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OCT Angiography-Derived Retinal Capillary Perfusion Measures in the Framingham Heart Study

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Purpose: To report baseline demographic associations and novel intereye correlations of retinal perfusion in the Framingham Heart Study (FHS).

Design: Cross-sectional study.

Participants: One thousand eighteen participants were recruited. Of these, 962 participants (mean age 75 ± 7, 59% female, 1720 undilated eyes) had any OCT angiography (OCTA) data.

Methods: Participants from the community-based FHS were recruited between 2020 and 2022. Foveal-centered 3 × 3 mm^2 OCTA scans were used to noninvasively measure retinal capillary perfusion in both undilated eyes of each subject. Retinal capillary perfusion measures, including vessel skeleton density (VSD), vessel area density (VAD), and flux, were calculated in the superficial retinal layer, deep retinal layer (DRL), and full retinal thickness. Multivariate mixed-effect models were used to examine the association between retinal perfusion measures and eye laterality, sex, image quality, axial length (AL), and age. Correlation of retinal perfusion measures between 2 eyes of individual participants was assessed.

Main Outcome Measures: Vessel skeleton density, VAD, and flux.

Results: One thousand two hundred forty-four eyes (73%) had usable OCTA data with 52% acquired from the right eye. Although there was a significant correlation of retinal perfusion measures between 2 eyes of an individual, this was only moderate in magnitude (R = 0.6, $P < 0.000$). There was also a significant decrease in retinal perfusion with age ($P < 0.001$) after controlling for sex, image quality, eye laterality, and AL. A potential interaction between age and layer-specific retinal perfusion was found ($P = 0.058$). Similar findings were observed with all measures of retinal perfusion (VAD, VSD, and flux). Projection artifact removal accounted for 9% to 34% ($P < 0.050$) of the variation in capillary perfusion measures in the DRL.

Conclusions: Retinal capillary perfusion measures between 2 eyes of an individual share only moderate correlation even after adjusting for image quality and scan level artifacts. This has important implications in study design and interpretation of data from unilaterally performed studies on the retinal circulation. These data suggest that intereye differences in retinal perfusion have physiological and disease-related causes that warrant further investigation.

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OCT angiography (OCTA) is a noninvasive, rapid, and low-cost method of assessing perfusion at the capillary level in the human retina. Since its United States Food and Drug Administration approval in 2015, there has been an abundance of evidence demonstrating changes in retinal capillary density or perfusion in diseases such as diabetes mellitus,[1–3] hypertension,[4] uveitis,[5] glaucoma,[6] and vascular cognitive impairment,[7] among others.[8–10] Most recently, a decline in retinal and choroidal vascularity independently correlates with estimated glomerular filtration rate decline in patients with chronic kidney disease and is partially reversible after kidney transplantation.[11] In parallel, the improvement in OCTA devices over the past decade has increased the resolution of images and sophistication of analysis methods (e.g., projection artifact [PA] removal, segmentation methods).[8,9] These data suggest there are promising, measurable, and cumulative subclinical changes in retinal capillaries that can serve as unique biomarkers of retinal and systemic vascular disease.

To realize this promise, several challenges need to be addressed for broader acceptance and clinical utility of OCTA-derived parameters.[12] There are very few large,

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community-based studies of normative OCTA parameters that can serve as a reference database to detect incremental capillary changes leading to disease.[6,13] In addition, relatively basic aspects of retinal perfusion are still unexplored. For example, the correlation of perfusion between 2 eyes of an individual is unknown at a population level. This has important implications in designing and interpreting bilateral versus unilateral retinal perfusion data. The lack of this kind of data is related to several factors including the presumed need for pupillary dilation to obtain reliable and high-quality OCTA images. Although dilation poses a very small risk, it is also perceived as a significant barrier to retinal imaging outside ophthalmology, where it precludes OCTA imaging or is limited to 1 eye in some studies.[6,14,15] Dilation is also inconvenient for participants and may impact other tests that require normal visual function. Therefore, development of robust nonmydriatic OCTA imaging paradigms and datasets that can be adopted or used outside ophthalmology clinics would be helpful. In addition, systematic measures of retinal capillary perfusion need to be developed and validated in large populations to understand the normative range of OCTA measures and relevant confounders.

To address these concerns, here we report nonmydriatic OCTA-derived measures of retinal capillary perfusion in the Framingham Heart Study (FHS), a large community-based study that has been ongoing since 1948.[16] The objectives of this study were to (1) demonstrate that high-quality, nonmydriatic OCTA measures of retinal capillary perfusion can be reliably obtained in a community-based setting with at least equal success to mydriatic studies, (2) examine the baseline demographic associations of retinal perfusion measures in the FHS population, and (3) characterize the intereye symmetry of retinal perfusion measures.

Methods

Subject Recruitment

This was a cross-sectional, institutional review board–approved study of adult human participants conducted from November 2020 to August 2022 on the Offspring Generation (Exam 10) and OMNI-1 (Exam 5) cohorts of the FHS (Framingham, MA). The study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. The study protocol was approved by the Institutional Review Board of Boston University Medical Center and all participants provided written informed consent. Figure 1 illustrates the recruitment scheme.

Data Collection

Data collection was attempted on both undilated eyes of participants in mesopic conditions (door closed, lights off, neutral density screen on all monitors) to maximize physiologic mydriasis. OCT angiography scans were acquired using commercially available, United States Food and Drug Administration-approved spectral domain Cirrus Angioplex 6000 (Carl Zeiss Meditec, Inc). Foveal-centered 3×3 mm^2 OCTA were attempted in quadruplicate in fixed order (right eye then left eye). To maximize success of imaging attempts in nonmydriatic eyes, 3×3 mm^2 OCTA were acquired due to its higher resolution and smaller scan size when compared with 6×6 mm^2 or larger OCTA scans. All imaging was completed within 15 minutes. Axial length (AL) and keratometry were attempted using IOL Master 500 (Carl Zeiss Meditec, Inc).

