Influence of Refractive Error and Axial Length on Retinal Vessel Geometric Characteristics

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Purpose. To evaluate the influence of refractive error and axial length (AL) on retinal vascular network geometry measurements in an adult Asian population.

Methods. This was a population-based, cross-sectional study on 2882 persons with diabetes in the Singapore Malay Eye Study. Spherical equivalent refraction was assessed using an autokeratorefractometer and subjective refraction. AL retinal vascular caliber, tortuosity, and branching characteristics were quantified from retinal fundus photographs using a semiautomated computer-assisted program according to a standardized protocol.

Results. In multivariate analyses adjusting for age, sex, education, smoking, blood pressure, diabetes status, and antihypertensive medication use, longer AL and more myopic refraction were associated with narrower retinal arterioles and venules ($P \leq 0.001$ for all) and less tortuous (straighter) arterioles ($P < 0.001$ for both). Longer AL and more myopic refraction were also associated with increased branching coefficients in both arterioles ($P < 0.001$ for both) and venules ($P = 0.02$ and $P < 0.001$, respectively). Longer AL and more myopic refraction were associated with more acute branching angles in arterioles ($P < 0.001$ for both) but not venules.

Conclusions. Myopic refractive errors and longer AL are associated with narrower retinal arterioles and venules, less tortuous arterioles, and increased branching coefficients in both arterioles and venules. These findings provide insights into ocular blood flow in myopia and also suggest that future studies evaluating these retinal parameters should account for the influence of AL and refractive error. (*Invest Ophthalmol Vis Sci.* 2011;52:669–678) DOI:10.1167/iovs.10-6184

Changes in the retinal vasculature have been shown to be biomarkers of systemic vasculopathy as well as major eye diseases, including diabetic retinopathy,[1,2] glaucoma,[3] and age-related macular degeneration.[4] In particular, the associations with measurements of retinal vessel caliber have been extensively studied in several large epidemiologic studies.[5–11]

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More recently, new computer-based programs have been developed to perform quantitative assessments of geometric parameters of the retinal vasculature, such as retinal vascular tortuosity and branching angles.[12–17] The vascular network is believed to be organized to minimize shear stresses and work across the system,[18–21] and these parameters may provide an indication of how closely a given network conforms to the geometrical ideal. In contrast with retinal vessel caliber measurements, which are generally performed at a fixed location relative to the optic disc and which measure only a single property of the retinal vessels, geometric network measures provide a better indication of the overall health of the vascular system. For example, Chapman et al.[16] have demonstrated that retinal vascular diameters at bifurcation points deviated significantly from the optimal in subjects with peripheral vascular disease but conformed to optimal values in healthy controls. Low-birth-weight men have also been shown to have narrower bifurcation angles,[22] suggesting a mechanistic link between impaired fetal development and the subsequent development of cardiovascular diseases like hypertension. Other studies in type 1 diabetes have also shown that changes in retinal tortuosity are related to key diabetes-related factors such as HbA1c levels and duration of disease, suggesting a potential role as a predictor of retinopathy.[23]

The relationship of refractive errors and axial length (AL) with retinal vascular network geometry has not been studied but merits investigation for two reasons. First, measurements of retinal vessel geometry are known to be influenced by refractive error.[24,25] Second, AL may also directly influence retinal vascular topographic features,[26] which may reflect retinal blood flow.[27–29] To date, however, data on the possible associations of refractive error and AL with retinal vascular network geometry have been limited. The Blue Mountains Eye Study reported that myopic refraction is associated with a reduction in the retinal vascular fractal dimension, a related parameter that measures the complexity of the vascular network, but have not examined branching or tortuosity.[30] In a small study of 52 adult subjects, Patton[31] did not find any associations between AL and branching angles or junctional exponents.

The aim of this study was to evaluate the influence of refractive error and AL on retinal vascular network geometry measurements in an adult Asian population.

Methods

Study Population

The Singapore Malay Eye Study (SiMES) is a population-based, cross-sectional study of urban Malay adults aged 40 to 80 years residing in Singapore. Study design and population details have been described elsewhere.[32,33] In brief, Malay subjects were selected from a national database using an age-stratified random sampling process. Of those eligible, 3280 (78.7% participation rate) were examined between 2004 and 2006.

All study procedures were performed in accordance with the tenets of the Declaration of Helsinki as revised in 1989. Written informed consent was obtained from the subjects, and the study was approved by the Institutional Review Board of the Singapore Eye Research Institute.

Assessment of Refractive Error and Axial Length

Each subject's refractive error was obtained with an autorefractor machine (Canon RK-5 Auto Ref-Keratometer; Canon Inc., Tokyo, Japan), after which subjective refraction by trained, certified study optometrists was performed to achieve the best corrected visual acuity. The final subjective refraction result was used in the analysis. Spherical equivalent (SE) was defined as sphere plus half negative cylinder. AL was measured using noncontact partial coherence laser interferometry (IOL Master v.3.01, Carl Zeiss; Meditec AG, Jena, Germany).[34]

