Relationship of Retinal Vascular Caliber with Retinal Nerve Fiber Layer Thickness: The Singapore Malay Eye Study

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Purpose. To describe the relationship of retinal arteriolar and venular caliber with retinal nerve fiber layer (RNFL) thickness.

Methods. A population-based, cross-sectional study of Malay persons aged 40 to 80 years residing in Singapore was conducted from 2004 to 2006. Retinal arteriolar and venular calibers were measured with a computer-based technique according to a validated, standardized protocol. RNFL was assessed with retinal tomography.

Results. There were 2706 persons with gradable retinal photographs and HRT images of acceptable quality available for analysis. In linear regression models that adjusted for age, sex, diabetes, body mass index, intraocular pressure, and other factors, each standard deviation decrease in arteriolar and venular caliber was associated with a 5.81- and 8.37-μm decrease, respectively, in mean global RNFL thickness (both $P < 0.001$). These associations remained similar after persons with glaucoma were excluded. In persons with glaucoma, retinal venular caliber was independently associated with the temporal and temporal-to-inferior region of mean RNFL thickness in multivariate analysis; each SD decrease in retinal venular diameter was associated with an 8.54-μm decrease in the temporal region ($P = 0.022$), and a 38.32-μm decrease in the temporal-to-inferior region ($P = 0.006$) of mean RNFL thickness.

Conclusions. Narrower retinal vessel caliber was associated with reduced RNFL thickness in this Asian population. (*Invest Ophthalmol Vis Sci.* 2009;50:4091–4096) DOI:10.1167/iovs.09-3444

There is increasing evidence of an association between vascular risk factors and glaucoma.[1] Studies report relationships between glaucoma and vascular diseases such as systemic hypertension, atherosclerosis, vasospasm, and migraine.[2–5] New prospective studies have provided further evidence, with lower systolic perfusion pressure and a history of cardiovascular disease shown to be independent predictors of glaucoma progression.[6,7]

Quantitative measurements of retinal vascular caliber from fundus photographs are now possible with new imaging software.[8] Retinal vascular caliber is a structural marker of microvascular changes in the fundus and is strongly associated with hypertension, diabetes, and other systemic vascular diseases.[9–11] Another study has also shown that narrower retinal vascular caliber correlates with thinner neuroretinal rim tissue,[12] larger vertical cup-to-disc ratio (VCDR)[13,14] larger nerve fiber layer visibility,[12] and the presence of glaucoma,[15–17] although it remains unclear whether these retinal vascular changes are causal or secondary to glaucoma.

Reduced retinal nerve fiber layer (RNFL) thickness is an early hallmark of glaucomatous optic neuropathy. There are few data describing the relationship of retinal vascular caliber and RNFL thickness in adult populations. In a study of school-aged children, narrower retinal vascular caliber was associated with reduced RNFL thickness, measured with optical coherence tomography (OCT).[18] However, it is unclear whether these associations are also present in older adults, who are at greater risk of glaucoma.

The purpose of this study was to determine the relationship of retinal vascular caliber and RNFL thickness, as determined by retinal tomography (HRT II; Heidelberg Engineering, Heidelberg, Germany) in a population-based sample of adult Asians. Understanding this relationship may provide new insights into the role of retinal vessel narrowing on glaucomatous optic neuropathy.

Methods

Study Population

The Singapore Malay Eye Study examined 3280 (78.7% response) persons aged 40 to 80 years of Malay ethnicity between August 2004 and June 2006, as described in detail elsewhere.[19,20] Ethics approval was obtained from the Institutional Review Board of the Singapore Eye Research Institute, Singapore, and the study was conducted in accordance with the World Medical Association's Declaration of Helsinki. Informed, written consent was obtained from each participant.

