Distances From Capillaries to Arterioles or Venules Measured Using OCTA and AOSLO

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Multidisciplinary Ophthalmic Imaging

Purpose. To investigate distances from retinal capillaries to arterioles or venules noninvasively.

Methods. An adaptive optics scanning laser ophthalmoscope (AOSLO) and optical coherence tomography angiography (OCTA) imager acquired detailed maps of retinal vasculature. Using OCTA, we quantified the distance from the edge of an arteriole or venule to the middle of the nearest capillaries (periarteriole or perivenule capillary-free zones, respectively) within the superficial vascular plexus of 20 young healthy subjects with normal axial lengths. These distances were compared to AOSLO images for three subjects. We tested the relation between the peripheral capillary-free zones and FAZ horizontal, vertical, effective diameters, and asymmetry indices in the deep vascular plexus. We examined enlargement with OCTA of capillary-free zones in a type 2 diabetic patient.

Results. The periarteriole capillary-free zone (67.2 ± 25.3 μm) was readily visible and larger than the perivenule capillary-free zone (42.7 ± 14.4 μm), P<0.0001. The distance from foveal center (P = 0.005) and diameter (P = 0.048) were predictive of perivenule capillary-free zone values. OCTA and AOSLO corresponded for arterioles. FAZ effective diameter was positively associated with asymmetry indices, r = 0.49, P = 0.028, but not peripheral capillary-free zones, although focal enlargements were found in a diabetic patient.

Conclusions. For normal retinas, periarteriole and perivenule capillary-free zones are readily visible with OCTA and AOSLO. Periarteriole capillary-free zones were larger, consistent with arterioles carrying oxygen rich blood that diffuses to support the retina.

Keywords: foveal avascular zone, periarteriole capillary-free zone, perivenule capillary-free zone, OCT angiography, adaptive optics scanning laser ophthalmoscopy

Retinal capillaries are the key interface for the exchange of nutrients, oxygen, and metabolites between retinal neurons and the vascular system.1 The retinal neurovascular unit consists of blood vessel endothelial cells, pericytes, astrocytes, Müller cells, and neurons that are intimately connected to form the inner blood-retinal barrier that controls nutrient flow to the neural retina.2 A capillary-free zone, represents the maximum distance that nutrients and oxygen must travel to reach the retinal neurons. Thus, the diffusion distance would be half or less depending on the location of the neural element or Müller cell to the capillary, arteriole or venule.

Capillary-free zones in the retina can now be mapped with noninvasive methods, using the motion over time of the red blood cells computed across a series of images, using adaptive optics scanning laser ophthalmoscopy (AOSLO)3 or optical coherence tomography angiography (OCTA).4,5 The comparison of AOSLO maps of the foveal avascular zone (FAZ) to noninvasive retinal thickness measurements from OCT shows that in the normal retina, the inner retinal layers have a relatively constant thickness at the margins of the FAZ; and for this particular neural and glial tissue, retinal capillaries are needed to sustain an inner retinal thickness greater than 60 μm.6 Greater distances may indicate potential ischemia or a very sick retina with a decreased oxygen demand, found in diseases such as diabetic retinopathy (DR) when the distribution and structure of capillaries are altered including poor perfusion, or dropout of capillaries and vessel remodeling.7–11 Slowed perfusion or dropout of capillaries are both well-documented in vivo in DR by the early phase measurements of fluorescein angiography (FA). These measurements show changes for both FAZ and parafoveal regions for diameter, asymmetry, pore size, or other measurements of capillary spacing.12–18 Invasive methods for visualizing capillaries include histology,19 FA,12,18,20,21 and indocyanine green angiography (ICGA).22 Noninvasive methods include OCTA3,23,24 and AOSLO,2 the adaptive optics digital light ophthalmoscope (AODLO),26 and the subjective blue field entoptoscopy technique that agrees well with FA.29 The FAZ is also quantified noninvasively using transverse analysis of layer reflectivity and geometry of the foveal pit, using spectral domain OCT (SD-OCT).30 Due to the lack of axial resolution for all techniques other than AOSLO, AODLO, and OCTA, assessment of more peripheral capillary-free zones can be problematic unless the retina is thin or there is minimal dye leakage.

Regardless of measurement method, there is large inter-individual variability in the FAZ size6,12,18,20,23–26,30 that is related to large variability in foveal morphology. As a result, FAZ size measured for even a diabetic patient with capillary loss is...
not always significantly greater compared to controls. Some studies on the interaction of FAZ size with aging show an increase, but with differing quantitative characterization for FA and OCTA. In contrast, other studies show no significant increase, perhaps due to insufficient statistical power, or lack of analysis with FA and OCTA. In normal eyes, FAZ width is horizontally elongated with a large FAZ size and vertically elongated with a small FAZ size.

