record (EMR). Patients in the user group were contacted directly via MyChart message (Epic) and/or telephone and offered additional information about the research study under the guidelines of Duke University's Cold Call Policy.

All participants were 18 years or older with a history of term birth (≥37 weeks gestational age) and had no known ocular pathologies. A history of prematurity (≤37 weeks gestational age), any pathology known to affect the retinal microvasculature (eg, hypertension, diabetes mellitus, or sickle cell disease), and eyes with a high refractive error (worse than +6.00 D or −6.00 D) that might prevent the acquisition of focused OCTA images were exclusion criteria for all volunteers. Inclusion criteria for the user group were current daily use, had smoked within the past 12 hours, and a minimum of 1 year of total daily e-cigarette use. Exclusion criteria for the user group included any current traditional cigarette, marijuana, or hookah use (>once a week). Healthy nonusers in the control group had no history of e-cigarette, traditional cigarette, marijuana, or hookah use.

Image Acquisition

Undilated OCTA and spectral-domain optical coherence tomography (SD-OCT) scans were obtained of all enrolled eyes using a standard tabletop Spectralis HRA+OCT device with an integrated OCTA technology unit (Heidelberg Engineering). Using the device, 10° × 10° scan angle OCTA images centered on the macula, consisting of 512 A-scans at 512 B-scan positions, were acquired in the right eye and left eye maculas in the user group and right eye maculas in the control group. The OCTA device's TruTrack Active Eye Tracking feature was used for all scans to minimize motion artifacts and fixation errors, and artificial tears were used before imaging. Only OCTA images in which the vessels could be traced throughout were used; images in which there was noticeable artifact were excluded from analysis and retaken immediately after initial acquisition. The quality output of the OCTA device averages across the entire scan and can result in a high score even if there are patches of signal dropout that obscure view of vessels in an otherwise high-quality image; therefore, this value was not used as a criterion for inclusion.

OCTA Analysis

A custom algorithm (MATLAB, MathWorks) previously validated by our group and described by Hsu et al[16] was used to calculate the retinal microvasculature parameters of FAZ, VAD, and VLD for the superficial vascular complex (SVC) and the deep vascular complex (DVC) for all OCTA images acquired. The SVC was automatically segmented from the DVC, and en face images of each vascular complex were automatically generated using the OCTA software. Correct segmentation was manually confirmed.

The upper bound of the SVC was defined as the internal limiting membrane, and the lower bound was defined as 17 μm above the inner plexiform layer. The upper bound of the DVC was defined as 17 μm above the inner plexiform layer to the lower boundary of the outer plexiform layer. The FAZ was defined as the foveal area through which there is no blood flow. The VAD was defined as the percentage of the total image area occupied by perfused vasculature. The VLD was defined as the total length of skeletonized perfused vasculature per unit area.

The OCTA images were first binarized, from which the VAD was calculated for both the SVC and DVC. This was done by comparing the intensity of each pixel against the average intensity of pixels from a 7×7 pixel box centered around the pixel of interest. These images were then skeletonized, from which the VLD in vessel length per millimeter (mm^{-1}) was calculated for both the SVC and DVC. Last, Hessian multiscale filtering was used to enhance the OCTA images so that the

vascular and avascular areas could be better distinguished to allow measurement of the FAZ from the DVC. In the calculation of the FAZ area, scaled x-dimensions and y-dimensions for each image provided from the OCTA software were used to convert pixels to square millimeters. The OCTA software was used to automatically calculate the CRT measurements of the foveal thickness, superior inner macula, inferior inner macula, temporal inner macula, and nasal inner macula from the OCT B-scans used to compose each OCTA image.

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.4, SAS Institute) and JMP Pro software (version 15.0, SAS Institute). The significance of the difference in the means between the user group and control group for continuous measures was assessed using generalized estimating equations (GEEs) using the Z-statistic to account for the correlation between eyes in the user group. Multivariable models assessed the contribution of former smoking status, menthol use, e-liquid concentration, and years of use to the continuous outcome variables using the score statistic from the GEEs.

Table 1. Descriptive Data of User Group and Age-Matched Control Group.