Imaging was performed by nonophthalmic technicians who were trained in image acquisition using training videos, as well as through in-person and on-site training. Images were reviewed weekly by trained graders (A.D.C., A.S., A.H.K.) not involved in data acquisition. Image quality was assessed by $\geq$2 trained graders not involved in image acquisition based on signal strength (provided by commercial device), decentration, presence of motion artifacts, and media opacities (Fig 2). Feedback on image quality was provided to the imaging technicians. Images rated as "Excellent" had a signal strength $\geq$7, decentration less than or equal to half of the foveal diameter, minimal or no motion artifacts that displace the large vessels by more than one vessel width, and minimal or no media opacities. Images rated as "Good or Usable" had a signal strength $\geq$7, decentration $<$1 foveal diameter, few motion artifacts that displace the large vessels by more than one vessel width, and/or some media opacities. Images rated as "Suboptimal or Unusable" had a signal strength $<$7, were decentered by $>$1 foveal diameter, had $\geq$5 large motion artifacts, and had large media opacities. When 2 graders disagreed on the quality assessment, a senior grader (A.H.K.) adjudicated the grading. OCT angiography (and underlying OCTs) images were reviewed for abnormal findings by a board-certified ophthalmologist and fellowship-trained retinal specialist (A.H.K.) and classified as either "reportable" or "unreportable." Reportable findings included any notable pathology on macular scans including but not limited to the following: vitreomacular traction, drusen, macular edema, subretinal fluid, pigment epithelial detachment, inner or outer retinal atrophy, epiretinal membrane, and subretinal fibrosis. Participants with no notable lesions on scans were classified as having an "unremarkable" examination for their age. Participants with reportable findings were further classified as "urgent" or "nonurgent," and appropriate letters were sent to participants with reportable findings. Participants with "urgent" findings were also called by a local physician to discuss relevant findings and any necessary course of action.

OCTA Image Analysis

The superficial retinal layer (SRL), deep retinal layer (DRL), and full retinal thickness slabs were segmented using commercially available segmentation software with PA removal for scans rated as "Excellent" or "Good" (Fig 2). Superficial retinal layer was defined from the internal limiting membrane to the outer boundary of the inner plexiform layer. Deep retinal layer was defined from the outer boundary of inner plexiform layer to the outer boundary of the outer plexiform layer. Full retinal thickness was defined from internal limiting membrane to external limiting membrane. Segmentation was manually reviewed, and images where automatic segmentation was not reliable were not used for further analyses as described in Figure 1. A previously validated, custom, semiautomated program (MATLAB R2019a; The MathWorks, Inc) was used to calculate the retinal perfusion measures including vessel area density (VAD), vessel skeleton density (VSD), and flux.[1,9,17,18] Vessel skeleton density and VAD are both binarized measures of retinal capillary density. These measures grossly indicate the presence or absence of flow in capillary segments. Vessel skeleton density measures the ratio of the cumulative length of perfused capillaries.[9] Vessel area density measures the ratio of the 2-dimensional surface area of perfused retinal capillaries over the scan area.[9] Flux is sensitive to the retinal capillary red blood cell content.[18,19] Large vessels (arterioles and venules) were removed from images before quantitation as previously reported.[20] The foveal avascular zone area was included in the quantified values. Results are shown for VSD analyses, and other metrics are shown in supplemental figures.

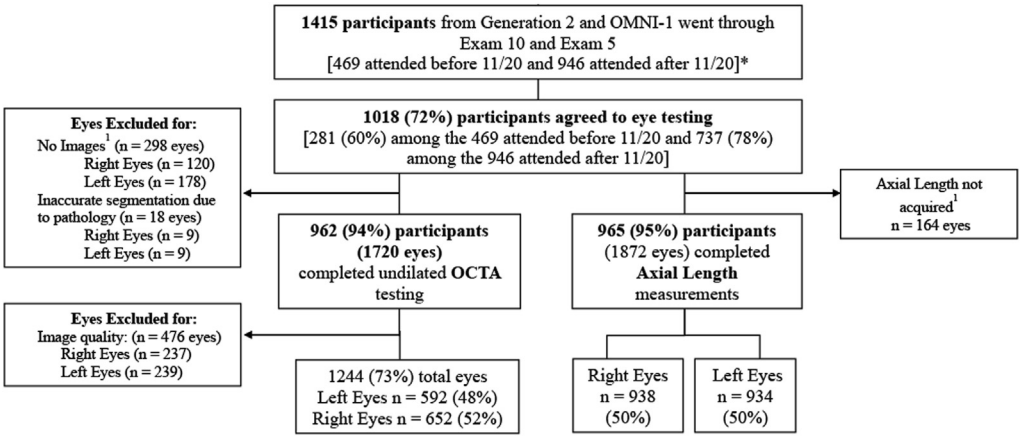

Figure 1. Flowchart summary of data collection. This figure summarizes the data collected, included, and excluded as described in the methods section. Asterisks (*) indicate that the eye examination portion of the study became available in 11/2020, and participants who were recruited before this date did not have an opportunity to participate in the eye examination portion at their original evaluation but were given an opportunity to return for the eye examination. Approximately 469/1415 participants were evaluated before eye examinations were available, and 946/1415 were evaluated after eye examinations became available. Of these, 281 out of 469 participants elected to return for eye examination because it was not available at the time of their initial visit, and 737 out of 946 participants who attended after 11/2020 agreed to participate in eye examination at their initial visit. In some cases, denoted (1), measurements were not acquired due to poor fixation, media opacities, or participant fatigue. OCTA = OCT angiography.

PAs

To assess the impact of PA removal on OCTA metrics, a sample of 100 unremarkable right eyes from 100 participants was exported with and without PA removal and analyzed as described earlier and in Figure S1 (available at www.ophthalmologyscience.org).