Another difficulty in using FAZ size to assess the status of the fovea or manage a diabetic patient is that the measurement per se is limited to only a few capillaries. In the case of severe DR, a metric of size increase may saturate, with no possibility of further size increase with increasing disease. FAZ capillaries are limited to the deeper layers because of the foveal pit, and are located only near the fovea where there is little influence of the ganglion cells. In contrast, assessing the status of the peripheral retina and the featureless retina, and not limiting assessment to visible vascular changes have been important clinical concepts for decades. Hence, in order to help in the diagnosis and management of DR, the use of salient features, such as black regions on OCTA next to a clearly visible and bright arteriole or venule, and more peripheral features could produce a more sensitive biomarker when used either alone or combined with FAZ metrics.

There are numerous capillary-free zones around larger blood vessels outside the fovea that could be used to help detect early damage to retinal vasculature. These have been demonstrated in living adult retinas using FA and histology when resolved in 3D. Capillary-free zones outside the fovea were imaged noninvasively, shown to be salient features, and comparable when acquired with AOSLO and OCTA. These capillary-free zones are a part of the vascular development process, when there is retraction or atrophy of the capillaries surrounding developing larger retinal vessels (i.e., arterial and venous channels develop while some of the side branches retract and atrophy). The presence of a capillary-free zone around the arterioles, the periarteriole capillary-free zone, may develop as a result of a vasoinhibitory effect of oxygen diffusing out of the retinal arterioles. The oxygen saturation within retinal arteries (77.8%–94.3%) is higher than in veins (55.5%–71.4%), but nevertheless for veins, the oxygenation when measured as the linear distance between the edge of an arteriole or venule and the nearest capillaries. The presence of a capillary-free zone around the arterioles, the periarteriole capillary-free zone, may develop as a result of a vasoinhibitory effect of oxygen diffusing out of the retinal arterioles. The oxygen saturation within retinal arteries (77.8%–94.3%) is higher than in veins (55.5%–71.4%), but nevertheless for veins, the oxygenation when measured as the linear distance between the edge of an arteriole or venule and the nearest capillaries. In normal eyes, FAZ shape is horizontally elongated with a large FAZ size and vertically elongated with a small FAZ size.

In this paper, we quantify the width of periarteriole and perivenule capillary-free zones using OCTA and AOSLO in young healthy subjects who do not have high refractive errors, and investigate the effect of sex as well as provide evidence of potential remodeling in diabetic patients. We examined the association between the metrics for the more peripheral periarteriole and perivenule capillary-free zones and the central macular FAZ, which are hypothesized to provide similar information; the association is expected to be weakened in normal subjects due to interindividual differences in foveal development.

### METHODS

#### Subjects

Young, healthy subjects (10 males and 10 females) free of ocular disease were recruited for this study. The estimated sample size was computed with a GPower 3.1 calculator using the following input parameters: effect size (partial eta squared) of 0.28, 9 of 0.05, power of 0.80, 3 independent variables (sex, vessel type, distance from fovea), and two levels for sex and vessel type. All subjects had refractive error <±3.00 diopters (D) to prevent significant differences in retinal magnification. Further exclusion criteria were lack of systemic disease or uncontrolled blood pressure. Axial length measurements were taken with the biometer (IOLMaster, version 5; Carl Zeiss Meditec, Dublin, CA, USA). The mean age of males (27.0 ± 3.02 years) and females (25.6 ± 3.13 years) did not differ, t(18) = 1.02, P = 0.32. There was no statistically significant difference between the mean axial length of males (23.8 ± 0.824 mm) and females (23.5 ± 0.847 mm), t(18) = 0.83, P = 0.42. To show remodeling of the capillary-free zones in diabetics, we also included data from a type 2 diabetic female, 54 years, duration of 11 years, and HbA1c of 8.0. Visual acuity was 20/20 – 2 OD, but the patient demonstrated that improvement could be made with pinhole OS from 20/60 + 2.
to 20/20 – 1. This patient had a thin retina despite having small cysts and vitreoretinal traction, e.g., the central macular thickness on OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany) was only \(229 \pm 0.18 \mu m\), with the inner ETDRS ring, 275, 244, 236, 209 and outer ETDRS ring, 257, 271, 243, and 243 \(\mu m\) for temporal, superior, nasal, and inferior retina, respectively. As the superficial vascular plexus was under study for controls for the peripheral capillary-free zones, but the patient had the potential for segmentation error because of the traction, we analyzed the sum of both the plexuses. This provides a conservative estimate of the size of the peripapillary arteriole and venule capillary-free zones, (i.e., the lower bound of the capillary-free zones). The study adhered to the tenets of the Declaration of Helsinki, and informed consent from all subjects was obtained prior to experimental data collection after explanation of the nature and possible consequences of the study. The study was approved by the Indiana University Institutional Review Board (IRB).