Characteristic	User Group (n = 26)	Control Group (n = 25)
Mean age (y) ± SD	27.8 ± 6.72	28.04 ± 4.53
Sex, n (%)		
Female	13 (50.0)	14 (56)
Male	13 (50.0)	11 (44)
Years of e-cigarette use		—
Mean ± SD	3.77 ± 1.94	
Range	1, 9	
e-liquid nicotine concentration (mg/mL)		—
Mean ± SD	25.8 ± 17.3	
Range	0, 50	
Menthol e-liquid use, n (%)		—
Yes	7 (26.9)	
No	19 (73.1)	
Former smoking status, n (%)		—
Former smokers[a]	4 (15.4)	
Never used cigarettes	22 (84.6)	

[a]Traditional cigarettes.

Results

Seventy-seven 10° × 10° scan angle OCTA images were acquired of 52 eyes of 26 e-cigarette users (user group) and 25 eyes of 25 age-matched nonusers (control group). Table 1 shows the mean age of the 2 groups and the following data for the user group: the mean consecutive number of years e-cigarette use, the mean nicotine concentration of e-liquid used, and the number and percentage of patients who used menthol liquid and who had previously used traditional cigarettes before quitting. The mean age of the e-cigarette users was 27.8 ± 6.715 years, and the mean age of e-cigarette nonusers was 28.04 ± 4.53 years. The mean consecutive number of years using e-cigarettes in the user group was 3.77 ± 1.94 years. The mean nicotine concentration of e-liquid used by the e-cigarette user group was 25.80 ± 1.62 mg/mL. Four of the 26 e-cigarette users had previously used traditional cigarettes before quitting. Seven of the 26 e-cigarette users used an e-liquid containing menthol.

Each enrolled eye was imaged once at the standard tabletop Spectralis SD-OCT OCTA unit. No manual resegmentation of vascular complexes was required for any of the successfully obtained images. The FAZ, SVC VAD, DVC VAD, SVC VLD, DVC VLD, and CRT parameters were measured in all 77 eyes. Figure 1 shows the DVC and SVC microvasculature parameter findings in the user group and control group. Figure 2 shows the CRT measurements of the foveal thickness, superior inner macula, inferior inner macula, temporal inner macula, and nasal inner macula in both groups.

The GEEs using the Z-statistic found no statistically significant difference between the user group and control group in the FAZ (P=.867), SVC VAD (P=.531), SVC VLD (P=.703), or DVC VAD (P=.083). However, a statistically significant difference was found in the DVC VLD (P=.002), with the user group having a slightly higher DVC VLD on average (3.17% higher).

No statistically significant difference was found in the central foveal thickness (P=.277) or nasal inner macula (P=.174) between the user group and control group. However, the inferior inner macular thickness, temporal inner macular thickness, and superior inner macular thickness were significantly thinner in the user group than in the control group (P=.012, P=.035, and P=.038, respectively). On average, the inferior inner macular thickness was 2.73% thinner, the temporal inner macular thickness was 2.36% thinner, and the superior inner macular thickness was 2.14% thinner in the user group than in the control group. Former smoking status, menthol use, e-liquid concentration, and years of use had no significant correlation to the DVC VLD or CRT findings.

Conclusions

The prevalence of e-cigarette use has increased notably in recent years, especially among teenagers and young adults, presenting a pressing public health concern given the largely unknown long-term health effects. The determination of whether there are chronic macular perfusion and structural changes in long-term e-cigarette users is necessary given the increasing use in younger patients. Whether e-cigarette use might lead to earlier effects in delicate tissues and vascular networks, such as the retina, can be better understood through the use of OCT and OCTA.

In this study we assessed whether there were differences at baseline between e-cigarette users (user group) and age-matched nonsmokers (control group) in the FAZ, retinal VAD, retinal VLD, and CRT measures. Overall, we found no significant negative differences in the retinal microvasculature