Statistical Analysis

Statistical analyses were performed using R version 4.1.3 (R Foundation for Statistical Computing). Statistical significance level was defined as $\alpha = 0.05$. McNemar test was used for statistical comparisons of image acquisition frequency and image quality rating between eyes. Paired t tests, linear regressions, and Pearson correlations were used to compare retinal perfusion values between the right eyes and left eyes. A chi-squared test was used to assess eye pathology asymmetry. Welch 2-sample t tests were used to compare retinal perfusion measures between sexes and between quality measurements. Paired t tests were used to compare images with and without PA removal. Pearson correlations and simple linear models were used to examine associations between retinal perfusion, AL, and age. To standardize image acquisition, our imaging protocol stipulated that the right eye of participants be imaged first, followed by the left eye. In order to minimize potential bias that may have resulted from this, we also categorized eyes as "higher" or "lower" VSD for each eye of an individual. R package nlme was used to perform linear mixed-effect models to estimate the effects of age, sex, AL, and retinal layer (SRL and DRL) on retinal perfusion values VSD, VAD, and flux.[21] Participant-specific random effects were used to account for correlations of 2 retinal layer measurements within 1 eye of individuals. Participants who had AL measurements >3 standard deviations from the mean AL (23.93 mm) were not included in models.

Results

In total, 1018 participants agreed to participate in undilated imaging (Table 1 and Fig 1). OCT angiography images were not acquired in 298 eyes due to poor fixation, media opacities, or participant fatigue. Eighteen eyes were excluded because of severe retinal pathology that impacted segmentation of retinal layers. Therefore, 962 participants (1720 eyes) completed undilated OCTA imaging. Manual review of images revealed 697 (41%) eyes with some retinal pathology on underlying OCT (Table 1).

Image Quality and OCTA Metrics

Previous studies have demonstrated that obtaining reliable, high-quality, nonmydriatic OCTA images in a large cohort of subjects is challenging and often limits analyses of retinal capillary measures.[22] Therefore, we designed an image acquisition protocol with high-intensity training and frequent quality control feedback to address this limitation. In our cohort of 962 participants (1720 eyes), the mean manufacturer-defined signal strength of all right eyes and all left eyes were 10 ± 0.0 and 9.93 ± 0.46, respectively ($P = 0.46$). A signal strength of 10 is the highest quality rating on the OCTA device. In addition to this automated signal strength metric, trained graders performed a manual quality assessment on all images that were obtained from 1720 eyes. This assessment classified scans of 717 eyes as "Excellent" (42%), 527 eyes as "Good or Usable" (31%), and 476 eyes as "Suboptimal or Unusable" (27%). The latter category of scans was not used for further analyses to minimize the potential confounding impact of imaging artifacts. In summary, 1244 (73%) undilated eyes were included in analyses described below.

Sex and Retinal Capillary Metrics

Table 2 summarizes OCTA metrics for the entire cohort. Women showed slightly higher average VSD in the SRL ($P = 0.013$) and full retinal thickness ($P = 0.020$) when compared to men. In contrast, the VSD in the DRL was slightly higher in men compared with women ($P = 0.015$).