Ophthalmic Examination

Standardized ocular examinations were performed in the Singapore Eye Research Institute for all participants.[20] Intraocular pressure (IOP) was measured with Goldmann applanation tonometry (Haag-Streit, Köniz, Switzerland), before pupil dilation. After pupil dilation, the optic disc was evaluated with a +78-D lens, at ×16 magnification, and vertical cup-to-disc ratio (CDR) was determined.[16] Automated perimetry (SITA 24-2; Humphrey Visual Field Analyzer II; Carl Zeiss, Meditec, Oberkochen, Germany) was performed with near refractive correction. Cases of glaucoma were defined according to the ISGEO (International Society of Geographical and Epidemiological Ophthalmology)

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criteria based on three categories.[20,21] Category 1 included subjects with optic disc abnormality (VCDR/VCDR asymmetry ≥97.5th percentile or NRR width between 11 and 1 o'clock or 5 and 7 o'clock <0.1 VCDR) with a corresponding glaucomatous visual field defect. Category 2 contained subjects with severely damaged optic discs (VCDR or VCDR asymmetry ≥99.5th percentile) in the absence of an adequate visual field test. In diagnosing category 1 or 2 glaucoma, it was required that there be no other explanation of the VCDR finding (e.g., dysplastic disc or marked anisometropia) or visual field defect (e.g., branch retinal vein occlusion, macular degeneration, or cerebrovascular disease). Category 3 included subjects without visual field or optic disc data who were blind (corrected visual acuity, <3/60) and who had undergone glaucoma surgery or had IOP >99.5th percentile.

HRT Imaging

The retinal tomography (HRT II; Heidelberg Engineering) measurement was performed in the subjects after pupil dilation in a dim room. HRT II cylindrical lenses were adapted for subjects with astigmatism ≥1.0 D. Corneal curvature data were used to correct for magnification. All examinations were performed by two operators. After the baseline images were captured by the system, the optic disc margin was manually defined by a trained ophthalmologist (SCL). This critical step was accomplished by plotting a series of dots around the margin of the disc on the reflectance image provided by the computer. The disc margin was defined as the inner edge of Elschnig's ring. The HRT II provided indirect measurement of RNFL thickness, which was defined as the mean height of the 360° disc contour line from the reference plane. The RNFL thicknesses in the global region and in six individual sectors (temporal, temporal-to-superior, temporal-to-inferior, nasal, nasal-to-superior, and nasal-to-inferior) were calculated by the tomograph system software. Each image was coupled with a standard deviation to reflect image quality; a standard deviation higher than 50 μm was used as the exclusion criterion.

Measurement of Retinal Vascular Caliber

Digital fundus photography (Canon CR-DGi with a 10-D SLR back; Canon, Tokyo, Japan) was performed after pupil dilation. Two retinal images were obtained: one centered on the optic disc and the other centered on the fovea (Early Treatment for Diabetic Retinopathy Study [ETDRS] standard fields 1 and 2). Retinal vascular caliber was measured by computer-assisted software (IVAN, University of Wisconsin, Madison) according to a standardized protocol at the Retinal Vascular Imaging Centre, University of Melbourne.[22,23] A trained grader, masked to participant characteristics, performed all vessel measurements using the optic disc-centered image of the right eye and of the left eye in those without gradable images. All arterioles and venules coursing through a specified zone, 0.5 to 1 disc diameter away from the optic disc margin, were measured in micrometers and combined into summary measures (referred to as central retinal arteriolar equivalent [CRAE] and central retinal venular equivalent or [CRVE]) using the improved Parr and Hubbard formulas as described by Knudtson et al.[22,24]

Retinal vessel measurements were corrected for ocular magnification by using the Bengtsson formula (1– 0.017 × spherical equivalent refraction).[25] Quality control procedures were implemented during the retinal grading. Intragrader reliability was assessed in 200 randomly selected retinal photographs, and the intraclass correlation coefficient (95% confidence interval) was 0.99 (0.98–0.99) for CRAE and 0.94 (0.92–0.96) for CRVE, respectively.