Measurement of Retinal Vascular Caliber

The methods for obtaining digital fundal photographs and for measuring retinal vascular caliber from these photographs have been described in SiMES and other publications.[35–37] Retinal photographs centered on the optic disc were obtained with a digital fundal camera (Canon CR6-NM45, EOS-D60, 6.3 megapixel; Canon Inc., Lake Success, NY) through a dilated pupil. A computer-based program was then used to measure the caliber of all retinal vessels located between one-half to one disc diameter from the optic disc margin. Using this program, the vessel edges were located by the points at which the vessel became uniformly darker than the adjacent retinal pigment epithelium. This method effectively measures the width of the column of formed blood particles. A pair of indices, the central retinal arteriolar and venular equivalents (CRAE and CRVE), representing the average arteriolar and venular calibers for each eye, was then calculated using formulas described previously.[38,39] Correction of CRAE and CRVE for ocular magnification was performed using the Bengtsson formula.[24,25] All retinal measurements were performed by a single grader who was masked to the subjects' identity and other measured parameters. Remeasurement of 50 images 2 weeks later showed high reproducibility, with intraclass correlation coefficients of 0.85 for arteriolar caliber and 0.97 for venular caliber.[35]

Measurement of Retinal Vascular Network Geometry

Digital fundus photography was taken using a 45° digital retinal camera (Canon CR-DGi with a 10D SLR digital camera backing; Canon) after pupil dilation using tropicamide 1% and phenylephrine hyprochloride 2.5%. Two retinal images of each eye were obtained, one centered at the optic disc and another centered at the fovea. Of the 3280 participants, 3267 subjects (99.6%) had fundus photographs taken for both eyes. We used an optic disc-centered photograph of the right eye of each participant; if the photograph of the right eye was ungradable, the measurement was performed on the left eye.

We used a new semiautomated computer-assisted program (Singapore I Vessel Assessment [SIVA], v.1.0, National University of Singapore, Singapore) to quantitatively measure a range of retinal vascular parameters, including retinal vascular caliber, retinal vascular tortuosity, and retinal branching measures, from digital fundus images.[23,40] Trained graders, masked to participant characteristics, executed the SIVA program to measure the retinal vasculature, according to a standardized protocol. Images of poor quality, including those due to media opacities (e.g., dense lens opacity), small size of the pupil, or images that were out of focus or that had poor contrast, were excluded.

The SIVA program automatically identifies the optic disc, projects a grid referenced to the optic disc, identifies the vessel types (arterioles and venules), and performs the vessel measurements detailed below. The measured area of retinal vascular tortuosity and branching measures was standardized and defined as the region from 0.5 to 2.0 disc diameters away from the disc margin. Trained graders follow a standardized protocol and perform visual evaluations of the automated measurements with corrections made manually if necessary.[23] Retinal vascular tortuosity is defined as the integral of the curvature square along the path of the vessel, normalized by the total path length.[14,23,41] All vessels coursing through the measured zone with a width larger than 40 μm were measured; these measures do not have units. A smaller tortuosity value indicates a straighter vessel. The estimates were summarized as retinal arteriolar tortuosity and retinal venular tortuosity, representing the average tortuosity of arterioles and venules of the eye, respectively. Retinal branching angle (BA; ω, which is the sum of $\theta_1 + \theta_2$, $\theta_1 < \theta_2$) is defined as the angle subtended between two daughter vessels at each vascular bifurcation.[20] The mean widths of the parent vessel (d_0) and the two daughter branching vessels (d_1 and d_2, $d_1 < d_2$) were also measured. Retinal branching coefficient (BC) is defined as the sum of the square of the two branching vessel widths divided by the square of the parent vessel width ($d_1^2 + d_2^2/d_0^2$). All vessels with the first bifurcation within the measured zone were measured.

Assessment and Definitions of Risk Factors

Blood pressure was measured with the patient seated after 5 minutes of rest, using an automated sphygmomanometer (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies, Milwaukee, WI). Systolic and diastolic blood pressure (SBP and DBP, respectively) readings were taken.[42] Two readings were taken 5 minutes apart, with a third reading taken if the two differed by >10 mmHg systolic or >5 mmHg diastolic. The mean of the two closest readings was then used for the analysis. Hypertension was defined as a systolic pressure of >140 mmHg, a diastolic pressure >90 mmHg, or a self-reported history of hypertension. The mean arterial blood pressure (MABP) was calculated as ([2 × DBP] + SBP)/3.

All participants underwent a standardized interview[33,43,44] that covered socioeconomic measures (e.g., income, education), lifestyle risk factors (e.g., smoking), medication use, and self-reported history of systemic diseases. Nonfasting venous blood samples were drawn and sent for analysis of glucose at the National University Hospital Reference Laboratory on the same day. Diabetes mellitus was identified from plasma glucose ≥200 mg/dL (11.1 mmol/L), self-reported use of diabetic medication, or physician-diagnosed diabetes. Here 718 (21.9%) of subjects met these criteria and were included in the analysis.

Statistical Analysis and Definitions

Statistical analysis was performed (SPSS v.16.0; SPSS Inc., Chicago, IL). The linear trend in the mean characteristics and proportions across the refractive error categories was tested using ANOVA or χ^2, respectively. Multivariate linear regression models were constructed with CRAE or CRVE, curvature tortuosity and branching coefficients and branching angles, for retinal arterioles and venules, respectively, as the dependent variable to assess the relationship with AL or SE, initially adjusting for age and sex. Education as an indicator of socioeconomic status, smoking, MABP, diabetes status, and antihypertensive medication use were added in the subsequent multivariate models. AL and SE were analyzed as continuous variables and by quartiles. Refractive errors were defined as emmetropia (-0.5 D ≤ SE ≤ $+0.5$ D), hyperopia (SE > $+0.5$ D), and myopia (SE < -0.5 D).

