

# Normative data of retinal arteriolar and venular calibre measurements determined using confocal scanning laser ophthalmoscopy system – Importance and implications for study of cardiometabolic disorders

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**Purpose:** To determine and validate retinal vascular caliber measurements by using the confocal scanning laser ophthalmoscopy system. Retinal vasculature changes are often regarded as clinical markers for systemic disease. **Methods:** It was a prospective observational study conducted on 600 eyes of 300 normal subjects with no systemic or ocular illness from January 1, 2016 to June 30, 2017 in a tertiary referral eye center. Non-mydratic infrared reflectance, blue reflectance, and blue peak blue autofluorescence fundus imaging were done on the confocal scanning laser ophthalmoscopy system. The dimensions of the retinal vessels were measured using inbuilt calipers at 1800  $\mu\text{m}$  from the center of the optic disc. Internal and external dimensions were measured. Observer variation and its comparison using Image J software were assessed. **Results:** The median age was 29 years (18–50 years). Mean internal and external diameters for arterioles were  $85.1 \pm 12.4 \mu\text{m}$  and  $105.0 \pm 12.0 \mu\text{m}$ , and for venules were  $133.8 \pm 16.6 \mu\text{m}$  and  $145.4 \pm 16.1 \mu\text{m}$ , respectively. The mean internal and external wall thicknesses were  $19.7 \pm 8.0 \mu\text{m}$  and  $11.0 \pm 5.6 \mu\text{m}$ , and wall thickness-to-lumen ratios were  $0.3 \pm 0.1$  and  $0.1 \pm 0.1$ , respectively. Arteriolar-to-venular ratio for lumen and vessel was  $0.66 \pm 0.1$  and  $0.74 \pm 0.1$ , respectively. There was no statistically significant difference between age groups. Both inter- and intra-observer reproducibility was >95%. The Bland–Altman plot showed that the difference between measurements using both confocal scanning laser ophthalmoscopy and Image J software lies within the limits of agreement approximately 95% of the time. **Conclusion:** This is the first effort to develop a normative database by using a simple non-invasive confocal scanning laser ophthalmoscopy system with high observer reproducibility.

**Key words:** Confocal scanning laser ophthalmoscopy, ocular imaging, retinal vasculature, vascular retinopathy, vessel diameter, vessel dimensions

Since the development of the first method for funduscopy by Hermann von Helmholtz in 1851,<sup>[1]</sup> there has been a persistent interest in evolving a technique for the assessment of retinal vessel dimensions.<sup>[2]</sup> The retinal vasculature is often regarded as a representation of the systemic microvasculature, providing information about changes in vascular morphology and function in a natural and non-invasive manner. Given that retinal, cerebral, and coronary blood vessels share similar anatomy and physiology, retinal blood vessels have routinely been evaluated as a part of the clinical ocular investigation, especially in hypertensive and metabolic disorders that lead to systemic small vessel disease.<sup>[3]</sup> The retinal vessel changes were described as “markers” of systemic disease by the Scottish physician, Robert Marcus Gunn.<sup>[3,4]</sup>

The clinical arteriolar-to-venular ratio on funduscopy is nearly 2:3 and depends on normal light reflex from the blood column rather than the outer vessel wall.<sup>[5]</sup> It was widely used

to negate the magnification differences, although it may not be as important as previously thought.<sup>[6]</sup> Its clinical evaluation by ophthalmoscopy involves no cost, but being a subjective test requires significant experience and good diagnostic skills. Thus, poor interobserver reproducibility is an issue of concern and reduces its utility while performing scientific studies. Liew *et al.*<sup>[7]</sup> demonstrated that the clinical arteriolar-to-venular ratio was not as informative as the calibers of the retinal vessel. This emphasizes the importance of having a quantitative measure of the retinal vasculature caliber.

Previous clinical and epidemiological studies have reported retinal vascular changes in systemic and ocular diseases such as hypertension, diabetes, obesity, stroke, coronary heart disease, primary open-angle glaucoma, central retina vein occlusion, and branch retinal vein occlusion.<sup>[8]</sup> Arterioles and venules are affected differently by different diseases.