Other Factors

Height was measured with a wall-mounted tape and weight with a digital scale (SECA, model 782 2321009; Vogel & Halke, Hamburg, Germany), to determine body mass index (BMI). Systolic and diastolic blood pressures were measured with an automated sphygmomanometer to determine mean arterial pressure. Nonfasting blood samples were drawn to determine serum lipids, serum glucose, and glycosylated hemoglobin (HbA_{1c}). Diabetes mellitus was defined as nonfasting glucose ≥11.1 mM, use of diabetic medication or self-reported history of diabetes. A detailed interviewer-administered questionnaire was used to collect information about cigarette smoking (current, past, or never). Axial length was measured with noncontact partial coherence laser interferometry (IOL Master V3.01; Carl Zeiss Meditec).

Table 1. Characteristics of Persons Included in and Excluded from the Study

	Included	Excluded	*P**
Persons without glaucoma (*n*)	2599	531	
Persons with glaucoma (*n*)	107	43	
Age (y)	56.8	67.6	<0.001
Men (%)	48.8	44.6	0.07
Any cataract (%)	48.4	56.5	<0.001
Diabetes (%)	23.1	24.3	0.567
Hypertension (%)	69.0	66.3	0.210
Serum glucose (mg/dL)	6.79	6.80	0.964
Glycosylated hemoglobin (%)	6.45	6.49	0.557
Systolic blood pressure (mm Hg)	146.6	149.4	0.009
Diastolic blood pressure (mm Hg)	79.7	79.8	0.896
Body mass index (kg/m^2)	26.4	26.2	0.576

Data are age-adjusted means or percentage.

* Based on difference in means or proportions, adjusted for age (except for age variable) in ANCOVA models.

Statistical Analysis

Statistical analysis was performed with commercial software (Stata, ver. 8.2; Stata Corp., College Station, TX). Retinal vascular calibers (CRAE and CRVE) were analyzed as continuous variables. Analysis of covariance (ANCOVA) and linear regression models were used to estimate the association between retinal vascular calibers and RNFL thicknesses. Multiple linear regression models were used to estimate the difference in RNFL thickness for each standard deviation change in arteriolar and venular diameters, adjusted for age, sex, BMI, glycosylated hemoglobin, mean arterial blood pressure, diabetes status, total and HDL cholesterol levels, smoking status, axial length, and IOP.

Results

Of all 3280 participants, 224 persons did not undergo HRT examination for logistical reasons in the first 2 weeks of field work, 195 had the HRT test but with results of unacceptable quality, 114 had no gradable retinal photographs, and 41 gave a history of cataract or intraocular surgery, leaving 2706 (82.5%, including 107 cases of glaucoma) persons for analysis. Both retinal vessel caliber and RNFL thickness had normal distribution.

Table 1 shows that compared with the excluded persons, those with gradable retinal photographs and acceptable HRT results were more likely to be younger, be less affected by cataract, and have lower blood pressure. Other baseline characteristics were similar.

Figures 1 and 2 show that narrower retinal arteriolar and venular calibers were associated with thinner mean global RNFL thickness, after adjustment for age and sex.

Table 2 shows associations of retinal vascular caliber with mean global RNFL thickness in persons stratified by age and sex. In general, narrower retinal venular caliber was associated with reduced RNFL thickness in persons older than 60 years, but not in persons younger than 60 years. Compared with retinal venules, retinal arteriolar caliber had weaker and less consistent associations with RNFL thickness.