**Imaging Session with AOSLO**

Videos (30 frames/second) of paired arterioles, venules, and surrounding capillaries of the SVP of 3 subjects (ages 27, 29, and 30 years) were acquired with a point scanning AOSLO at 0.93 \(\mu m/pixel\) and wavelengths of 775 \(\pm 20\) and 827 \(\pm 20\) nm. Different confocal apertures were used in the two optical imaging channels to visualize vessel walls versus lumen to obtain accurate vessel widths or emphasize reflectivity of flowing particles versus absorption in the arterioles for motion mapping for capillary density. Videos were processed using custom software (MATLAB; MathWorks, Natick, MA, USA) and perfusion maps were generated using the variance of scatter from moving blood flow. The variance maps were montaged in image editing software (Photoshop CS6; Adobe, Inc., San Jose, CA, USA) to generate a wider field of view of the paired arterioles, venules, and surrounding capillaries, which provides the potential for numerous regions of interest to be measured (Fig. 1). For illustrative purposes only, vessels were manually traced using a single pixel pencil tool in image editing software (Adobe, Inc.), using a 27-inch graphics tablet and electronic pen (Cintiq, WACOM, Portland, OR, USA) to better illustrate the relative locations of all vessels, and the peripapillary and perivenule capillary-free zones (Fig. 2). Distances between capillaries and the arterioles and lumen widths were computed manually from the images, not the tracing, using the image editing software (Adobe, Inc.) measurement tool and compared with OCTA measurements.

**Imaging Session with OCTA**

OCTA images of paired arterioles and venules with their surrounding capillaries were obtained (Spectralis HRA + OCT; Eye Explorer version 1.9.14.0 software with upgrades through 1.9.17.0; Heidelberg Engineering). The signal quality values of all our OCTA images from the vendor software ranged from 30 to 40 with 40 being the highest possible value that can be obtained. For all subjects, we analyzed the SVP in an approximately linear pattern evenly along each sampled arteriole or venule. The average distances from the fovea of the regions of interest were 14.2° \(\pm\) 3.22° (range: 8.28°–19.6°) and 14.6° \(\pm\) 2.39° (range: 10.7°–20.4°), respectively for arterioles and venules. The distance from the foveal center for arterioles (14.7° \(\pm\) 2.57°) and venules (14.4° \(\pm\) 3.03°) for males did not differ from the arteriole (13.8° \(\pm\) 3.85°) and venule (14.7° \(\pm\) 1.71°) distance from the foveal center for females, \(t(18) = 0.619, p = 0.54, t(18) = -0.221, P = 0.83\) for arterioles and venules, respectively. The SVP was defined as the retinal vasculature from the inner limiting membrane (ILM) to the inner plexiform layer (IPL)/inner nuclear layer (INL) boundary while the DVP consisted of the retinal vasculature from the IPL/INL boundary to the outer plexiform layer (OPL)/outer nuclear layer (ONL) boundary. Each OCTA image was 10 \(\times\) 10° consisting of 512 b-scans, 512 A-scans per b-scan, 6\(\mu m\) spacing between the b-scans and seven frames averaged per each b-scan location. Images were then manually montaged...
using image editing software (Adobe, Inc.) to generate a wider field of view of the paired vessels (Fig. 3).

**Image Analysis**

*Computation of Periarteriole and Perivenule Capillary-Free Zones in OCTA Images.* To compute the distance from the vessel to the capillary, we calculated the length of a line, drawn perpendicular to the arteriole or venule, between the nearest edge of the larger vessel and the center of the capillary, using the SVP (Fig. 4). The middle of the nearest capillary and not the edge was chosen because OCTA does not have sufficient lateral resolution to accurately delineate the edge of a capillary. Multiple distance samples were collected by manual marking at close spacing, so that orientation or curvature artifacts were negligible. A custom computing program (MathWorks, Inc.) then automatically recorded the x and y coordinates of each marked point and saved these values in spreadsheet software (Microsoft Excel 2013; Microsoft Corp., Redmond, WA, USA) files. Each distance sample was then computed as distance $= \sqrt{(X_{\text{large vessel edge}} - X_{\text{capillary}})^2 + (Y_{\text{large vessel edge}} - Y_{\text{capillary}})^2}$. The number of pixels computed was then converted into microns by multiplying the Euclidean distances by the micron-to-pixel ratio in the x and y directions, as computed from the fiducial marks (Heidelberg Engineering). The periarteriole and perivenule capillary-free zones were then computed as the average of 50 Euclidean distance samples.

**FIGURE 2.** Tracing of the AOSLO montage from Figure 1 for a 30-year-old healthy female showing arterioles, venules, and capillaries of the SVP using a single pixel pencil in an image editing program (Adobe, Inc.). Regions free of capillaries are better delineated around the arteriole (periarteriole capillary-free zone) and venule (perivenule capillary-free zone). The periarteriole capillary-free zone can be observed as wider than the perivenule capillary-free zone. Paired arteriole and venule are labeled “A” and “V” respectively.