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In hypertension, all grading systems had defined arteriolar narrowing as the first grading to hypertensive retinopathy, which is highly dependent on the chronicity of hypertension. On the contrary, a recent meta-analysis reported that retinal arteriolar narrowing may precede the development of hypertension; they also mentioned that there is a  $3.07\text{-}\mu\text{m}$  ( $2.40\text{--}3.73\text{ }\mu\text{m}$ ) decrease in the arteriolar caliber per 10 mm Hg increase in systemic arterial blood pressure. Further, both Atherosclerosis Risks in Communities and Beaver Dam Eye Study demonstrated the inverse relationship between increasing hypertension and decreasing arteriolar-to-venular ratio.<sup>[8]</sup>

In diabetes, there is microangiopathy associated with endothelial dysfunction. A smaller arteriolar-to-venular ratio has been reported with an increased risk of diabetes incidence. Previously, the wider arteriolar caliber has also been noted with chronic diabetes though they lacked the data on the exact threshold and individual caliber of vessels with diabetes and its severity.<sup>[8]</sup> Cerebral vasculature changes are also represented via retinal vasculature. A small arteriolar-to-venular ratio is also associated with an increased risk of stroke. The same has been concluded for the larger venular caliber in previous literature.<sup>[8]</sup>

Similarly, retinal vasculature also provides insights into coronary heart disease. Both increasing venular caliber and decreasing arteriolar caliber were found to be associated with coronary heart disease independent of each other or cardiovascular risk factors.<sup>[8]</sup> However, as recently as 2013, it has been highlighted that the lack of normative data was a limitation toward understanding the vast amount of literature available about retinal vasculature dimensions.<sup>[8]</sup>

In an era of advancement and constantly improving technology, digital and semi-automated fundus imaging allow more precise measurements of the retinal vascular caliber as compared to fundus examination. This becomes more prudent given the definitive role of artificial intelligence and teleophthalmic imaging in the near future. Confocal scanning laser ophthalmoscopy camera is a digital confocal fundus camera by which images can be analyzed by various software or manually using caliper-based computation. Measurements using this device are also considered to be very accurate, though never evaluated or proven in the context of retinal vasculature.

Literature review of PubMed and Medline utilizing the keywords “normal retinal vessel diameter,” “confocal scanning laser ophthalmoscopy,” “Image J software,” “retinal vasculature,” “vessel caliber,” and “relation between retinal vasculature and systemic diseases” revealed the lack of normative data of retinal vasculature dimensions. Herein, we have utilized the non-invasive confocal scanning laser ophthalmoscopy system to establish a normative database of retinal vessel diameter by independent observers. We have also studied observer variation to evaluate its reproducibility and compared the reliability of the normative database on Image J software.

## Methods

### Ethics approval

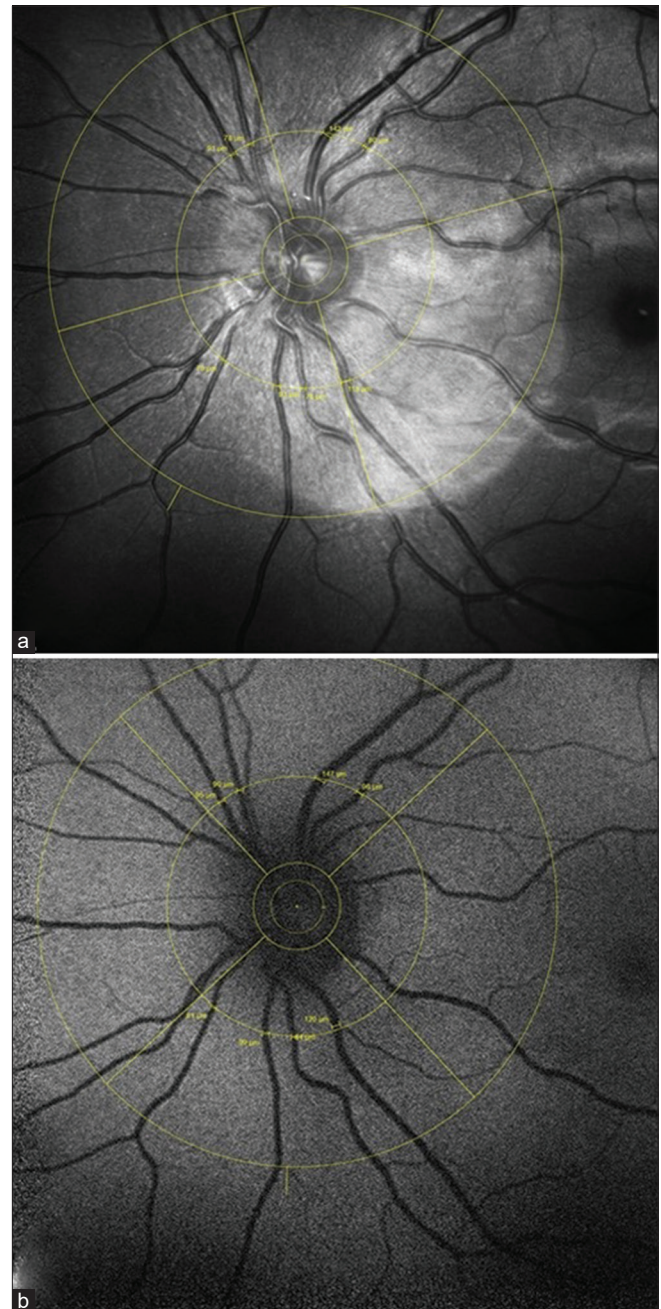
This was a prospective observational study and was approved by the institute’s ethics committee (Ref. No.- IECPG- 44/27.11.2015). This study adhered to the Declaration of Helsinki.