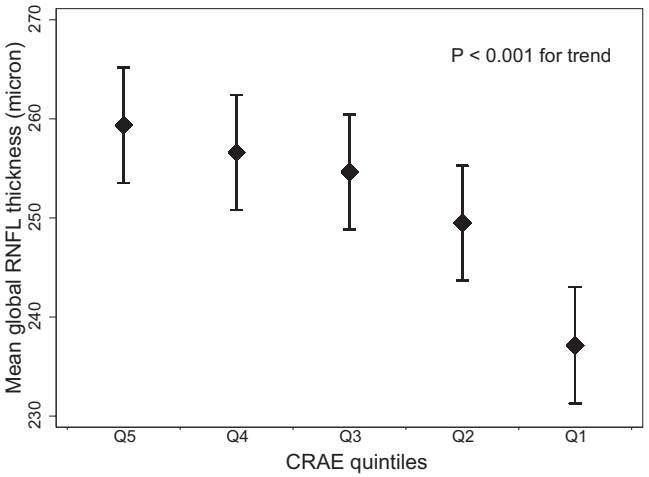

FIGURE 1. Relationship of retinal arteriolar diameter (CRAE) by quintile and mean global RNFL thickness, adjusted for age and sex.

Table 3 shows the associations of retinal arteriolar caliber and RNFL thickness. Among all subjects, retinal arteriolar caliber was associated with RNFL thickness in all retinal segments, after adjustment for age and sex. In multivariate analyses, the associations between retinal arteriolar caliber and RNFL thickness in all retinal segments remained consistent after adjustment for age, sex, BMI, glycosylated hemoglobin, mean arterial blood pressure, diabetes, cholesterol level, smoking status, optic disc area, axial length, and IOP. Excluding any one or combination of these confounding factors did not change the results (data not shown). Each SD decrease in arteriolar caliber was associated with a 5.81-μm decrease in mean global RNFL thickness ($P < 0.001$). Subgroup analysis showed that retinal arteriolar caliber was not associated with RNFL thickness in persons with glaucoma, a finding probably due to reduced sample size ($n = 107$) in this subgroup.

Table 4 shows the associations between retinal venular caliber and RNFL thickness. In multivariate analyses, each SD decrease in venular caliber was associated with an 8.37-μm decrease in mean global RNFL thickness ($P < 0.001$). The magnitude of association was stronger in the inferior segments of the retina compared with other regions. These associations remained unaltered after excluding persons with glaucoma. In persons with glaucoma, retinal venular caliber was independently associated with RNFL thickness in the temporal and temporal-to-inferior region, and was marginally associated with RNFL thickness in the nasal-to-inferior region. Each SD decrease in retinal venular diameter was associated with an 8.54-μm decrease in mean RNFL thickness in the temporal region ($P = 0.022$), and a 38.32-μm decrease in the temporal-to-inferior region ($P = 0.006$). After excluding cases of glaucoma other than primary open angle glaucoma (11 with pseudophakic glaucoma, 8 with glaucoma of unspecified cause, 4 with pseudoexfoliation glaucoma, 2 with primary angle-closure glaucoma, 1 with rubeotic glaucoma, and 1 with developmental glaucoma), the relationship between retinal vessel caliber and RNFL thickness remained similar (data not shown).

DISCUSSION

This study extends our previous report on the association between retinal vascular caliber and the presence of clinical glaucoma.[16] The use of the HRT II allowed a quantitative and more objective method for assessing early changes in the RNFL. We found strong independent associations between narrower retinal vessel caliber and thinner RNFL in all segments, while controlling for age, sex, diabetes, hypertension, BMI, axial length, and other factors. Furthermore, these associations remained largely unchanged in persons without evidence of clinical glaucoma.

Our results should be compared with the only other study we are aware of in which retinal vessel caliber and RNFL thickness were examined. In a population-based study in Australian school-aged children, reduced RNFL thickness was also associated with narrower vessels, with each SD decrease in OCT-measured RNFL thickness associated with a 0.62-μm (95% CI: 0.47–0.76) decrease in retinal arteriolar caliber and a 0.99-μm (95% CI: 0.80–1.18) decrease in retinal venular caliber.[18] Our findings are also consistent with those of Jonas and Schiro[12] that retinal arteriolar narrowing is associated with RNFL visibility, a subjective surrogate for RNFL thickness. It is also informative to compare our findings with those of previous studies that have examined the relationship of retinal vessel caliber and glaucoma. Some studies, including the Blue Mountains Eye Study, have reported associations between narrower retinal vessel caliber and the presence of glaucoma,[16,17,26] although others have not found such relationships.[27,28] Some of the discrepancies may be related to differences in study design, measurement methods, and definitions of glaucoma.