**FIGURE 3.** OCTA montage of $10^5 \times 10^5$ images plus enlargement of the venule portion of a 30-year-old healthy male showing paired arteriole (red) and venule (blue) at 13.7° and 11.9°, respectively, from the foveal center in the SVP. (A) Periarteriole and perivenule capillary-free zones indicated by blue and red arrows, respectively. SVP consists of retinal vasculature from the ILM to the IPL/INL boundary. (B) Magnified binarized image of the venule showing well-defined narrow perivenule capillary-free zones. Periarteriole capillary-free zone can be observed as wider than the perivenule capillary-free zone in (A).
Computation of Arteriole Diameter, Venule Diameter, and Distance From Foveal Center in OCTA Images.
The paired arteriole or venule lumen diameter was computed as $\left[\left(\frac{X_{vessel\ top}}{C_0} - \frac{X_{vessel\ bottom}}{C_0}\right)^2 + \left(\frac{Y_{vessel\ top}}{C_0} - \frac{Y_{vessel\ bottom}}{C_0}\right)^2\right]^{1/2}$. To compute arteriole and venule distance from the center of the fovea, the center of the FAZ was marked and corresponding $x$ and $y$ coordinates generated. The vessel distance from the center of the fovea was then computed as $\left[\left(\frac{X_{vessel\ top}}{C_0} - \frac{X_{fovea}}{C_0}\right)^2 + \left(\frac{Y_{vessel\ top}}{C_0} - \frac{Y_{fovea}}{C_0}\right)^2\right]^{1/2}$. The number of pixels computed was then converted into microns by multiplying them by the micron to pixel ratio in the $x$ and $y$ directions from the vendor’s fiducial marks and then converted into degrees.

Computation of FAZ Size, and Asymmetry Index in OCTA Images. Using vendor software, the FAZ of all subjects in the DVP was marked and the area covered recorded (Fig. 5A). The horizontal FAZ diameter (FAZH) and the vertical FAZ diameter (FAZV) were also computed (Fig. 5B). Asymmetry indices were then computed as the ratio of FAZH to FAZV (FAZH/FAZV). A second method for FAZ diameter was computed from the FAZ area values, defined as the diameter of the circle whose area was equivalent to the area of the FAZ (the effective diameter). Hence, the FAZ effective diameter = $(4\pi FAZ\ area/\pi)^{1/2}$.

Statistical Analysis
All statistical analyses were performed using statistical software (IBM SPSS Statistics for Windows, Version 24.0; IBM Corp., Armonk, NY, USA). All values are presented as mean ± SD. Samples included 50 points of periarteriole and perivenule capillary-free zones for each subject, sampled evenly at regular intervals or equally spaced along a linear distance of 1.78 ± 1.12 mm and 1.38 ± 0.717 mm for arterioles and venules, respectively, for statistical analysis. A $2 \times 2$ mixed model ANOVA was performed with sex (male and female) and vessel type (arteriole and venule) as independent variables and width of capillary-free zone as the dependent variable. A multiple linear regression of periarteriole capillary-free zone as the dependent variable versus arteriole distance from foveal center, arteriole diameter, and sex as predictors was performed. A multiple linear regression of perivenule capillary-free zone as the dependent variable versus venule distance from foveal center, venule diameter, and sex as predictors was also performed. The mean periarteriole and perivenule capillary-free zones were computed. The associations between the mean periarteriole capillary-free zone versus FAZ effective diameter, mean perivenule capillary-free zone versus FAZ effective diameter, mean periarteriole capillary-free zone...
versus mean perivenule capillary-free zone, asymmetry indices versus FAZ effective diameter, and FAZ size versus age were examined using Pearson product moment correlation. Independent t-tests were used to compare the means of the FAZ metrics and the various covariates between males and females, assuming Levene’s test for equality of variance was not statistically significant. An F test for two sample variances compared the intersubject variability of periarteriole vs. perivenule capillary-free zones, periarteriole capillary-free zone versus FAZ size, and perivenule capillary-free zone versus FAZ size. A value of $P < 0.05$ was considered statistically significant.

**Results**

The periarteriole and perivenule capillary-free zones were readily computed for both AOSLO and OCTA methods for all 3 subjects. The AOSLO, which has the spatial resolution to provide measurements of the walls as well as the lumens, gave an average width of the arteriole lumens of 68.0 ± 5.00, 69.0 ± 3.00, and 53.0 ± 2.00 $\mu$m, with a wall: lumen ratio of 0.265, 0.688, and 0.781, similar to previous findings.

The flow through the lumen is visualized for both AOSLO and OCTA, but walls are visualized by AOSLO only. The AOSLO and OCTA values agreed for the periarteriole capillary-free zones. Measuring at similar distances from the fovea (Fig. 1) (12.5°, 15.3°, and 13.7°, respectively), the distance from the lumen to the nearest capillary loop for AOSLO was 59.0 ± 21.0, 50.0 ± 17.0, and 55.0 ± 15.0 $\mu$m and from the edge of the vessel to the capillary with OCTA was 53.0 ± 16.0, 51.0 ± 20.0, and 52.0 ± 15.0 $\mu$m, for each of the three subjects, respectively. Subsequent results are reported only from the OCTA images, which provided data across a wide field in a time efficient manner.