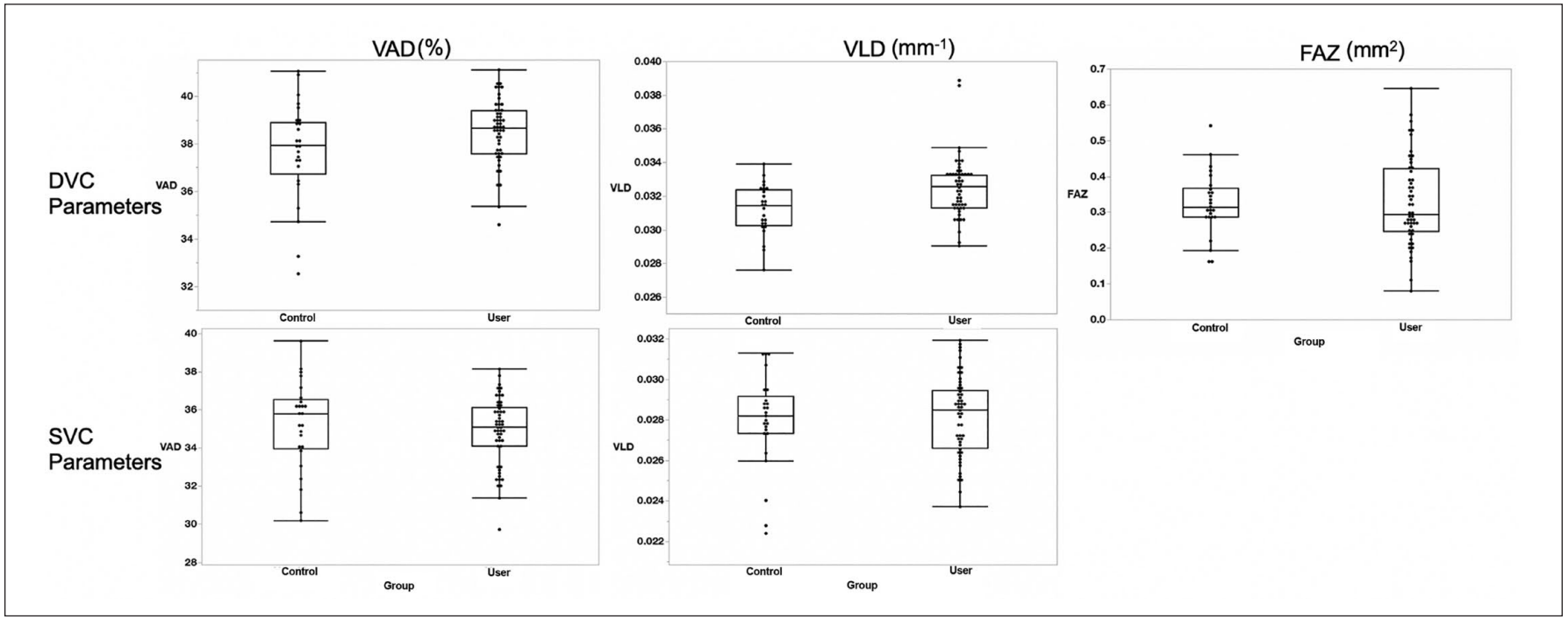

Figure 1. Mean ± SD of retinal microvasculature parameters. Control group: FAZ, 0.324 ± 0.09; DVC VAD, 37.70 ± 2.11; DVC VLD, 31 ± 1.12; SVC VAD, 35.19 ± 2.32; SVC VLD, 28 ± 2.00. User group: FAZ, 0.328 ± 0.12; DVC VAD, 38.52 ± 1.33; DVC VLD, 32 ± 2.00; SVC VAD, 34.84 ± 1.79; SVC VLD, 28 ± 2.00.
Abbreviations: DVC, deep vascular complex; FAZ, foveal avascular zone; SVC, superficial vascular complex; VAD, vessel area density; VLD, vessel length density.