The relationship of narrower retinal vessels and reduced RNFL thickness may reflect the effects of early vascular dysregulation on the nerve fiber layer.[29] Although our study was not designed to determine the exact mechanisms, narrowing of retinal vessels may be caused by dysfunction of vascular endothelium. Such defects could lead to disproportionate release of endogenous vasodilators (e.g., nitric oxide and prostaglandin I2) and/or vasoconstrictors (e.g., endothelin). Hence, the optic nerve head microcirculation could be disrupted, resulting in optic nerve ischemia and, ultimately, glaucomatous optic neuropathy.[1,30] In keeping with this hypothesis, studies have shown that markers of nitric oxide (NO) activity decrease, but endothelin increases, in patients with glaucoma.[31] Furthermore, peripheral endothelial dysfunction has reportedly been associated with normal-pressure glaucoma.[32] The dysregulation of vasodilators and vasoconstrictors may therefore offer potential pathophysiologic links between retinal vessel narrowing, reflected by a decrease in retinal vascular caliber, and retinal ganglion cell (RGC) loss in glaucoma, reflected by thinning of the RNFL. However, the cross-sectional

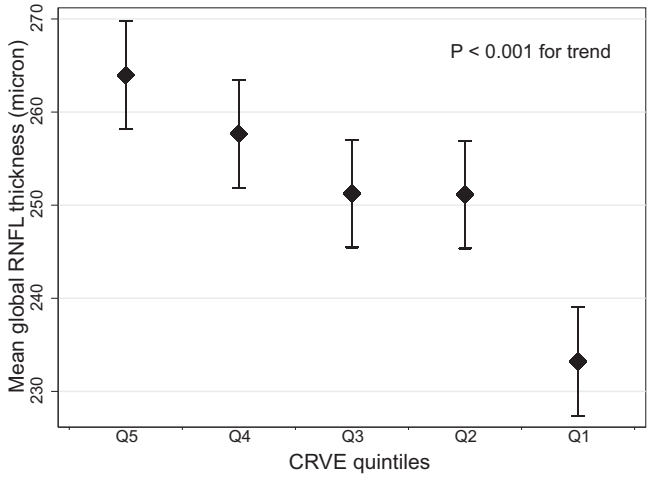

FIGURE 2. Relationship of retinal venular diameter (CRVE) by quintile and mean global RNFL thickness, adjusted for age and sex.

TABLE 2. Relationships of Retinal Arteriolar Caliber and Venular Caliber and RNFL Thickness

	n	Age-Adjusted Mean (95% CI)*	*P*	Multivariable-Adjusted Mean (95% CI)†	*P*
Retinal arteriolar caliber (per SD decrease)					
All persons	2706	−7.46 (−10.10 to −4.83)	<0.001	−5.81 (−8.77 to −2.84)	<0.001
Men	1320	−6.94 (−10.55 to −3.32)	<0.001	−4.24 (−8.45 to −0.03)	0.048
Women	1386	−7.73 (−11.50 to −3.97)	<0.001	−7.27 (−11.38 to −3.15)	<0.001
Age group					
40–49	774	−1.90 (−6.58 to 2.77)	0.424	−2.22 (−7.34 to 2.90)	0.395
50–59	888	−6.34 (−10.86 to −1.82)	0.006	−6.52 (−11.50 to −1.54)	0.010
60–69	640	−9.49 (−15.11 to −3.86)	<0.001	−5.89 (−12.42 to 0.65)	0.077
70–79	404	−16.01 (−23.15 to −8.87)	<0.001	−9.91 (−19.10 to −0.73)	0.035
Retinal venular caliber (per SD decrease)					
All persons	2706	−10.28 (−12.91 to −7.65)	<0.001	−8.37 (−11.19 to −5.54)	<0.001
Sex					
Men	1320	−12.06 (−15.69 to −8.44)	<0.001	−9.04 (−12.97 to −5.11)	<0.001
Women	1386	−8.49 (−12.29 to −4.69)	<0.001	−8.05 (−12.13 to −3.97)	<0.001
Age group					
40–49	774	−4.88 (−9.50 to −0.26)	0.039	−4.13 (−8.92 to 0.67)	0.092
50–59	888	−4.64 (−9.10 to −1.80)	0.041	−2.81 (−7.43 to 1.80)	0.232
60–69	640	−12.59 (−18.09 to −7.09)	<0.001	−10.70 (−16.69 to −4.71)	<0.001
70–79	404	−25.33 (−32.21 to −18.45)	<0.001	−25.44 (−34.23 to −16.64)	<0.001