For measurements using OCTA, in the SVP, the width of the periarteriole capillary-free zone (67.2 ± 25.3 $\mu$m) was significantly greater than that of the perivenule capillary-free zone (42.7 ± 14.4 $\mu$m), $F_{(1,998)} = 771, P < 0.0001$ (Fig. 6). No difference was found in the sample for the mean arteriole diameter of males (99.8 ± 11.5 $\mu$m) and females (105 ± 15.2 $\mu$m), $t(18) = -0.805, P = 0.43$. Similarly, the mean venule diameter of sampled vessels did not differ for males (129 ± 18.9 $\mu$m) and females (129 ± 20.7 $\mu$m), $t(18) = 0.056, P = 0.96$. There was no significant interaction between vessel type and sex on the width of the capillary-free zone, $F_{(1,998)} = 0.684, P = 0.41$ (Fig. 6). Males had similar periarteriole capillary-free zone (67.8 ± 26.0 $\mu$m) compared to females (66.7 ± 24.5 $\mu$m), as well as similar perivenule capillary-free zone (42.5 ± 13.8 $\mu$m, 42.9 ± 15.0 $\mu$m, respectively), the distance from the lumen to the capillary loop across the vascular territories was 26.0 ± 17.0, 21.0 ± 13.7, and 14.8 ± 12.5 $\mu$m for AOSLO and 25.3 ± 17.0, 20.7 ± 13.7, and 13.7 ± 12.5 $\mu$m for OCTA, for each of the three subjects, respectively.

For our normal subjects, the regression between retinal eccentricity and the periarteriole capillary-free zone was not significant ($F_{(3,996)} = 0.834, P = 0.48$ with an $R^2$ of 0.003) for a multiple linear regression predicting periarteriole capillary-free zone based on arteriole distance from foveal center, arteriole diameter, and sex. Within the multiple linear regression, the influence of arteriole distance from foveal center ($P = 0.16$), arteriole diameter ($P = 0.98$), and sex ($P = 0.65$) were all not significant. The regression weights and subgroup means are summarized in the Table.

![Figure 6](image-url)  
*Figure 6.* The effect of vessel type and sex on the width of capillary-free zone. The periarteriole capillary-free zone is larger than the perivenule capillary-free zone for both males and females ($P < 0.0001$). There is no significant interaction between vessel type and sex on the width of the capillary-free zones ($P = 0.41$): Males had similar periarteriole and perivenule capillary-free zones compared to females ($P = 0.72$). Error bars represent standard deviation.

| TABLE. | Multiple Linear Regression Between Periarteriole and Perivenule Capillary-Free Zones and Vessel Distance from Fovea, Vessel Diameter, and Sex |
|--------|--------------------------------------------------------------------------------------------------------------|
|        | $\beta$ | **Mean ± SD (\(\mu m\))** | $t$ | $P$ Value |
| Periarteriole capillary-free zone | Arteriole distance from fovea | 0.36 | 68.6 ± 11.9 | 1.41 | 0.16 |
| | Close (8°–12°) | | 64.6 ± 12.7 | 0.62 | 0.53 |
| | Middle (12°–16°) | | 70.8 ± 14.8 | 0.57 | 0.57 |
| | Far (16°–20°) | | 65.9 ± 13.7 | -0.025 | 0.98 |
| | Greater than 102 \(\mu m\) (average) | | 68.5 ± 12.5 | -0.737 | 0.48 |
| | Less than 102 \(\mu m\) (average) | | 65.9 ± 13.7 | -0.025 | 0.98 |
| | Sex | -0.737 | -0.452 | 0.65 |
| Perivenule capillary-free zone | Venule distance from fovea | 0.571 | 41.8 ± 0.38 | 2.93 | 0.003 |
| | Close (8°–12°) | | 40.2 ± 5.75 | 1.98 | 0.048 |
| | Middle (12°–16°) | | 46.6 ± 3.38 | 1.98 | 0.048 |
| | Far (16°–20°) | | 45.5 ± 5.35 | 1.98 | 0.048 |
| | Greater than 129 \(\mu m\) (average) | | 41.1 ± 4.95 | 1.98 | 0.048 |
| | Less than 129 \(\mu m\) (average) | | 41.1 ± 4.95 | 1.98 | 0.048 |

Subgroup means are shown.
In contrast, the regression between retinal eccentricity and perivenule capillary-free zone was significant ($F_{(5, 996)} = 5.62$, $P = 0.001$ with an $R^2$ of 0.017) for a multiple linear regression predicting perivenule capillary-free zone based on venule distance from the foveal center, venule diameter, and sex. Predicted perivenule capillary-free zone $= 29.1 + 0.571$ (venule distance from the foveal center) $+ 0.037$ (venule diameter) $+ 0.259$ (sex). Venule distance from the foveal center ($P = 0.003$), and venule diameter ($P = 0.048$) were significant, but sex ($P = 0.78$) was not significant.