Results

Retinal fundus photographs were available in 3266 out of 3280 subjects. We excluded eyes with poor image quality ($n = 154$), those without at least six large gradable arterioles or venules ($n = 159$), images without an adequate measured area (optical artifact) at the measured zone ($n = 24$), retinal images with the wrong camera setting ($n = 14$), or those without vascular bifurcation within the measured zone ($n = 33$), leaving 2882 subjects for the final analysis (87.9% of 3280 participants). There were 1382 (48.0%) male subjects, and the mean age was 57.67 ± 10.68 years. The mean AL was 23.53 ± 1.03 mm, and the mean SE was −0.03 ± 1.94 D. In terms of refractive categories, 1215 (42.2%) subjects were classified as emmetropic, 1004 (34.8%) were hypermetropic, 503 (17.5%) were mildly myopic, and 160 (5.6%) were moderately myopic. Table 1 describes the characteristics of the included participants, stratified by refractive error category. Myopic subjects were more likely to be younger and female and to have higher educational qualifications, but were also less likely to smoke or to have hypertension.

CRAE and CRVE were associated with both AL and SE ($P \leq 0.001$ for all; Table 2). Longer AL and more myopic refraction were associated with narrower CRAE (regression estimate B [95% CI] = −3.20 [−3.68 to −2.72], $P < 0.001$, and B [95% CI] = 0.42 [0.17 to 0.67], $P = 0.001$, respectively) and CRVE ($B = -5.21$ [−5.88 to −4.53], $P < 0.001$; B [95% CI] = 0.69 [0.34 to 1.04], $P < 0.001$, respectively) with multivariate adjustment.

Table 3 describes the associations with retinal arteriolar and venular curvature tortuosity. In age–sex adjusted models, reduced arteriolar curvature tortuosity was associated with increasing AL (regression estimate B [95% CI] = −0.14 [−0.19 to −0.09], $P < 0.001$) and more myopic SE (B [95% CI] = 0.067 [0.04 to 0.093], $P < 0.001$). These relationships persisted with multivariate adjustment for age, sex, education, smoking, MABP, diabetes status, and antihypertensive medication use (B [95% CI] = −0.15 [−0.20 to −0.10] and 0.065 [0.039 to 0.092], $P < 0.001$ and $P = 0.001$, respectively). Stratification by AL and SE quartiles revealed consistent relationships with AL. In age–sex adjusted as well as multivariate adjusted models, venular curvature tortuosity was associated with AL (B [95% CI] = −0.124 [−0.217 to −0.031], $P < 0.01$, and B [95% CI] = −0.122 [−0.216 to −0.028], $P = 0.01$, respectively) but not SE. A diagnosis of myopia was also associated with reduced arteriolar curvature tortuosity in both age–sex and multivariate adjusted models (P for trend <0.01 for both).

Arteriolar BC and BA were significantly associated with AL and SE (Tables 4 and 5) Longer ALs were associated with larger BC (B [95% CI] = 0.027 [0.018 to 0.036], $P < 0.001$) and smaller BA (B [95% CI] = −1.29 [−1.72 to −0.87], $P < 0.001$), while more myopic refraction was correspondingly associated with higher BC (B [95% CI] = −0.011 [−0.015 to −0.006], $P < 0.001$) and smaller BA (B [95% CI] = 0.56 [0.34 to 0.77], $P < 0.001$). A diagnosis of myopia was also associated with larger BC and smaller BA. Consistent associations were seen when AL and SE were stratified and analyzed into quartiles. Venular BC but not the BA was associated with SE, AL, and the refractive error category. Longer ALs were associated with higher BC (B [95% CI] = 0.008 [0.001 to 0.015], $P = 0.02$), more myopic refraction was correspondingly associated with higher BC (B [95% CI] = −0.006 [−0.01 to −0.003], $P < 0.001$), and a diagnosis of myopia was also associated with larger BC ($P = 0.03$).

Sample photographs showing the vascular patterns in myopic and hyperopic eyes are shown in Figure 1 (panels A and B, respectively).

Discussion

Our population-based study shows that SE and AL are significantly associated with retinal vascular caliber and retinal tortuosity as well as branching characteristics. Specifically, more myopic SE and longer AL were associated with narrower CRAE

Table 1. Characteristics of Included Participants from the Singapore Malay Eye Study, by Refractive Error Status