Data are the difference in mean global RNFL thickness (in micrometers).

* Mean (95% CI) difference in retinal vessel diameter adjusted for age (except for subgroup analysis stratified by age groups)

† Mean (95% CI) difference in retinal vessel diameter adjusted for age, sex (except for stratification by sex), BMI, glycosylated hemoglobin, mean arterial pressure, diabetes status, total and HDL cholesterol levels, smoking status, optic disc area, axial length, and IOP.

design of our study limits our ability to verify such hypotheses. It remains unknown whether the relationship between retinal vessel narrowing and RNFL thickness is causal. We note that narrow retinal vessels are not only seen in eyes with glaucoma, but also identified in eyes with nonglaucomatous optic nerve damage such as nonarteritic anterior ischemic optic neuropathy.[33] In light of these findings, further investigations, particularly prospective ones, are needed to confirm or refute the relationship between early vascular dysfunction and glaucoma damage.

TABLE 3. Relationships of Retinal Arteriolar Caliber with RNFL Thickness

Retinal Arteriolar Caliber (per SD decrease)	Age and Sex-Adjusted Mean (95% CI)	*P*	Multivariable-Adjusted Mean (95% CI)*	*P*
All persons				
Location				
Global	−7.46 (−10.10 to −4.83)	<0.001	−5.81 (−8.77 to −2.84)	<0.001
Temporal	−18.73 (−28.90 to −0.85)	<0.001	−1.22 (−2.37 to −0.06)	0.039
Temporal-to-superior	−10.01 (−13.53 to −6.50)	<0.001	−8.25 (−12.25 to −4.24)	<0.001
Temporal-to-inferior	−11.69 (−15.24 to −8.15)	<0.001	−8.13 (−12.06 to −4.19)	<0.001
Nasal	−6.95 (−11.13 to −2.77)	<0.001	−6.36 (−10.98 to −1.74)	0.007
Nasal-to-superior	−9.01 (−13.13 to −4.89)	<0.001	−7.93 (−12.54 to −3.32)	0.001
Nasal-to-inferior	−12.21 (−16.43 to −7.99)	<0.001	−9.45 (−14.14 to −4.76)	<0.001
Persons without glaucoma				
Location				
Global	−7.29 (−9.96 to −4.62)	<0.001	−5.60 (−8.58 to −2.62)	<0.001
Temporal	−1.98 (−3.02 to −0.95)	<0.001	−1.33 (−2.50 to −0.16)	0.026
Temporal-to-superior	−9.61 (−13.17 to −6.05)	<0.001	−7.79 (−11.84 to −3.73)	<0.001
Temporal-to-inferior	−11.78 (−15.37 to −8.19)	<0.001	−8.41 (−12.40 to −4.41)	<0.001
Nasal	−7.34 (−11.59 to −3.09)	<0.001	−7.13 (−11.83 to −2.43)	0.003
Nasal-to-superior	−9.01 (−13.18 to −4.83)	<0.001	−8.05 (−12.73 to −3.36)	0.001
Nasal-to-inferior	−12.59 (−16.88 to −8.30)	<0.001	−10.07 (−14.85 to −5.30)	<0.001
Persons with glaucoma				
Location				
Global	−8.71 (−23.94 to 6.51)	0.259	−5.12 (−22.56 to 12.31)	0.566
Temporal	−1.15 (−4.52 to 6.82)	0.689	2.18 (−4.45 to 8.81)	0.431
Temporal-to-superior	−19.02 (−40.32 to 2.33)	0.080	−17.70 (−43.64 to 8.24)	0.178
Temporal-to-inferior	−5.68 (−25.82 to 14.51)	0.577	4.05 (−19.36 to 27.45)	0.731
Nasal	3.41 (−19.82 to 26.71)	0.772	6.62 (−20.00 to 33.25)	0.621
Nasal-to-superior	−8.81 (−32.81 to 15.13)	0.468	−8.87 (−36.58 to 18.85)	0.526
Nasal-to-inferior	−0.95 (−23.9 to 22.02)	0.935	7.28 (−18.81 to 33.37)	0.580