Thus, there was no significant association between periarteriole capillary-free zone and arteriole distance from foveal center (Fig. 7A) but there was a positive association between perivenule capillary-free zone and venule distance from the foveal center. For a $300 \mu m$ ($\sim 1\frac{1}{2}$) increase in venule distance from foveal center, the perivenule capillary-free zone increases by $0.571 \mu m$ holding all other variables constant (Fig. 7B). There was also no significant association between periarteriole capillary-free zone and arteriole diameter (Fig. 8A), but there was a positive association between perivenule capillary-free zone and venule diameter. For a $1\mu m$ increase in venule diameter, perivenule capillary-free zone increases by $0.037 \mu m$ holding all other variables constant (Fig. 8B).

There was no significant association between mean perivenule capillary-free zone and FAZ effective diameter, $r = -0.167$, $P = 0.48$ (Fig. 9A). There was also no significant association between mean perivenule capillary-free zone and FAZ effective diameter, $r = -0.196$, $P = 0.41$ (Fig. 9B). Mean perivenule capillary-free zone was not significantly associated with mean perivenule capillary-free zone, $r = 0.244$, $P = 0.30$. However, there was a significant positive association between asymmetry indices and FAZ effective diameter, $r = 0.49$, $P = 0.028$ (Fig. 9C). There was also no significant association between FAZ size and age, $r = 0.189$, $P = 0.43$, although our age range was so narrow that this would be expected. The average FAZ of all subjects was $0.368 \pm 0.114$ mm$^2$. The mean asymmetry indices and effective diameter of all subjects was $0.957 \pm 0.130$, and $674 \pm 120 \mu m$ respectively. The FAZ of males ($0.350 \pm 0.142$ mm$^2$) did not differ from that of females ($0.386 \pm 0.082$ mm$^2$), $t(18) = 0.50$, as would be expected for a small sample size. The coefficient of variation (CV) of the FAZ ($0.311$) did not differ from that of the perivenule ($0.376$) and perivenule ($0.337$) capillary-free zones, $F_{(19,19)} = 1.47$, $P = 0.21$, $F_{(19,19)} = 1.18$, $P = 0.36$, respectively. A similar trend was found for the CV of the periarteriole versus perivenule capillary-free zones, $F_{(19,19)} = 1.25$, $P = 0.32$.

In the individual with diabetes, we also observed focal remodeling of the capillary-free zones, including the FAZ, periarteriole capillary-free zone, and perivenule capillary-free zone (Figs. 10A, 10B). As expected, the FAZ had an irregular shape. The FAZH was $860 \mu m$ and the FAZv was $781 \mu m$, larger than the mean from the effective diameter of the controls ($674 \mu m$) but not outside the 95% 1-tailed confidence limit of $881 \mu m$. There are clear microaneurysms and small regions of vessel remodeling, which were not limited to the FAZ (Fig. 10A). There were also regions of nonperfused or poorly perfused perifoveal capillaries, as well as regions near both inferior arterioles and venules that were conspicuously dark (i.e., lacking capillaries with perfusion rates above the criterion for OCTA; Figs. 10A, 10B).

The mean periarteriole capillary-free zone is $106 \mu m$, which is greater than the average periarteriole capillary-free zone of the controls ($67.2 \mu m$) and outside the right sided one-tailed 95% confidence limit ($68.6 \mu m$) of the healthy controls. This was computed for an arteriole distance from the fovea of $15.5 \mu m$ and an arteriole diameter of $81 \mu m$, which are both less than or within the one-tailed 95% confidence limit of $15.4 \mu m$ and $107 \mu m$, respectively, for arteriole distance and diameter for the healthy controls. The linear distance of sampling at regular or evenly spaced intervals is $3.30 \mu m$.

The mean perivenule capillary-free zone is $79.6 \mu m$, which is greater than the average perivenule capillary-free zone of the controls ($42.7 \mu m$) and outside the one-tailed 95% confidence limit ($43.5 \mu m$) of the healthy controls. This was computed for a venule distance from the fovea of $15.2 \mu m$ and a venule diameter of $134 \mu m$, which are both less than or within the one-tailed 95% confidence limit of $15.4 \mu m$ and $136 \mu m$ respectively, for venule distance and diameter for the healthy controls. The linear distance of sampling at regular or evenly spaced intervals is $3.20 \mu m$.