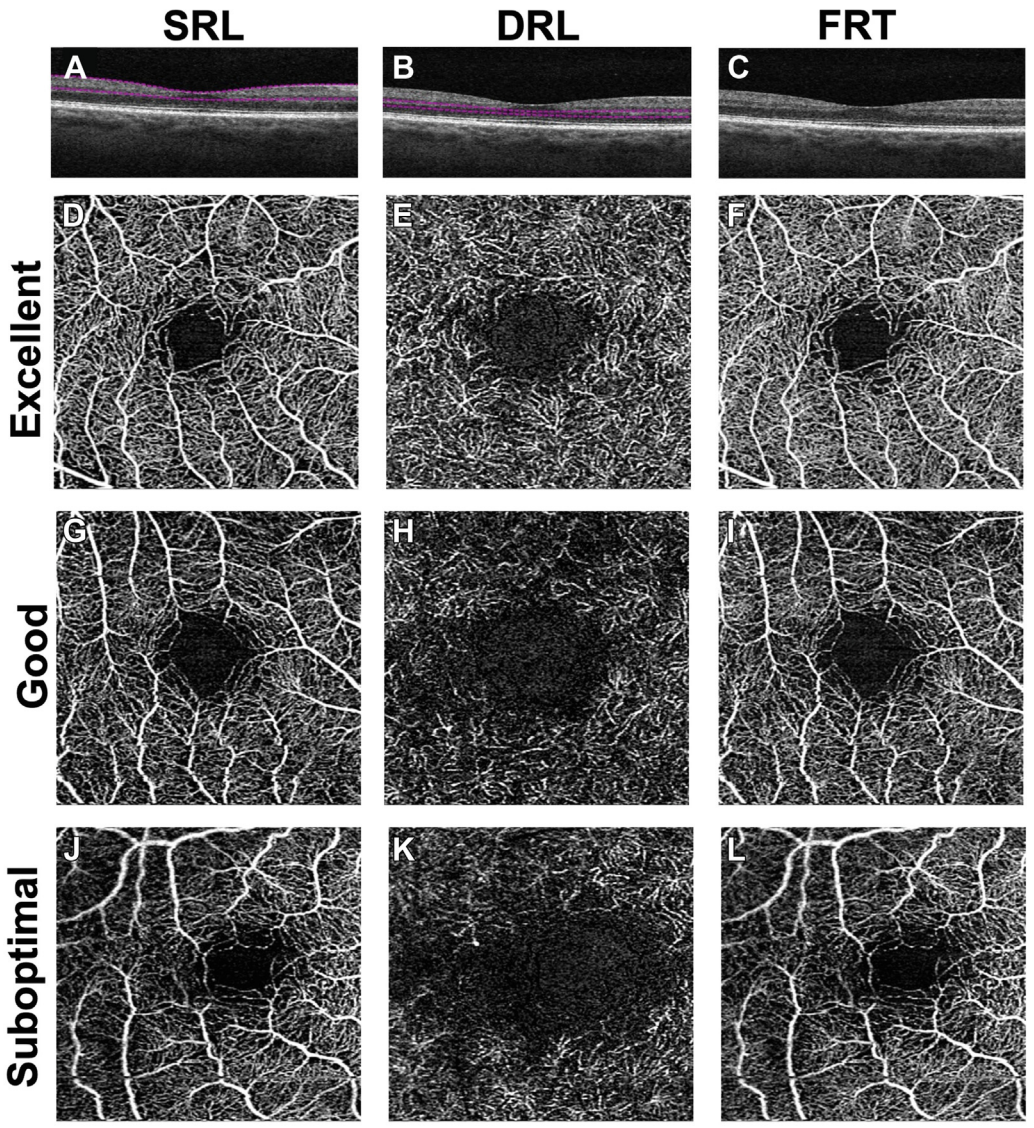

Figure 2. Representative OCTA images. The 3 × 3 mm^2 OCTA images centered on the fovea were acquired for this study. **A–C**, Illustrate representative B-scans through the fovea from the underlying OCTA images. Panels A and B show segmentation for the superficial retinal layer (SRL) and deep retinal layer (DRL), respectively. Panel C illustrates an unsegmented panel for comparison. **D–F**, Illustrate representative en face OCTA images with minimal or no apparent artifacts and graded as "excellent" quality for the study purposes. Panels D and E illustrate the en face images of the SRL and DRL, respectively. Panel F illustrates a full retinal thickness (FRT) en face slab. **G–I**, Illustrate en face OCTA images with mild or moderate levels of artifact and graded as "good or usable" quality. **J–L**, Illustrate en face OCTA images with severe artifacts graded as "suboptimal or unusable" quality. OCTA = OCT angiography.

Similar findings were observed for VAD and flux (Table S1, available at www.ophthalmologyscience.org).

Eye Laterality and Retinal Capillary Metrics

Although there was no significant difference in the mean OCTA signal strength for right and left eyes, the frequency of successful OCTA acquisition (defined as OCTA of any quality) was significantly higher for right eyes (n = 898, 52%) compared with left eyes (n = 840, 48%) (McNemar test, $P = 1.29 \times 10^{-8}$). Manual review of OCTA image quality assessed by trained graders also demonstrated a significantly higher frequency of "Excellent" or "Good" quality images when compared with "Suboptimal" or "not acquired (NA)" for the right versus the left eye (McNemar test, $P = 0.0002$). For the right eyes, 661/962 (69%) had an "excellent/good" rating, and 301/962 (31%) had a "suboptimal/NA" rating. For the left eyes, 601/962 (62%) had an "excellent/good" rating, and 361/962 (38%) had a "suboptimal/NA" rating. In the entire dataset, the mean measures of capillary perfusion were significantly greater in the right compared with left eyes for the SRL and full retinal thickness measures but not in the DRL (Table 2 and Table S1, available at www.ophthalmologyscience.org).

There was a significant but only moderate correlation between retinal capillary perfusion in the right and left eyes of individuals (Fig 3). This correlation persisted even when

Table 1. Demographics and Characteristics of Study Data

Characteristic	Value
No. of participants in parent FHS study*	1415
No. of participants who agreed to eye examination (n)†	1018
No. of participants with OCTA (n)‡	962
No. of eyes with OCTA (n)	1720§
Mean age, yrs (SD) [range]	75 (7) [52–98]
Female n (%)	566 (59%)
Race/Ethnicity	
White	848 (88%)
Black/African American	51 (5%)
>1 race	63 (7%)
Hispanic or Latino	36 (4%)
Not Hispanic or Latino	926 (96%)
Total number of eyes with retinal findings, n (%)‖	697 (41%)
Drusen	230 (13%)
Epiretinal membrane	196 (11%)
Outer retinal disruption	45 (3%)
Abnormal foveal contour	41 (2%)
Retinal distortion	41 (2%)
Other	144 (9%)
OCTA signal strength (n = 1720 eyes), mean (SD) [range]	
All eyes	10 (0.44) [2–10]
Right eyes	10 (0) [6–10]
Left eyes	9.93 (0.46) [2–10]
OCTA image quality (n = 1720 eyes), n (%)	
Excellent	717 (42%)
Good	527 (31%)
Suboptimal	476 (27%)
Mean axial length (n = 1872 eyes), mm (SD) [range]	
All eyes	23.93 (1.20) [18.23–30.29]
Males¶	24.23 (1.45) [21.06–29.32]
Females¶	23.72 (1.19) [18.26–30.29]
Right eyes	23.94 (1.19) [20.64–30.29]
Left eyes	23.91 (1.21) [18.26–29.32]

FHS = Framingham Heart Study; OCTA = OCT angiography; SD = standard deviation.
*Total number of participants from Generation 2 and OMNI-1 who attended Exam 10 and Exam 5 (see Fig 1 for details).
†Total number of participants who attended Exam 10 and Exam 5 and agreed to complete the eye exam (see Fig 1 for details).
‡Total number of participants who completed OCTA in ≥1 eye.
§Data were not acquired for 186 eyes (see Fig 1 for details).
‖Eye pathology percentages were calculated from the total number of eyes n = 1720 eyes.
¶The mean axial length difference between males and females was statistically significant (*P*-value < 2.2e-16).

comparing right and left eyes classified as "Excellent" quality, as well as in right and left eyes without any retinal pathology (Table S2, available at www.ophthalmologyscience.org). This suggests that image quality and retinal pathology were not responsible for this finding. Similar correlations were observed for all measures of retinal perfusion (Fig S2, available at www.ophthalmologyscience.org). Additionally, chi-squared statistic to test for asymmetric prevalence of incidental pathology was not statistically significant ($P = 0.399$).