Characteristic	Total (n = 2882)	Hyperopia (SE > +0.5 D) (n = 1004)	Emmetropia (−0.5 D ≤ SE ≤ +0.5 D) (n = 1215)	Myopia (SE < −0.5 D) (n = 663)	P*
Age, y	57.53 (10.64)	60.98 (8.54)	55.2 (10.71)	56.56 (11.94)	<0.001
Sex, male	1382 (48.0)	476 (47.4)	631 (51.9)	275 (41.5)	<0.001
Education					
No formal education	547 (18.8)	222 (22.2)	197 (16.2)	112 (17.0)	<0.001
Elementary or less	258 (8.9)	96 (9.6)	112 (9.2)	46 (7.0)	
Elementary	1340 (46.0)	489 (48.9)	569 (46.9)	272 (41.2)	
High school	561 (19.2)	151 (15.1)	242 (20.0)	166 (25.2)	
College/university	200 (6.9)	43 (4.3)	93 (7.7)	64 (9.7)	
Systolic blood pressure, mmHg	145.90 (23.48)	148.71 (23.33)	143.81 (23.64)	144.80 (24.32)	<0.001
Diastolic blood pressure, mmHg	79.57 (11.15)	80.11 (11.01)	79.59 (11.12)	78.74 (11.28)	0.048
Hypertension	1950 (66.9)	735 (73.2)	758 (62.4)	427 (64.4)	<0.001
Diabetes	664 (22.8)	247 (24.6)	258 (21.2)	149 (22.5)	0.17
Current smoker (current vs. past/never)	605 (20.8)	180 (18.0)	311 (25.6)	110 (16.7)	<0.001
Spherical equivalent (D), right eye	−0.03 (1.94)	+1.51 (0.86)	+0.03 (0.30)	−2.47 (2.34)	<0.001
Axial length (mm), right eye	23.53 (1.03)	23.14 (0.77)	23.44 (0.74)	24.38 (1.36)	<0.001
CRAE	133.74 (13.50)	133.98 (12.78)	133.93 (13.00)	133.01 (15.35)	0.28
CRVE	202.28 (18.48)	201.97 (17.90)	203.64 (17.69)	200.28 (20.49)	0.001
Curvature tortuosity					
Arteriole, $\times 10^4$	3.00 (1.41)	2.95 (1.43)	3.09 (1.37)	2.89 (1.42)	0.005
Venule, $\times 10^4$	4.65 (2.40)	4.55 (2.30)	4.70 (2.49)	4.70 (2.38)	0.27
Retinal branching coefficient					
Arteriole	1.41 (0.23)	1.41 (0.24)	1.40 (0.21)	1.43 (0.25)	0.015
Venule	1.23 (0.18)	1.24 (0.17)	1.22 (0.17)	1.25 (0.20)	0.01
Branching angle					
Arteriole	76.79 (11.20)	76.49 (11.08)	77.48 (10.93)	75.95 (11.78)	0.01
Venule	79.70 (10.39)	79.21 (10.38)	79.21 (10.38)	79.18 (9.73)	0.18

Data are presented as numbers (proportions) or means (standard deviation), as appropriate for variable. CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent.

* P for the difference in characteristics by refractive error status, based on chi-square (categorical variables) and one-way ANOVA (continuous variables).

Table 2. Multiple Linear Regression Models of the Factors Associated with Retinal Vessel Caliber

	CRAE					CRVE				
	Age-Sex Adjusted		Multivariate Adjusted*			Age-Sex Adjusted		Multivariate Adjusted*		
	B (95% CI)	*P*	*B* (95% CI)	*P*	R^2	*B* (95% CI)	*P*	*B* (95% CI)	*P*	R^2
AL, mm	−3.25 (−3.74 to −2.75)	<0.001	−3.20 (−3.68 to −2.72)	<0.001	0.15	−5.37 (−6.04 to −4.70)	<0.001	−5.21 (−5.88 to −4.53)	<0.001	0.10
SE, D	0.46 (0.21 to 0.72)	<0.001	0.42 (0.17 to 0.67)	0.001	0.08	0.76 (0.40 to 1.11)	<0.001	0.69 (0.34 to 1.04)	<0.001	0.03

	CRAE			CRVE		
	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2
AL, mm						
First quartile, ≤22.89	137.66 (136.65 to 138.67)	138.58 (137.36 to 139.80)	0.13	209.30 (207.92 to 210.67)	211.25 (209.54 to 212.96)	0.09
Second quartile, 22.89 to 23.45	135.01 (134.02 to 135.99)	135.70 (134.48 to 136.91)		204.59 (203.24 to 205.93)	206.80 (205.10 to 208.50)	
Third quartile, 22.45 to 24.04	133.23 (132.22 to 134.23)	134.16 (132.96 to 135.36)		201.76 (200.40 to 203.13)	203.93 (202.25 to 205.61)	
Fourth quartile, >24.04	129.77 (128.76 to 130.77)	130.80 (129.57 to 132.02)		195.70 (194.34 to 197.07)	198.13 (196.41 to 199.84)	
P for trend	<0.001	<0.001		<0.001	<0.001	
SE, D						
First quartile, ≤−0.5	132.98 (132.02 to 133.93)	133.54 (132.39 to 134.69)	0.08	200.44 (199.13 to 201.75)	202.26 (200.64 to 203.88)	0.03
Second quartile, −0.5 to +0.25	134.13 (133.23 to 135.03)	134.78 (133.66 to 135.89)		203.50 (202.27 to 204.74)	205.13 (203.56 to 206.70)	
Third quartile, +0.25 to +1.0	133.74 (132.69 to 134.80)	134.45 (133.22 to 135.68)		203.08 (201.63 to 204.53)	204.70 (202.96 to 206.43)	
Fourth quartile, >+1.0	133.76 (132.69 to 134.84)	134.47 (133.19 to 135.75)		202.06 (200.58 to 203.54)	204.07 (202.26 to 205.87)	
P for trend	0.44	0.28		0.17	0.12	
Refractive error category						
Myopia, <−0.5 D	132.79 (131.77 to 133.82)	133.50 (132.29 to 134.71)	0.08	200.15 (198.74 to 201.56)	202.08 (200.37 to 203.78)	0.03
Emmetropia, −0.5 to +0.5 D	134.22 (133.46 to 134.98)	134.77 (133.76 to 135.77)		203.29 (202.25 to 204.34)	204.87 (203.45 to 206.29)	
Hyperopia, >0.5 D	133.56 (132.71 to 134.40)	134.25 (133.16 to 135.35)		202.49 (201.33 to 203.65)	204.35 (202.80 to 205.89)	
P for trend	0.26	0.25		0.01	0.02	

CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent.