Using the multiple regression parameters from controls (Table), we computed the expected perivenule capillary-free zone, perivenule $CFZ = 29.1 + 0.571 (15.2) + 0.037 (134) + 0.259 (2) = 43.3 \mu m$. The 95%, 1-tail confidence limit (18 degrees of freedom) based on the standard deviation of the residuals from the multiple regression gives a cutoff of $58.9 \mu m$. Thus, the measured perivenule capillary-free zone ($79.6 \mu m$) of the diabetic subject in Figure 10 is significantly greater.
Figure 8. Mean periarteriole and perivenule capillary-free zones plotted as a function of mean arteriole and venule diameter, respectively. (A) Plot showing no association between mean periarteriole capillary-free zone and mean arteriole diameter, \( \beta = -0.001, P = 0.98 \). (B) Plot showing a positive association between mean perivenule capillary-free zone and mean venule diameter, \( \beta = 0.037, P = 0.048 \). Closed and open symbols represent male and female, respectively.

Figure 9. Mean periarteriole capillary-free zone, mean perivenule capillary-free zone, and asymmetry indices plotted as a function of FAZ effective diameter. (A) Plot of mean periarteriole capillary-free zone versus FAZ effective diameter showing lack of significant association, \( r = -0.167, P = 0.48 \). (B) Plot of mean perivenule capillary-free zone versus FAZ effective diameter showing lack of significant association, \( r = -0.196, P = 0.41 \). (C) Plot of asymmetry indices versus FAZ effective diameter showing a significant positive association, \( r = 0.49, P = 0.028 \) indicating that the shape of the FAZ area is horizontally elongated with a large FAZ area, but vertically elongated with a small FAZ area. Closed and open symbols represent male and female, respectively.
However, the healthy retina extracts oxygen and the need for capillaries to exist immediately around the arterioles is consistent with the retinal arterioles carrying wider than the perivenule capillary-free zone in the healthy range of measurement locations (Figs. 1, 2), unlike the FAZ.

Measurements of capillary-free zones provide a wide range of axial measurements, or may have measured too close to the contrast to delineate perivenule capillary-free zones, particularly in previous studies.32–34 Periarteriole capillary-free zones were clearly visualized and quantified. Perivenule capillary-free zones were also measured, consistent with one previous study34 but in contrast to other studies failing to measure it.32,33 Our finding of periarteriole capillary-free zones as being wider than perivenule capillary-free zones is also consistent with the results of a previous study.33 Our results also show that venule distance from foveal center, and diameter are significantly predictive of the perivenule capillary-free zones. Thus, previous studies may have had insufficient resolution or contrast to delineate perivenule capillary-free zones, particularly with FA and the inability to restrict measurements to a narrow axial range, or may have measured too close to the fovea. Measurement of capillary-free zones provides a wide range of measurement locations (Figs. 1, 2), unlike the FAZ.

Our finding of the periarteriole capillary-free zone being wider than the perivenule capillary-free zone in the healthy retina is consistent with the retinal arterioles carrying oxygenated blood in the healthy retina and hence a reduced need for capillaries to exist immediately around the arterioles.32 However, the healthy retina extracts oxygen and the venules are at a lower level of oxygenation and hence there is an increased need for the capillaries to exist closer to the venules.33 The retinal veins however do carry oxygen, and the larger veins provide a larger reservoir, as well as a large surface area, for oxygen diffusion and hence, the increase in the perivenule capillary-free zones with increasing lumen diameter of the venules was observed. Also, the larger perivenule capillary-free zones observed at larger distances from the fovea may be due to lower cell density farther away from the fovea and hence less metabolic demand in these regions.