AL and Retinal Capillary Metrics

It is widely recognized that AL can impact OCTA-based measures. Mean AL in our cohort was 23.93 ± 1.20 mm. There was a modest but statistically significant difference in AL between men and women (Table 1). As expected, there was a significant association between AL and VSD (R = 0.12, $P = 0.002$, n = 699 eyes). Flux also showed a significant but negative association with AL. Figure S3 (available at www.ophthalmologyscience.org) summarizes these findings. We adjusted for AL as a covariate in all subsequent analyses. We also performed a secondary analysis with AL-adjusted VSD measures that were derived using Littman's method.[23–25] For all analyses, both methods showed similar results; therefore, only the statistical adjustments for AL are presented in the main text (see supplementary figures for AL-adjusted VSD measure analyses).

Age and Retinal Capillary Perfusion

In a univariate analysis, there was a significant decrease in VSD with age in both the SRL (R = −0.33, $P < 2.2e-16$) and the DRL (R = −0.16, $P < 1.3e-05$). As Figure 4 shows, this association appeared to be slightly more robust for the SRL than the DRL. Similar results were also observed when we categorized eyes based on higher or lower

Table 2. Summary of Layer-Specific Retinal Capillary Density in the Framingham Cohort

Vessel Skeleton Density	Mean (SD)	Min, Max	N
Superficial retinal layer			
All eyes	0.143 (0.00803)	0.102, 0.166	1244
Males	0.142 (0.00796)*	0.112, 0.166	508
Females	0.144 (0.00805)*	0.102, 0.166	736
Right eyes	0.144 (0.00781)†	0.111, 0.166	652
Males	0.143 (0.00793)	0.122, 0.166	271
Females	0.145 (0.00768)	0.111, 0.166	381
Left eyes	0.142 (0.00819)†	0.102, 0.161	592
Males	0.142 (0.00794)	0.112, 0.158	237
Females	0.143 (0.00834)	0.102, 0.161	355
Deep retinal layer			
All eyes	0.138 (0.0111)	0.106, 0.168	1244
Males	0.139 (0.0113)*	0.108, 0.168	508
Females	0.138 (0.0109)*	0.106, 0.167	736
Right eyes	0.139 (0.0112)	0.106, 0.168	652
Males	0.139 (0.0117)	0.108, 0.168	271
Females	0.138 (0.0108)	0.106, 0.164	381
Left eyes	0.138 (0.0109)	0.106, 0.167	592
Males	0.139 (0.0109)	0.108, 0.162	237
Females	0.137 (0.0109)	0.106, 0.167	355
Full retinal thickness			
All eyes	0.152 (0.00791)	0.106, 0.174	1244
Males	0.152 (0.0083)*	0.106, 0.174	508
Females	0.153 (0.00759)*	0.119, 0.172	736
Right eyes	0.153 (0.00771)†	0.119, 0.174	652
Males	0.152 (0.00815)	0.119, 0.174	271
Females	0.154 (0.00735)	0.129, 0.172	381
Left eyes	0.152 (0.00806)†	0.106, 0.172	592
Males	0.151 (0.00843)	0.106, 0.169	237
Females	0.152 (0.00778)	0.119, 0.172	355

SD = standard deviation.
*Denotes statistical significance in comparison between retinal perfusion parameters in males vs. females ($P < 0.05$).
†Denotes statistical significance in comparison between retinal perfusion parameters when comparing right and left eyes of participants where both eyes were acquired and analyzed (n = 494 participants, 988 eyes, $P < 0.001$).

retinal capillary perfusion measures to minimize potential bias related to standardized imaging of right eyes followed by left eyes in our protocol (Fig S4, available at www.ophthalmologyscience.org).

Linear Mixed Effects Model

Age, AL, image quality, and retinal layer were all significantly associated with retinal capillary perfusion in multivariable models (Table 3). As described above, we noted a significant trend for right eyes to have significantly higher image acquisition frequency, significantly higher measures of retinal capillary perfusion, and higher signal strength than left eyes. Since these trends persisted even when we only evaluated eyes with "Excellent" quality data, it does not seem likely that this is due to image quality. However, we cannot exclude the possibility that our standardized imaging protocol (in which right eyes were always imaged first) somehow introduced a bias underlying this pattern. Therefore, in our linear mixed effects model, we also separated eyes of each subject into "higher" and "lower" capillary perfusion (VSD). Retinal capillary perfusion in the SRL and DRL significantly decreased with age even when accounting for participant-specific biological factors (sex and AL), image quality, and relative retinal capillary density of one eye versus the other ("Higher" VSD eye or "Lower" VSD eye).

In models using only the measures from participant's eyes with higher VSD, the SRL perfusion decreased by 0.00033 ($P < 0.001$) per year of age and increased by 0.00086 for every 1 mm increase in AL ($P = 0.002$). Also, images rated as "Excellent" had 0.00201 higher SRL VSD when compared with images rated as "Good" at average age ($P = 0.001$). The DRL had a lower VSD of −0.01246 when compared with the SRL at average age ($P = 0.001$) in this same group. There was a potential interaction in which SRL perfusion decreases more than the DRL per year of age in those eyes with higher VSD (Table 3, $P = 0.058$). Similar findings were present for multivariable models of all OCTA metrics (Table S3, available at www.ophthalmologyscience.org). The DRL was consistently lower in VSD than SRL at all ages, while the SRL had a significantly greater rate of decline in VSD with each year of age compared with DRL.