### Patient database, security, and protection

Data entry, storage, patient privacy, and statistical analysis were done in accordance with international standards. Data were secured by omitting any personal patient information.

### Eligibility criteria

Consecutive phakic subjects ( $n = 300$ ; 600 eyes) between 18 and 50 years of age with clear media were included in the study from January 1, 2016 to June 30, 2017. All samples had visual acuity of  $\text{LogMAR} \leq 0.2$ , the axial length between 22.0



**Figure 1:** (a) showing blue reflectance (red-free) images in which arteriole and venular pairs were identified and measured by an inbuilt caliper (b) showing blue peak blue autofluorescence images in which arteriole and venular pairs were identified and measured by an inbuilt caliper



and 24.5 mm with no significant systemic, ocular, or personal history. Subjects with general and potentially effect-modifying variables such as hypertension, diabetes, chronic smoking, alcoholism, obesity, coronary artery disease, cerebrovascular accident, myopia > -1D, hypermetropia > +1D, and IOP >21 mm Hg were excluded from the study.

### Imaging

Non-mydratic, 30° fundus images were captured, centered on the optic nerve head, by using the high-resolution Spectralis Heidelberg Retinal Angiography system (S3610-CIFP). For all subjects, the infrared reflectance image, blue reflectance image, and blue peak blue autofluorescence image were captured. The scaling was fixed at 6.1 µm/pixel. To ensure data quality, all three observers had received specific training (initial 20 images) after a technical discussion.

### Measurements

For measurements, a circular zone between half a disc diameter and one-disc diameter from the optic disc margin was used. This region was selected because its vessels are unequivocally arterioles instead of arteries.<sup>[9]</sup> Furthermore, in this region, there is less overlap between the vessels as compared to near or on the optic disc, making the measurements more reliable.<sup>[9]</sup> For uniformity, an inbuilt ETDRS grid was used whose center was placed at the root of the vessels in the optic disc. The three circular grids of ETDRS were at 600, 1800, and 3600 µm from the inside out. The second circular grid (1800 µm from the center of the optic disc) lies in the circular zone between half-a-disc diameter and one-disc diameter from the optic disc margin and was used to measure the dimension of the retinal vessels by using inbuilt calipers [Fig. 1]. The most prominent arteriole and venular pairs were identified using infrared reflectance images as a reference and measured using an inbuilt caliper. Blue reflectance images were used to determine the internal diameter (lumen) veins in which the laser is reflected from the blood column [Fig. 1a], whereas blue peak blue autofluorescence images were used to determine their external diameter (vessel) as the laser is reflected from RPE making it hyperreflective and leaving vessels (including lumen and wall) as hyporeflexive due to blocked reflectance by vessel [Fig. 1b].<sup>[10]</sup>

Measurements were done by a single observer for all 600 eyes. For inter-observer variation, three observers measured 200 eyes (33.3%, n = 600 eyes), and for intra-observer variation, the same three observers did repeat measurements for 50 eyes (25.0%, n = 200 eyes) in different sessions with an interval of more than 1 week. The values so obtained by each of the independent observers were noted for analysis. The following formulas were used in evaluating the above parameters: Wall thickness = External Diameter-Internal Diameter; Arteriolar-to-Venular Ratio = Arteriolar Diameter/Venular Diameter; and Wall thickness-to-Lumen Ratio = Wall thickness/Internal Diameter.

### Image J software

The normative database was compared using Image J 1.51J8 software (a Java-based image freeware processing program developed by the National Institute of Health (<http://rsb.info.nih.gov/ij>)) in a randomly selected sample of 40 eyes (6.7% of 600 eyes). Repeatability of the measurements using widely accepted and freely available software was undertaken to assess its utility for future teleophthalmic applications.