Data are the difference in RNFL thickness (in micrometers).

* Adjusted for age, sex (except for sex stratification), BMI, glycosylated hemoglobin, mean arterial pressure, diabetes status, total and HDL cholesterol levels, smoking status, optic disc area, axial length, and IOP.

TABLE 4. Relationships of Retinal Venular Caliber with RNFL Thickness

Retinal Venular Caliber (per SD Decrease)	Age and Sex-Adjusted Mean (95% CI)	*P*	Multivariable-Adjusted Mean (95% CI), Model 1*	*P*
All persons				
Location				
Global	−10.28 (−12.91 to −7.65)	<0.001	−8.37 (−11.19 to −5.54)	<0.001
Temporal	−2.57 (−3.59 to −1.56)	<0.001	−2.44 (−3.49 to −1.40)	<0.001
Temporal-to-superior	−10.75 (−14.28 to −7.23)	<0.001	−9.32 (−13.15 to −5.50)	<0.001
Temporal-to-inferior	−14.19 (−17.73 to −11.65)	<0.001	−11.61 (−15.36 to −7.86)	<0.001
Nasal	−11.50 (−15.67 to −7.32)	<0.001	−9.37 (−13.78 to −4.96)	<0.001
Nasal-to-superior	−11.49 (−15.61 to −7.37)	<0.001	−9.84 (−14.25 to −5.44)	<0.001
Nasal-to-inferior	−17.34 (−21.55 to −13.14)	<0.001	−13.71 (−18.18 to −9.24)	<0.001
Persons without glaucoma				
Location				
Global	−9.88 (−12.54 to −7.21)	<0.001	−8.04 (−10.87 to −5.20)	<0.001
Temporal	−2.39 (−3.43 to −1.36)	<0.001	−2.34 (−3.40 to −1.28)	<0.001
Temporal-to-superior	−10.58 (−14.14 to −7.02)	<0.001	−9.30 (−13.16 to −5.44)	<0.001
Temporal-to-inferior	−13.28 (−16.87 to −9.69)	<0.001	−10.72 (−14.52 to −6.92)	<0.001
Nasal	−11.56 (−15.80 to −7.32)	<0.001	−10.00 (−14.47 to −5.53)	<0.001
Nasal-to-superior	−11.79 (−15.96 to −7.62)	<0.001	−10.14 (−14.60 to −5.68)	<0.001
Nasal-to-inferior	−16.89 (−21.17 to −12.61)	<0.001	−13.32 (−17.86 to −8.78)	<0.001
Persons with glaucoma				
Location				
Global	−10.62 (−25.8 to 4.49)	0.166	−5.23 (−23.45 to 12.99)	0.569
Temporal	−5.65 (−11.22 to −0.10)	0.046	−6.00 (−12.32 to −0.34)	0.045
Temporal-to-superior	−14.22 (−35.61 to 7.24)	0.192	−6.57 (−33.99 to 20.85)	0.634
Temporal-to-inferior	−23.32 (−42.92 to −3.73)	0.020	−26.14 (−49.65 to −2.64)	0.030
Nasal	−7.53 (−30.72 to 15.6)	0.520	−0.50 (−27.35 to 28.34)	0.972
Nasal-to-superior	−4.57 (−28.54 to 19.4)	0.706	−2.48 (−31.29 to 26.34)	0.864
Nasal-to-inferior	−21.21 (−43.72 to 1.40)	0.066	−17.09 (−44.12 to 9.95)	0.212