PAs and Retinal Capillary Metrics

Projection artifacts are an imaging phenomenon that impact systematic measurements of retinal capillary perfusion, particularly in the DRL.[9,26] Figure S1 (available at www.ophthalmologyscience.org) illustrates representative OCTA images

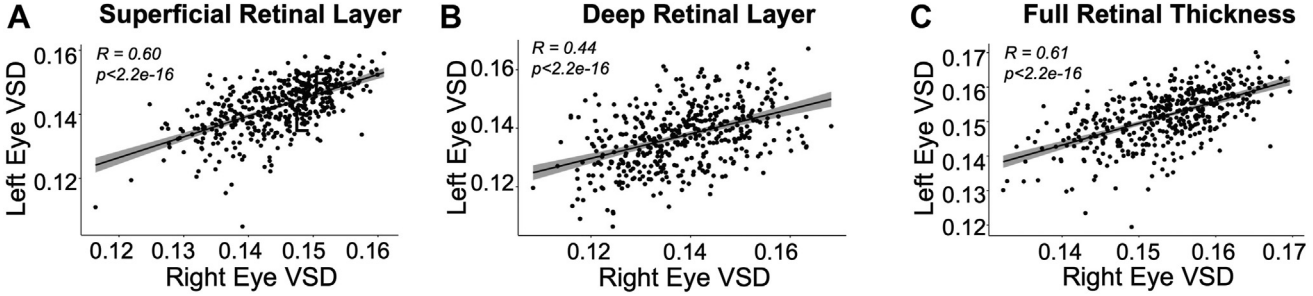

Figure 3. Correlation of retinal perfusion measures from 2 eyes of participants. Pearson correlations for right and left eyes in participants where images from both eyes were acquired and quantified (n = 494 participants, 988 eyes). Panel A shows correlations for the superficial retinal layer. Panel B shows correlations for the deep retinal layer. Panel C shows correlations for the full retinal thickness. VSD = vessel skeleton density.

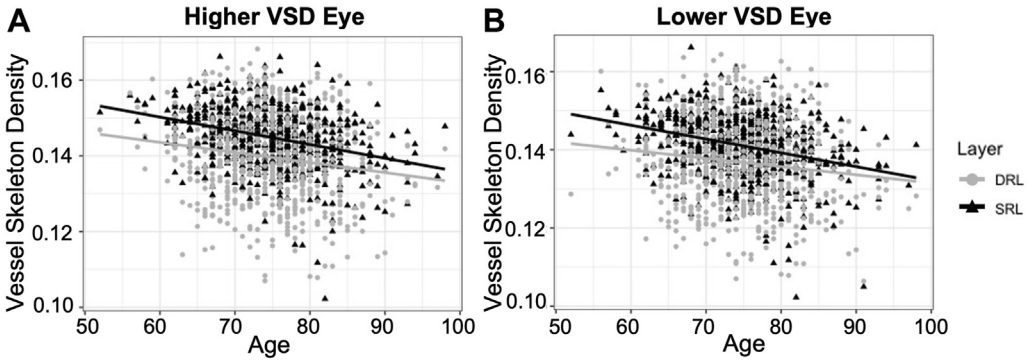

Figure 4. Univariate linear regression for vessel skeleton density (VSD) in the superficial retinal layer (SRL, black triangles) and deep retinal layer (DRL, gray circles) through age. Panel A shows regressions for the eye with higher VSD through age in years for the SRL and DRL (n = 699 participants). Panel B shows regressions for eyes with lower VSD through age in years for the SRL and DRL (n = 698 participants).

with and without PA removal from a single subject in our study. Although PAs are widely recognized, robust data on the magnitude and significance of their impact on OCTA metrics is limited.[27] Therefore, we examined the impact of PA removal on quantitation of OCTA metrics in 100 subjects from our cohort with unremarkable (no apparent pathology) images. When comparing OCTA images before and after PA removal, there were statistically significant differences in all retinal capillary perfusion metrics (VSD, VAD, and flux) in the DRL ranging in magnitude from 9.7% to 34% (all $P < 0.05$; Fig 5 and Table 4). As expected, no statistically significant changes were observed in retinal perfusion measures for the SRL.

Discussion

In this large, cross-sectional, community-based study of FHS participants, we make unique and significant contributions to the literature on both the imaging methodology, demographic associations, and intereye symmetry of noninvasive retinal capillary perfusion measurements using a United States Food and Drug Administration-approved OCTA device. These contributions are highly relevant to successful application of retinal capillary perfusion measures in studies of systemic health and disease. First, we demonstrate that undilated OCTA imaging is feasible in a community-based setting of elderly adults with limited or no on-site ophthalmology expertise and with >70% data utilization. This utilization rate is at least as good as, if not better than, data utilization in previous studies using OCTA data from dilated examinations in outpatient ophthalmology clinics.[22] This was a result of our technician training protocol and continuous image quality control. Second, we show that even in participants with "Excellent" image quality scans in both eyes and no retinal pathology, the intereye correlation of retinal capillary perfusion measures is only moderately strong (r∼0.6), thus suggesting a biological basis for this difference that needs to be further explored. Third, we demonstrate that PA removal is a critical factor in correctly assessing perfusion in the DRL and for the first time quantify its effect size on a population level. Lastly, we demonstrate a faster decline in the perfusion of the SRL when compared with the DRL with age. Collectively, these findings suggest that retinal capillary perfusion measurements in both undilated eyes of an

Table 3. Linear Mixed Models for Retinal Perfusion Values and Age

	Higher VSD Eye			Lower VSD Eye		
Predictor	*Beta*	*95% CI*	*P-Value*	*Beta*	*95% CI*	*P-Value*
Age (1 year older difference in SRL)	−0.00033	−4.32e-04, -0.00023	<0.001	−0.00032	−4.32e-04, -0.00022	<0.001
Sex (females when compared to males at average age)	−0.00013	−0.0014, 0.0011	0.844	0.00037	−8.462e-04, 0.00159	0.548
Axial length (1 mm greater length)	0.00086	0.00031, 0.0014	0.002	0.00094	0.00039, 0.00148	<0.001
Image quality (excellent when compared with good quality at average age)	0.00201	0.0008, 0.00322	0.001	0.00160	4.27e-04, 0.00160	0.008
Layer (DRL when compared to SRL at average age)	−0.01246	−0.0199, −0.0050	0.001	−0.01499	−0.0227, −0.00722	<0.001
Age × layer (1 year older difference in the SRL when compared to DRL)	0.000096	−3.17e-06, 0.0002	0.058	0.00014	3.98e-05, 0.000247	0.007