* Adjusted for age, sex, education (no formal education, less than elementary, elementary, high school and college/university), smoking (current vs. past/never), MABP, diabetes status, and use of antihypertensive medications.

Table 3. Multiple Linear Regression Models of the Factors Associated with Curvature Tortuosity

	Curvature Tortuosity								
	Arteriole, ×10^4					Venule, ×10^4				
	Age-Sex Adjusted		Multivariate Adjusted*			Age-Sex Adjusted		Multivariate Adjusted*		
	B (95% CI)	*P*	*B* (95% CI)	*P*	R^2	*B* (95% CI)	*P*	*B* (95% CI)	*P*	R^2
AL, mm	−0.14 (−0.19 to −0.09)	<0.001	−0.15 (−0.20 to −0.10)	<0.001	0.07	−0.124 (−0.217 to −0.031)	0.01	−0.122 (−0.216 to −0.028)	0.01	0.02
SE, D	0.067 (0.04 to 0.093)	<0.001	0.065 (0.039 to 0.092)	<0.001	0.05	0.012 (−0.034 to 0.058)	0.61	0.013 (−0.033 to 0.059)	0.58	0.01

	Curvature Tortuosity					
	Arteriole, ×10^4			Venule, ×10^4		
	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2
AL, mm						
First quartile, ≤22.89	3.08 (2.98 to 3.18)	3.11 (2.98 to 3.23)		4.69 (4.51 to 4.88)	4.89 (4.64 to 5.12)	
Second quartile, 22.89 to 23.45	3.07 (2.97 to 3.17)	3.09 (2.97 to 3.22)	0.07	4.74 (4.56 to 4.92)	4.93 (4.70 to 5.17)	0.02
Third quartile, 22.45 to 24.04	2.96 (2.86 to 3.06)	2.99 (2.86 to 3.11)		4.49 (4.30 to 4.67)	4.67 (4.44 to 4.90)	
Fourth quartile, >24.04	2.78 (2.68 to 2.88)	2.79 (2.66 to 2.92)		4.52 (4.33 to 4.71)	4.71 (4.48 to 4.95)	
P for trend	<0.001	<0.001		0.08	0.08	
SE (D)						
First quartile, ≤−0.5	2.88 (2.78 to 2.98)	2.91 (2.79 to 3.03)		4.66 (4.49 to 4.83)	4.82 (4.60 to 5.03)	
Second quartile, −0.5 to +0.25	3.05 (2.96 to 3.14)	3.10 (2.98 to 3.21)	0.05	4.63 (4.47 to 4.79)	4.79 (4.59 to 5.00)	0.02
Third quartile, +0.25 to +1.0	3.02 (2.91 to 3.13)	3.06 (2.93 to 3.19)		4.65 (4.47 to 4.84)	4.81 (4.58 to 5.03)	
Fourth quartile, >+1.0	3.03 (2.92 to 3.14)	3.07 (2.93 to 3.20)		4.62 (4.43 to 4.81)	4.81 (4.57 to 5.05)	
P for trend	0.08	0.07		0.84	1	
Refractive error category, D						
Myopia, <−0.5	2.86 (2.76 to 2.97)	2.89 (2.76 to 3.02)		4.67 (4.49 to 4.85)	4.83 (4.60 to 5.05)	
Emmetropia, −0.5 to +0.5	3.04 (2.96 to 3.11)	3.08 (2.97 to 3.19)	0.05	4.67 (4.53 to 4.80)	4.83 (4.64 to 5.02)	0.02
Hyperopia, >0.5	3.03 (2.95 to 3.12)	3.07 (2.95 to 3.19)		4.59 (4.44 to 4.74)	4.76 (4.56 to 4.96)	
P for trend	0.01	0.01		0.51	0.58	

* Adjusted for age, sex, education (no formal education, less than elementary, elementary, high school and college/university), smoking (current vs. past/never), MABP, diabetes status, and use of antihypertensive medications.

Table 4. Multiple Linear Regression Models of the Factors Associated with Branching Coefficients

	Branching Coefficient									
	Arteriole					Venule				
	Age-Sex Adjusted		Multivariate Adjusted*			Age-Sex Adjusted		Multivariate Adjusted*		
	B (95% CI)	*P*	*B* (95% CI)	*P*	R^2	*B* (95% CI)	*P*	*B* (95% CI)	*P*	R^2
AL, mm	0.025 (0.016 to 0.034)	<0.001	0.027 (0.018 to 0.036)	<0.001	0.03	0.008 (0.001 to 0.014)	0.02	0.008 (0.001 to 0.015)	0.02	0.03
SE, D	−0.010 (−0.015 to −0.006)	<0.001	−0.011 (−0.015 to −0.006)	<0.001	0.03	−0.007 (−0.01 to −0.003)	<0.001	−0.006 (−0.01 to −0.003)	<0.001	0.03