Data are the difference in RNFL thickness (in micrometers).

* Adjusted for age, sex, BMI, glycosylated hemoglobin, mean arterial pressure, diabetes status, total and HDL cholesterol levels, smoking status, optic disc area, axial length, and IOP.

It should be highlighted that narrowed retinal arterioles was associated with reduced RNFL thickness, although the magnitude of association was less marked than that of the narrowed venules. In fact, several hospital-based studies and the Beijing Eye Study have shown a stronger association of retinal arteriolar caliber with glaucoma compared with that of retinal venular caliber.[26,34–36] This finding is explicable on the ground that venules and arterioles have a different set of systemic risks and associations. For example, retinal venular caliber is more affected by hyperglycemia, inflammation, and BMI, whereas retinal arteriolar caliber is associated with hypertension.[9–11] The discrepancy between our findings and others may be attributable to various prevalence of systemic diseases in different studies. The magnitude of association between retinal vessel caliber and glaucoma may be affected by these systematic conditions, leading to a stronger association of retinal venular caliber with RNFL thickness in our study but not in the others.[26,34–36]

In persons with glaucoma, retinal venular narrowing was associated with RNFL thickness only in the temporal and temporal-to-inferior regions, and marginally associated with RNFL thickness in the nasal-to-inferior region. The implication of this location specific relationship remains unclear, but it coincides with the clinical observation that the neuroretinal rim is preferentially lost in the inferior, especially its temporal part, of the optic disc region.[37]

Our age-sex–stratified analyses showed that retinal vessel narrowing was associated with reduced RNFL thickness in persons older than 60 years, but not in those younger. These results somewhat contrast with our findings in school-aged children.[18] It is possible that the associations in the adults and those in the children represent two different processes, with the former reflecting a pathophysiological link, whereas the latter reflect a physiological relationship. We were unable to offer biological explanations for this potential age effect, although both retinal venular caliber and RNFL thickness have been shown to decrease with increasing age.[38,39] Nevertheless, less consistent associations in subgroup analysis could be related to reduced power as the number of participants in each subgroup was reduced.

Strengths of our study include its large-sample, population-based design with standardized definition for glaucoma, use of a validated computer-based technique to quantify retinal vascular calibers, and the use of the HRT instrument to provide objective RNFL thickness measurements. However, several important issues require consideration. First, the lack of association between retinal vessel calibers and RNFL thickness in persons with glaucoma may be due to the reduced sample size, and thus limited power, in this subgroup. Second, it is important to note that the HRT II does not provide a direct measurement of RNFL thickness.[40] The RNFL thickness measurement is calculated using the mean height contour measurements relative to an individual-based reference plane. Inevitably, there are some variations in the RNFL thickness measurement, given that the contour line is operator dependent. HRT measurements are also influenced by fluctuations in IOP. However, RNFL thickness measurements derived from the HRT II have been shown to be reproducible and highly associated with nerve fiber count in animal eyes.[41,42] We felt that the associations described by our study were unlikely to be confounded by these technical limitations. Last, contour lines were depicted without the aid of stereo photographs, which

could have affected the accuracy of the disc margin delineation.

In summary, using quantitative methods to measure retinal vessel caliber and RNFL thickness, we found significant associations between narrower retinal vessel caliber and RNFL thinning in this population-based study of Asian Malay persons aged 40 to 80 years.

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