CI = confidence interval; DRL = deep retinal layer; SRL = superficial retinal layer; VSD = vessel skeleton density.
Higher VSD eye includes 699 participants with 1398 observations (699 SRL scans and 699 DRL scans). Lower VSD eye included 698 participants with 1396 observations (698 SRL scans and 698 DRL scans). Higher VSD eyes were defined as the higher VSD value in the SRL. Lower VSD eye was defined as the lower VSD value in the SRL.

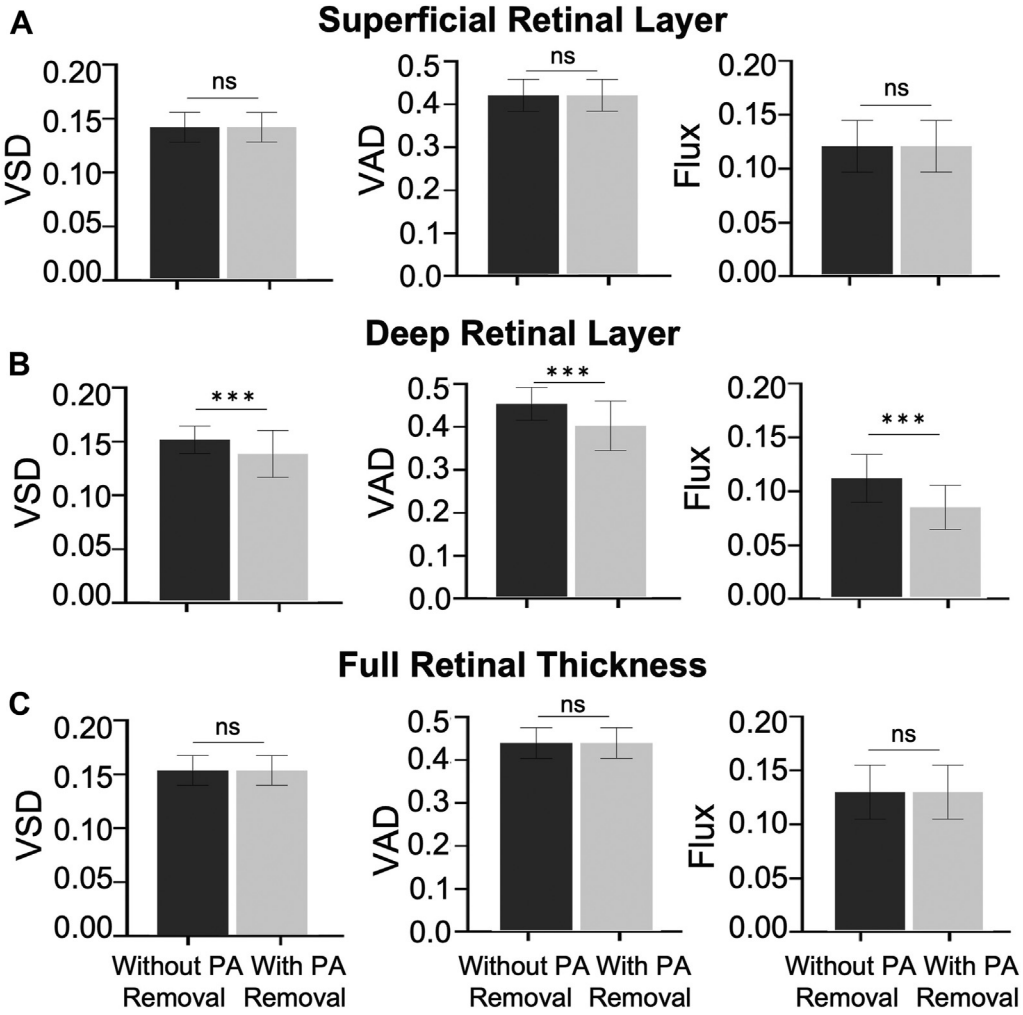

Figure 5. Impact of projection artifact (PA) removal on OCTA measures. Panel A shows measures for superficial retinal layer. Panel B shows measures for deep retinal layer. Panel C shows measures for full retinal thickness. Error bars show standard deviation of the mean. Black bars indicate measures without PA removal. Gray bars indicate measures with PA removal. *** indicates $P < 2.2e\text{-}16$. ns = not significant; OCTA = OCT angiography; VAD = vessel area density; VSD = vessel skeleton density.

individual can (and maybe should) be used in large-scale research and clinical studies to assess microvascular changes in health and disease.

Our study makes several important methodological contributions to OCTA imaging studies. Implementation of OCTA imaging in the absence of pupillary dilation stands to significantly benefit the ophthalmology and medical communities since the capillary-level data available through this modality can have significant systemic applications.[1,7,9,12–14,19,28–31] We demonstrate that undilated, bilateral OCTA imaging in approximately 15 minutes is possible with >70% data utilization in a community setting. This is important because it allows both implementation of OCTA in nonophthalmology settings as well as eliminates any potential confounding impact of pharmacologic dilation with vasoactive agents (phenylephrine, tropicamide, etc.) on retinal capillary blood flow. Notably, this utilization rate can be readily increased with a modest increase (5–10 minutes) in time for image acquisition. One limitation of this approach is that wide-field OCTA imaging (12 × 12 mm or greater) and even moderately large fields of view (e.g., 6 × 6 mm) will not be feasible to acquire without dilation. However, the 3 × 3 mm OCTA scan patterns used in this study have very good-to-excellent correlation with retinal vascular disease severity,[1,5,32] systemic diseases,[19,29,31,33] and are sufficient to evaluate retinal vascular physiology.[18,34,35] Notably, in addition to requiring dilation, larger scan sizes likely have insufficient resolution to detect subclinical changes in capillary measures.