	Branching Coefficient					
	Arteriole			Venule		
	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2
AL, mm						
First quartile, ≤22.89	1.38 (1.36 to 1.40)	1.38 (1.36 to 1.41)	0.03	1.22 (1.21 to 1.24)	1.22 (1.20 to 1.23)	0.03
Second quartile, 22.89 to 23.45	1.40 (1.38 to 1.41)	1.40 (1.38 to 1.42)		1.23 (1.21 to 1.24)	1.22 (1.20 to 1.23)	
Third quartile, 22.45 to 24.04	1.41 (1.39 to 1.43)	1.42 (1.39 to 1.44)		1.23 (1.21 to 1.24)	1.22 (1.20 to 1.24)	
Fourth quartile, >24.04	1.44 (1.42 to 1.45)	1.44 (1.42 to 1.46)		1.24 (1.22 to 1.25)	1.23 (1.21 to 1.25)	
P for trend	<0.001	<0.001		0.21	0.02	
SE, D						
First quartile, ≤−0.5	1.43 (1.41 to 1.45)	1.44 (1.42 to 1.46)	0.02	1.25 (1.24 to 1.26)	1.24 (1.22 to 1.25)	0.03
Second quartile, −0.5 to +0.25	1.40 (1.39 to 1.42)	1.41 (1.39 to 1.43)		1.23 (1.22 to 1.24)	1.22 (1.21 to 1.24)	
Third quartile, +0.25 to +1.0	1.40 (1.39 to 1.42)	1.41 (1.39 to 1.43)		1.23 (1.21 to 1.24)	1.22 (1.20 to 1.24)	
Fourth quartile, >+1.0	1.40 (1.38 to 1.41)	1.40 (1.38 to 1.43)		1.23 (1.22 to 1.24)	1.22 (1.21 to 1.24)	
P for trend	0.01	0.01		0.10	0.14	
Refractive error category, D						
Myopia, <−0.5	1.43 (1.41 to 1.45)	1.44 (1.42 to 1.46)	0.02	1.25 (1.24 to 1.26)	1.24 (1.22 to 1.26)	0.03
Emmetropia, −0.5 to +0.5	1.40 (1.39 to 1.41)	1.41 (1.39 to 1.43)		1.23 (1.22 to 1.24)	1.22 (1.21 to 1.24)	
Hyperopia, >0.5	1.40 (1.39 to 1.42)	1.41 (1.39 to 1.43)		1.23 (1.22 to 1.24)	1.22 (1.21 to 1.24)	
P for trend	0.02	0.01		0.02	0.03	

Table 5. Multiple Linear Regression Models of the Factors Associated with Branching Angles

	Branching Angle									
	Arteriole					Venule				
	Age-Sex Adjusted		Multivariate Adjusted*			Age-Sex Adjusted		Multivariate Adjusted*		
	B (95% CI)	*P*	*B* (95% CI)	*P*	R^2	*B* (95% CI)	*P*	*B* (95% CI)	*P*	R^2
AL, mm	−1.36 (−1.78 to −0.94)	<0.001	−1.29 (−1.72 to −0.87)	<0.001	0.06	−0.03 (−0.43 to 0.37)	0.89	0.02 (−0.38 to 0.43)	0.91	0.02
SE, D	0.58 (0.37 to 0.80)	<0.001	0.56 (0.34 to 0.77)	<0.001	0.05	0.08 (−0.12 to 0.29)	0.42	0.08 (−0.12 to 0.28)	0.44	0.01

	Branching Angle					
	Arteriole			Venule		
	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2	Age-Sex Adjusted Mean (95% CI)	Multivariate Adjusted* Mean (95% CI)	R^2
AL, mm						
First quartile, ≤22.89	77.62 (76.76 to 78.47)	78.15 (77.09 to 79.22)		79.23 (78.42 to 80.03)	79.52 (78.51 to 80.53)	
Second quartile, 22.89 to 23.45	77.87 (77.04 to 78.70)	78.46 (77.40 to 79.52)	0.05	80.09 (79.30 to 80.87)	80.40 (79.39 to 81.40)	0.02
Third quartile, 22.45 to 24.04	76.90 (76.06 to 77.75)	77.58 (76.53 to 78.63)		79.71 (78.91 to 80.51)	80.07 (79.08 to 81.06)	
Fourth quartile, >24.04	74.91 (74.06 to 75.76)	75.61 (74.54 to 76.69)		79.44 (78.64 to 80.24)	79.84 (78.82 to 80.85)	
P for trend	<0.001	<0.001		0.89	0.74	
SE, D						
First quartile, ≤−0.5	75.81 (75.02 to 76.60)	76.42 (75.44 to 77.40)		79.50 (78.76 to 80.24)	79.88 (78.95 to 80.80)	
Second quartile, −0.5 to +0.25	77.24 (76.50 to 77.99)	77.79 (76.84 to 78.74)	0.04	80.04 (79.34 to 80.73)	80.36 (79.46 to 81.25)	0.01
Third quartile, +0.25 to +1.0	76.46 (75.59 to 77.33)	76.96 (75.91 to 78.01)		79.21 (78.39 to 80.03)	79.62 (78.63 to 80.61)	
Fourth quartile, >+1.0	77.60 (76.72 to 78.49)	78.22 (77.13 to 79.31)		79.95 (79.11 to 80.78)	80.32 (79.30 to 81.35)	
P for trend	0.02	0.02		0.78	0.74	
Refractive error category, D						
Myopia, <−0.5	75.71 (74.86 to 76.55)	76.34 (75.31 to 77.38)		79.45 (78.65 to 80.25)	79.82 (78.85 to 80.79)	
Emmetropia, −0.5 to +0.5	77.05 (76.42 to 77.68)	77.55 (76.68 to 78.41)	0.04	79.99 (79.40 to 80.58)	80.30 (79.49 to 81.11)	0.01
Hyperopia, >0.5	77.14 (76.45 to 77.84)	77.68 (76.75 to 78.62)		79.50 (78.84 to 80.15)	79.88 (79.00 to 80.76)	
P for trend	0.01	0.02		0.93	0.91	