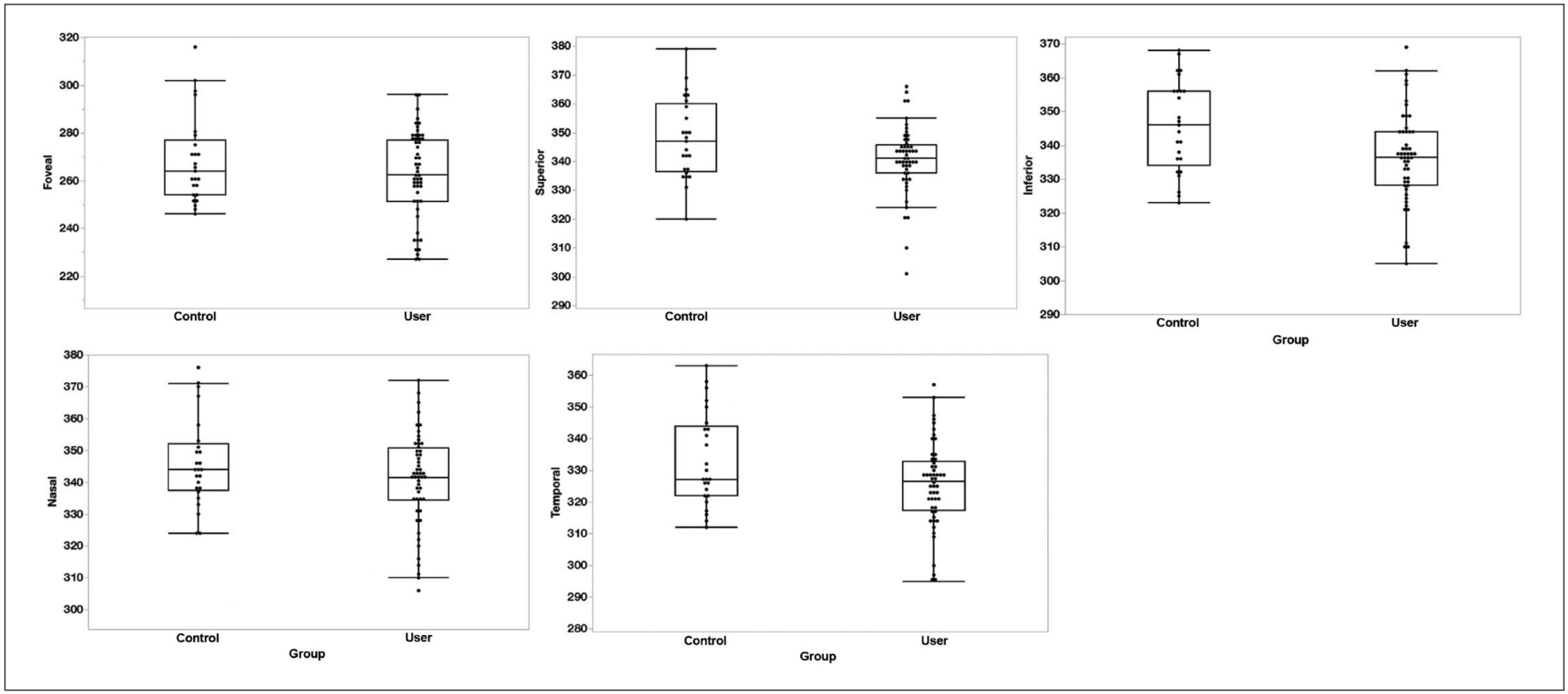

Figure 2. Mean ± SD of central retinal thickness parameters. Control group: central foveal thickness, 268.24 ± 18.25 μm; superior inner macular thickness, 347.76 ± 13.85 μm; inferior inner macular thickness, 345.76 ± 13.59 μm; nasal inner macular thickness, 346.08 ± 13.83 μm; temporal inner macular thickness, 333.24 ± 14.71 μm. User group: central foveal thickness, 262.79 ± 18.45 μm; superior inner macular thickness, 340.39 ± 11.96 μm; inferior inner macular thickness, 336.42 ± 13.50 μm; nasal inner macular thickness, 340.81 ± 14.62 μm; temporal inner macular thickness, 325.46 ± 13.48 μm.

parameters of FAZ and vessel density in either vascular complex between the user group and control group, implying no detectable long-term negative effect on retinal vasculature parameters detectable via OCTA. However, significant CRT parameter thickness differences, specifically in the superior inner macula, inferior inner macula, and temporal inner macula, were noted between the 2 groups.

The finding of increased DVC VLD in the user group in our study must be contextualized by the repeatability and reproducibility of microvasculature parameters from OCTA images on the Spectralis tabletop unit. Our group recently found that although the FAZ and CRT measures for the tabletop device demonstrate high repeatability and reproducibility, retinal vessel density measures had less. These retinal density measures could vary by as much as 6% across visits and 4% within a visit on repeat scans. As such, the slightly increased DVC VLD in the user group in our study likely falls within the range of baseline acceptable variance and as such is most likely not clinically relevant.

The overall lack of chronic effects of e-cigarette use on the microvasculature measures of FAZ, VAD, and VLD seems to fall in line with findings in studies of the chronic effects of traditional cigarette use on retinal health. Although acute decreases in macular blood flow have been observed immediately after cigarette use, significant differences in macular blood flow, FAZ measurements, or retinal vessel density measurements were not observed in the chronic period of smoking between traditional smokers with a mean pack a year history of 13.3 ± 9.0 years and nonsmokers.[10] This picture is complicated, however, by results in a recent 2020 study, which found decreased total vascular density, parafoveal vascular density, and perifoveal vascular density in the deep capillary plexus of chronic traditional cigarette users with a mean of 3.3 ± 1.0 pack years.[12] In addition to differing significantly in terms of pack year history, the more recent study, which found significant microvascular changes, separately evaluated density parameters for the superficial complex and the deep complex. The absence of differences appreciated within the superficial slab suggests that in the case of chronic smoking of traditional cigarettes, microvascular changes may present in the deep vascular network first. Our study also separately evaluated density measures by depth but did not find clinically significant differences in either the superficial complex or deep vascular complex.