The FAZ, periarteriole, and perivenule capillary-free zones varied from one normal subject to another. Our results show that smaller FAZ sizes are associated with vertically elongated FAZ while larger FAZ sizes are associated with a horizontally elongated FAZ (Fig. 9C), consistent with our previous AOSLO study.6 The human FAZ is not fully formed until after approximately 6 years of life.32,53 The development of the FAZ and lateral displacement of the inner retinal neurons during this period of development has been tied to mechanical stress caused by eye growth and intraocular pressure.54 The relation between shape and size of the FAZ has been suggested to arise as a result of variation in mechanical stress during the formation of the foveal pit.55 This interindividual variability, including the relation between the size and shape of the FAZ, may have led to our finding of no association of FAZ and the periarteriole or perivenule capillary-free zone (Figs. 9A, 9B). However, the area of the FAZ varies strikingly with DR, with patients having FAZ areas more than twice that of controls.15 The FAZ, periarteriole, and perivenule capillary-free zones varied from one normal subject to another. Our results show that smaller FAZ sizes are associated with vertically elongated FAZ while larger FAZ sizes are associated with a horizontally elongated FAZ (Fig. 9C), consistent with our previous AOSLO study.6 The human FAZ is not fully formed until after approximately 6 years of life.32,53 The development of the FAZ and lateral displacement of the inner retinal neurons during this period of development has been tied to mechanical stress caused by eye growth and intraocular pressure.54 The relation between shape and size of the FAZ has been suggested to arise as a result of variation in mechanical stress during the formation of the foveal pit.55 This interindividual variability, including the relation between the size and shape of the FAZ, may have led to our finding of no association of FAZ and the periarteriole or perivenule capillary-free zone (Figs. 9A, 9B). However, the area of the FAZ varies strikingly with DR, with patients having FAZ areas more than twice that of controls.15 Newer analyses approaches for FAZ are under development that incorporate a larger area of the retina, thus including more vessels and reducing the increased inter-subject variability contributed by developmental differences and a limited number of sampled vessels available in the FAZ inner loop.55 The mean FAZ of our young healthy subjects using OCTA (0.368 ± 0.114 mm²) was comparable to that measured with AOSLO in our previous study and another study; (0.32 ± 0.16 mm²)8 and (0.32 ± 0.107 mm²)26 as well as with another OCTA study (0.335 ± 0.113 mm²)26 and two other studies with FA; (median = 0.35 mm²),12 and (0.567 ± 0.090 mm²).57 The FAZ effective diameter of our young normal subjects (674 ± 120 μm) was also comparable to that found with AOSLO in our previous study and another study; (614 ± 184 μm),5 and (633 ± 103 μm).35 The good agreement between AOSLO and OCTA was found in normal subjects and thus in retinal vessels that did not
include vessel remodeling. We measured to the center of capillaries, not the edges. The OCTA measurements provided sufficiently narrow confidence limits to allow comparisons of a single individual to the model from a group of controls. However, in this population and with this method, the lack of higher or lower vessels, along with our present variables of vessel number of capillary layers, distance travelled for a given arteriole or venule, along with our present variables of vessel diameter and distance from the fovea that were statistically significant for venules. Further refinement of patient demographics (e.g., age), available oxygen (i.e., beyond classification of vessels as an artery or vein), and other factors are also needed for a complete model.

The periarteriole and perivenule capillary-free zones also offer the potential for measurements focally over a large region of retina. However, our OCTA system has remaining issues with projection artifacts. Hence, cleanly visualizing vascular plexuses at different depths without interference of neighboring plexuses was difficult. However, this is unlikely to affect our results, since we did not measure capillary density. Also, we measured the capillary-free zones in the SVP, which does not have overlying vasculature. Measuring capillary-free zones may not be accurate in the DVP with OCTA,35 plus there are not large retinal vessels to lead to such zones. Projection artifacts from more superficial arterioles and venules in the SVP as well as deeper capillaries having a more lobular pattern, make identification of zone more difficult in the DVP. Patient eye movement and alignment decreased contrast in two images, but in all subjects, periarteriole and perivenule capillary-free zones were clearly observed and quantifiable.

Retinal vascular changes in DR are complex and often unexpected by clinical classification, and include capillary nonperfusion, capillary tangles, and the formation of arteriovenous shunts.38 There can be a paradoxical higher oxygen saturation in venules due to reduced oxygen extraction from the retina.10,11 Vessel remodeling, as well as other vascular changes such as microaneurysms, can all reduce the ability to quantify the FAZ, the periarteriole, or perivenule capillary-free zones. Capillary nonperfusion or dropout around arterioles may also occur and hence lead to a larger FAZ, periarteriole, or perivenule capillary-free zones. Capillary nonperfusion or dropout around arterioles and venules may also occur and hence lead to a larger FAZ, periarteriole, or perivenule capillary-free zones in diabetics, as shown in Figure 10. Thus, the capillary-free zones around the arterioles and venules may serve as a biomarker which may aid in the diagnosis, management, and monitoring of disease progression in DR. The developed capillary-free zone is not limited to the perivenule or periarteriole region, but might be more readily detected due to the contrast. As the present study is a cross-sectional study in young control subjects, the next step would be to assess the mean periarteriole and perivenule capillary-free zones over time and in a wider range of control subjects, to serve as a baseline against which values in patients could be measured as indicative of diabetic retinopathy (DR) classification and progression. The qualitative grading as well as quantitative metrics such as width of the capillary-free zones may also aid in staging DR since it is well established that there can be retinal changes more severe than seen on clinical exam or fundus photography.7,58,44 The capillary-free zones, both periarteriole and perivenule, along with more advanced metrics concerning capillaries near the fovea,15,46 and other measures, may aid in bringing us closer to the needed reclassification of DR that is now possible with improved imaging modalities.

OCTA and AOSLO have the ability to generate vascular maps of a specific vascular plexus, and an emphasis in recent studies with OCTA concerns normative values and types of artifact removal for quantifying the vascular plexuses at different depths, such as the SVP and DVP.4,59–62 In our study, we reported data only for the SVP capillary-free zones outside of the fovea, since this is where the larger vessels occur, and capillary-free zones would be expected.55

To understand what sizes and locations of capillary-free zones indicate the highest risk for retinal damage, further research is needed to form a 3D model of oxygen demand and potential ischemia. A complete model would include such variables for each measurement location as retinal thickness, number of capillary layers, distance travelled for a given arteriole or venule, along with our present variables of vessel diameter and distance from the fovea that were statistically

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