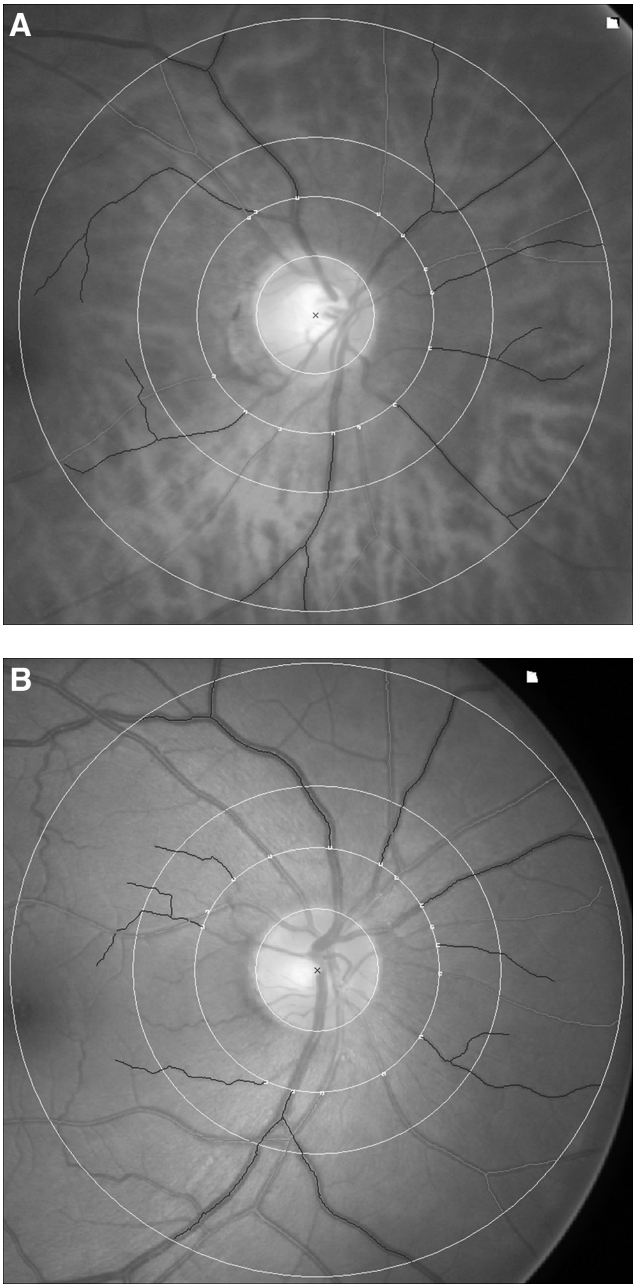

FIGURE 1. (**A**) Sample fundus image of myopic eye: CRAE = 105.0, CRVE = 102.2, branching angle (arteriole) = 68.1, branching angle (venule) = 73.3, tortuosity (arteriole) = 0.000142, tortuosity (venule) = 0.000468. (**B**) Sample fundus image of myopic eye: CRAE = 148.8, CRVE = 215.1, Branching angle (arteriole) = 86.9, branching angle (venule) = 77.4, tortuosity (arteriole) = 0.000308, tortuosity (venule) = 0.001049.

and CRVE, less tortuous (straighter) arterioles, and increased branching coefficients in both arterioles and venules. Longer AL was associated with more acute branching angles in arterioles but not venules. Consistent relationships were seen when subjects were categorized as hyperopes, emmetropes, and myopes.

The newer measures of retinal vessel geometry beyond simple measurements of caliber have the potential to provide a more "global" perspective on the health of the retinal circulation and, indirectly, the systemic microvasculature. We have previously reported on the reliability of the software we used for retinal vessel grading.[23,40] Retinal vascular tortuosity describes the course taken by the vessel as it meanders over the retinal surface.[45] Straighter or less tortuous vessels have been associated with various systemic conditions in which ischemia or hypoperfusion are thought to be contributory, including ischemic heart disease, higher blood pressure and body mass index, smoking, diabetes, and anemia.[14,46,47] In the eye, a recent publication from our cohort has also demonstrated an association between straighter vessels and a thinner neuroretinal rim.[40] Changes in retinal tortuosity have also been shown to be related to key diabetes-related factors such as HbA1c levels and duration of disease in diabetic subjects without retinopathy, suggesting a potential role as a predictor of retinopathy.[23] The concept that vascular network branching does not occur randomly but may instead be an organized geometric structure was first mooted by Murray in 1926.[18,19] "Murray's Law" states that the maximum circulatory efficiency with minimal energy losses across a vascular network can be achieved if blood flow is proportional to the cubed power of the vessel's radius.[18] Mathematically, the BC at a vascular bifurcation can be derived from BC = ([diameter of branch $1]^2$ + [diameter of branch $2]^2$)/diameter of main trunk^2. Deviations of the BC from an "opitmal" value[38] have been associated with cognitive impairment in the elderly, supporting the concept that the retinal microvasculature may be a visible surrogate for the cerebral microvasculature.[48] The angle subtended between two daughter vessels at a vascular junction has also been found to be an important variable, with an optimal value of approximately 75°.[18] Larger angles[14] have been related to decreased blood flow, whereas smaller angles are associated with aging and hypertension.[49]