Further acute effects have been chronicled, with reductions in retinal blood flow and autoregulation to hyperoxia of retinal vessels seen immediately after traditional cigarette smoking.[17] These acute effects are largely thought to be caused by the vasoconstrictive action of nicotine, and this effect of nicotine on ophthalmic blood flow has been isolated in subjects demonstrating reductions in ophthalmic artery blood circulation immediately after ingestion of nicotine, as appreciated via transcranial Doppler ultrasound.[18]

Studies of the acute effects of e-cigarette use have confirmed similar immediate endothelial dysfunction, and decreases in flow of large vessels can be seen after inhalation of aerosolized e-cigarette e-liquid containing nicotine.[6–8] Concerningly, effects on large vessels have been demonstrated after e-cigarette use even when the e-liquid contains no nicotine, with vessels showing severely blunted luminal flow-mediated dilation, reduced flow velocity, and increased arterial stiffness.[5] This implies that the translocation of free radicals generated through the heating of e-liquid into the systemic circulation coupled with the effect of nicotine in e-liquid might result in a compounded effect on vessels throughout the body. This additive effect may result in more marked long-term retinal effects, which can present sooner.

We found that e-cigarette users had significantly thinner inferior, temporal, and superior inner macular thicknesses than healthy age-matched controls. This may illustrate an early chronic structural effect of e-cigarette use that precedes detectable vessel changes. The pattern of thinning in the user group vs the control group in our study seems to demonstrate congruence with the chronology of thinning observed in other known retinal disease processes. Structural central retinal thinning has been observed in retinal diseases such as sickle cell retinopathy and incontinentia pigmenti (IP),[19–23] with patients with sickle cell disease having thinner temporal and superior inner maculas[19] and patients with IP having thinner temporal inner maculas. In patients with sickle cell disease, early structural changes can be seen even if proliferative or nonproliferative retinopathy cannot yet be appreciated.[23] This finding demonstrates how chronic structural changes can precede later retinal microvascular changes that follow.

Whether the underlying mechanism of the thinning seen in the e-cigarette users is a possible repeated acute microvascular vascular microinjury in the setting of possible acute changes in blood flow during e-cigarette use will require further study. Lack of a significant correlation of these findings to e-liquid nicotine concentration and years of use likely involves a multifactorial explanation. Of note, the lack of regulation of the ingredients in e-liquids has resulted in a substantial range of additives, with the number of additional compounds rarely correlating with the amount of nicotine. In addition, given the younger demographic of e-cigarette users and the relative lack of stratification of years of use, it is possible that longer term microvasculature and thickness effects have not yet had time to present.

It remains to be determined whether there are acute differences in retinal microvasculature measurements and CRT measurements immediately after e-cigarette use. Differences immediately after inhalation between e-cigarette users and age-matched nonsmoker controls must still be assessed in the delicate vascular networks of the retina, especially given the acute, marked changes seen in the larger vessels. Our participants were not asked to limit exercise or coffee intake before imaging; thus, it is possible these uncontrolled variables could have had small effects on the retinal microvasculature parameters[24,25]; however, given our findings it is unlikely that this had a substantial contribution to one group over the other. This study was also limited

by its size; all e-cigarette users in the Duke University health system whose providers had indicated use in their EMRs were contacted for possible participation.

Overall, in the comparison between daily e-cigarette users and healthy age-matched controls, we found no significant negative differences in retinal microvasculature parameters in the chronic time period. Decreased inferior, temporal, and superior inner macular thicknesses in e-cigarette users might be suggestive of an early chronic structural effect of e-cigarette use that may precede detectable vessel changes. Further assessment of retinal structural and microvasculature changes in e-cigarette users over more time as this population continues use is necessary to anticipate and evaluate for compromised function and so that the general public can better understand and weigh the dangers of e-cigarette use.

Meeting Presentations

Presented at the 2021 annual meeting of the Association for Research in Vision and Ophthalmology.

Ethical Approval

Ethical approval for this study was obtained from the Duke University Health System Institutional Review Board (approval # Pro00073505). The study protocol adhered to the US Health Insurance Portability and Accountability Act and all tenets of the Declaration of Helsinki.

Statement of Informed Consent

Informed consent was obtained prior to performing the procedure, including permission for publication of all data, photographs, and images herein.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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