Our study also demonstrates that the frequency of image acquisition was slightly, but significantly, greater in the right than left eye, and all OCTA measures were generally greater for the right than left eyes. There may be a few explanations for these patterns. First, in this study, imaging was performed on the right eye first. It is likely that the order of eye imaging impacts the success of image acquisition and explains the lower frequency of image acquisition in the left eye, which might be related to participant fatigue. However,

Table 4. Impact of PA Removal on Layer-Specific OCTA Metrics

Retinal Layers	Metrics	With PA Removal (Mean ± SD)	Without PA Removal (Mean ± SD)	Mean Differences (Mean ± SEM)	% Difference	*P*-Value
SRL	VSD	0.1452 ± 0.0071	0.1451 ± 0.0071	0.0000 ± 0.0000	0.07%	0.38
	VAD	0.4238 ± 0.0187	0.4237 ± 0.01892	0.0000 ± 0.0001	0.01%	0.67
	Flux	0.1197 ± 0.0121	0.1197 ± 0.0121	0.0000 ± 0.0001	0.00%	0.66
DRL	VSD	0.1387 ± 0.0111	0.1521 ± 0.0064	**−0.0133 ± 0.0008**	**−9.7%**	**<2.2e-16**
	VAD	0.4050 ± 0.0293	0.4572 ± 0.0194	**−0.0521 ± 0.0021**	**−12.88%**	**<2.2e-16**
	Flux	0.0839 ± 0.0103	0.1124 ± 0.0170	**−0.0284 ± 0.001**	**−33.96%**	**<2.2e-16**
FRT	VSD	0.1540 ± 0.0069	0.1540 ± 0.0069	0.0000 ± 0.0000	0.00%	0.70
	VAD	0.4428 ± 0.0181	0.4428 ± 0.0182	0.0000 ± 0.0001	0.00%	0.91
	Flux	0.1306 ± 0.0128	0.1306 ± 0.01279	0.0000 ± 0.0001	0.00%	0.83

DRL = deep retinal layer; FRT = full retinal thickness; OCTA = OCT angiography; PA = projection artifact; SD = standard deviation; SEM = standard error of the mean; SRL = superficial retinal layer; VAD = vessel area density; VSD = vessel skeleton density.
Means are based on 100 right eyes analyzed with PA removal and 100 analyzed without PA removal. Paired *t* tests were used to calculate *P*-values.
Bold text indicates statistical significance: $P < 0.05$.

the significantly higher retinal capillary perfusion measures in the right eyes compared with the left eyes in our study persist even in analyses with only the highest quality images and in subjects without any retinal pathology on OCT. This suggests that there is a biological basis to the difference in retinal perfusion between the 2 eyes of an individual. This interpretation is further supported by a study of color fundus photos from 54 813 UK Biobank participants that demonstrated higher fractal dimension and vascular density of large retinal arteries and veins in the right eyes than left eyes,[36] similar to our findings at the capillary level. Further investigation of this finding is warranted, and studies utilizing OCTA images should account for laterality and image acquisition order in analyses.

This study demonstrates a decrease in capillary density with age that is expected and in agreement with other data.[37–42] However, we demonstrate that PA can artificially inflate OCTA measures of retinal perfusion (VSD, VAD, and flux) from 9% to 34% in the DRL. This would likely obscure changes in retinal capillary perfusion due to aging or disease in the DRL. Using PA-resolved images, we found a significant age-dependent decrease in all retinal capillary perfusion measures in the SRL and DRL. The magnitude of this decrease is significantly less in the DRL than the SRL (Table S3, available at www.ophthalmologyscience.org). The reasons for this are unclear but may be related to the greater baseline capillary density of the SRL.

Our study has some limitations. First, since this is a cross-sectional study, we cannot infer causal relationships. Second, the FHS cohort is largely white, and therefore these data are not representative of multiple ethnicities or races. Nevertheless, many of the same principles used in this study may be applied to studies utilizing OCTA in other populations to obtain and analyze large datasets. In addition, the impact of PAs and dilation are likely to be similar across different populations, and this study can serve as a basis for future multi-ethnic studies. Adddditionally, studies have demonstrated that older age is correlated with smaller pupil size, which can impact OCT and OCTA quality.[43,44] We implemented rigorous image quality assessment and quality control; therefore, images with poor quality due to small pupil size were not included in the analyses. Lastly, ALs can impact retinal measurements from OCTA.[45] In our mixed-effects model analyses, we made statistical adjustments for AL, and we excluded AL outliers >3 standard deviation from the mean. In addition to this, as a secondary analysis, we also performed Littman formula[23–25] correction of VSD values for AL, and this did not change the significance or magnitude of our results or conclusions (Table S4, available at www.ophthalmologyscience.org).

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Analysis and interpretation: Collazo Martinez, Jiang, Beiser, Sobrin, Seshadri, Kashani, Wang

Obtained funding: Seshadri, Kashani

Overall responsibility: Collazo Martinez, Ting, Shahidzadeh, Vaidya, Kowalczyk, Alluwimi, Rijal, Jiang, Wang, Beiser, Sobrin, Seshadri, Kashani

Abbreviations and Acronyms:
AL = axial length; **DRL** = deep retinal layer; **FHS** = Framingham Heart Study; **OCTA** = OCT angiography; **PA** = projection artifact; **SRL** = superficial retinal layer; **VAD** = vessel area density; **VSD** = vessel skeleton density.

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