Meaningful interpretation and precise measurement of these new parameters, however, require an understanding of the possible interactions with AL and refractive error. Regarding retinal vessel caliber, refractive error and AL are known to influence these measurements. Patton et al.[31] reported a negative trend between AL and both CRAE and CRVE. The Beaver Dam Eye Study (BDES) likewise found that myopic refraction was associated with smaller retinal vessel diameters.[50] Our study results are in agreement with these published findings. Importantly, while the study by Patton[31] analyzed AL only without refraction data, and the BDES[51] had refraction but not biometric data, our study results indicate that both longer AL and more myopic refraction are associated with narrower CRAE and CRVE. It has also been suggested that these associations either represent magnification effects or indicate a true biological process. Refractive error and AL may affect the magnification and apparent dimensions of retinal structures on fundus photography.[25] For example, in the Singapore Cohort Study of the Risk Factors for Myopia,[25] AL and retinal vascular caliber were found not to be associated after correction for ocular magnification using the Bengtsson formula.[52] Alternatively, studies that have demonstrated decreased ocular pulse and retinal blood flow with increasing myopia[28,29,53] tend to support a direct biological association between longer AL and narrower retinal vessels.

Importantly, in contrast to retinal vessel caliber, the new measures of retinal network geometry are dimensionless descriptors of object shapes and angles that are likely to be unaffected by any magnification effects. We thus propose that the associations found with vessel tortuosity, BC, and BA represent an underlying biological association with globe elongation. However, limited data are available for comparison with our findings. In a cross-sectional study of 52 adult subjects by Patton et al.,[31] of which 30 had a history of cardiovascular disease and 17 were receiving cardio-active medications, AL was not significantly correlated with bifurcation angles or junctional exponents, the latter of which is known to be correlated with branching coefficients.[45] The reason for the discrepancies between our studies is unclear beyond the obvious racial differences, the much smaller sample size, and the inclusion of only pseudophakic subjects in Patton's cohort. The Blue Mountains Eye Study[32] has reported, however, that myopic refraction is associated with a reduction in the retinal vascular fractal dimension, a parameter that measures the complexity of the vascular network and that has been linked to microvascular pathology.[13] We speculate that the straighter (less tortuous) retinal vessels and correspondingly more acute bifurcations we found with longer AL may be consistent with mechanical stretch on the vessels in the posterior pole as the eye elongates posteriorly with myopia. These changes may in turn have implications for ocular blood flow in myopia. Several studies have demonstrated decreased ocular pulse and retinal blood flow with increasing myopia.[28,29,53] Narrower branching angles and increasing deviation from an optimal bifurcation coefficient have been associated with hypertension and aging,[49] and the suboptimal branching geometry implies energy losses, increased work of blood transport, and uneven distribution of shear forces across the vascular network. Our findings may help to explain some important clinical phenomena. We have recently shown in our cohort that myopic eyes are less likely to have diabetic retinopathy,[27] an effect that may be mediated through reduced blood flow as more severe nonproliferative diabetic retinopathy preceding proliferative stages of the disease has been associated with increased retinal blood flow.[26,54] On the other hand, in glaucomatous eyes, longer AL and reduced ocular blood flow in myopes might contribute to the development of glaucomatous damage in these eyes.[55]

Retinal vessel caliber (CRAE and CRVE) generally does not vary in tandem with the various risk factors and pathologies with which they have been correlated. The mechanisms underlying changes in CRAE and CRVE are believed to differ. Although the muscular wall of arterioles makes them more susceptible to luminal mechanical forces such as hydrostatic pressure,[56] retinal venule diameter is influenced primarily by factors such as metabolic syndrome, inflammation, and endothelial dysfunction.[14,57] The measures of network geometry that we analyzed in general, however, showed parallel associations for both arterioles and venules. This suggests that the as yet incompletely elucidated determinants of these network parameters differ from those affecting retinal vessel caliber, such that they provide fundamentally different information on the state of the retinal circulation.

The strengths of our study design include in vivo assessments of retinal vascular parameters in a large homogenous population by independent and masked observers, high reproducibility in retinal vessel measurements, and standardized assessment of cycloplegic refraction, biometry, and blood pressure. General limitations of our study regarding errors inherent in retinal photography and measurement,[39] as well as random errors associated with the timing of photography in relation to the cardiac cycle,[58] have been described previously. The measurement of retinal blood vessel caliber from retinal photographs also does not measure the true vessel luminal diameter but the width of the column of formed blood particles.[39] These nondifferential random errors, however, would likely bias our results to the null. Selection bias cannot be totally excluded, and our racially homogenous cohort may also limit the generalizability of our findings.

In conclusion, our study demonstrates significant associations between both refractive error and AL and retinal vessel caliber, tortuosity, and branching characteristics. Our results provide insights into the influence of axial globe elongation on retinal vascular geometry, possibly reflecting ocular blood flow in myopia. Our study also suggests that future studies evaluating these retinal parameters account for the influence of AL and refractive error.

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