**Table 1: Normative database**

Parameters	Mean (µm)	95% confidence interval (µm)
<b>Arteriolar</b>		
Internal dimension- lumen	85.10±12.40	59.58-109.18
External dimension	105.04±12.05	80.40-128.6
Wall thickness	19.71±8.04	2.92-35.08
Wall thickness-to-lumen ratio	0.26±0.12	0.01-0.53
<b>Venular</b>		
Internal dimension- lumen	133.84±16.62	99.26-165.74
External dimension	145.42±16.13	110.99-175.51
Wall thickness	11.02±5.65	0-22.03
Wall thickness-to-lumen ratio	0.10±0.05	0-0.2
<b>Arteriolar-to-Venular Ratio</b>		
AVR- Lumen*	0.66±0.1	0.46-0.86
AVR- External dimension*	0.74±0.09	0.56-0.92

\*AVR: Arteriolar-to-Venular Ratio

### Statistical analysis

Vessel diameter parameters, including wall thickness, internal (lumen) diameter, external (vessel) diameter, arteriolar-to-venular ratio, and wall thickness-to-lumen ratio, were analyzed based on age and gender [Tables 1 and 2]. These parameters were compared between OD and OS by using paired *t* tests. The intra-class correlation coefficient was used to determine inter- and intra-observer repeatability. Bland-Altman plot was used to compare measurements done by the confocal scanning laser ophthalmoscopy system with that done by Image J. For the statistical difference between measurement two-sample *t* test, two-sample Wilcoxon rank-sum (Mann-Whitney) test, analysis of variance (ANOVA), and Kruskal-Wallis equality-of-populations rank test were used appropriately. All statistical tests were two-sided at a 95% confidence interval, and *P* < 0.05 was considered statistically significant. SPSS Statistics 20 software released in 2015 was used (IBM, Armonk, New York, USA).

## Results

### Demographics

In total, 600 eyes of 300 subjects were studied. The median age was 29 years (range: 18–50 years). Among these, 166 cases (55.3%) were between 18 and 30 years, 102 (34.0%) were between 31 and 40 years, and 32 (10.7%) were between 41 and 50 years. Males (63.7%) predominated the sample. The normative database has been summarized in Table 1. Arterioles were found to be thinner with a higher wall thickness-to-lumen ratio.

A comparison between OD and OS did not reveal any significant differences (*P* = 0.09). However, significant differences were noted on comparisons between the vascular calibers of different retinal quadrants. Supero-temporal arterioles were found to be the thickest arterioles (*P* < 0.001), while infero-temporal venules were found to be the thickest venules (*P* < 0.001). The arteriolar-to-venular ratio (*P* < 0.001) and the wall thickness-to-lumen ratio (*P* < 0.001) were found to be significantly more for the nasal retina in comparison to the temporal retina.

**Table 2: Normative database based on age and gender**

Parameters	Mean (µm)			P for Age	Mean (µm)		P for Gender	Remark
	Category 1 18-30 years	Category 2 31-40 years	Category 3 41-50 years		Male	Female		
<b>Arteriolar</b>								
Internal dimension- lumen	86.17±18.23	83.54±17.92	82.64±17.63	0.54	84.41±17.93	85.76±18.46	0.13	No significant difference
External dimension	106.09±17.45	103.72±17.84	101.75±17.90	0.783	104.04±17.83	106.19±17.38	0.015	Male < Female
Wall thickness	19.72±14.01	19.92±13.58	18.98±13.06	0.544	19.4±13.88	20.26±13.54	0.077	No significant difference
Wall thickness-to-lumen ratio	0.26±0.21	0.27±0.20	0.25±0.20	0.292	0.27±0.21	0.27±0.20	0.256	No significant difference
<b>Venular</b>								
Internal dimension- lumen	133.96±23.35	131.17±23.07	126.1±23.08	0.51	133.23±24.04	130.27±21.91	0.032	Male > Female
External dimension	145.77±22.11	142.42±22.29	138.46±22.80	0.81	145.05±23.20	141.70±20.56	0.015	Male > Female
Wall thickness	11.19±10.74	10.42±10.89	12.05±10.52	0.247	11.14±11.15	10.80±9.92	0.55	No significant difference
Wall thickness-to-lumen ratio	0.10±0.10	0.10±0.10	0.11±0.10	0.247	0.10±0.12	0.10±0.10	0.867	No significant difference
<b>Arteriolar-to-Venular Ratio</b>								
AVR- Lumen*	0.66±0.13	0.64±0.12	0.68±0.14	0.381	0.66±0.17	0.68±0.18	0.003	Male < Female
AVR- External dimension*	0.74±0.11	0.74±0.11	0.76±0.11	0.493	0.74±0.15	0.77±0.16	<0.0001	Male < Female

\*AVR: Arteriolar-to-Venular Ratio

Data were also categorized according to different age groups and gender [Table 2]. No significant statistical significance was detected between different age groups ( $P > 0.05$ ). Males were found to have slightly higher external and internal venular diameters, while females were found to have slightly larger arteriolar external diameters ( $P = 0.015$ ,  $P = 0.032$ , and  $P = 0.015$ , respectively).

**Observer variation**

For inter-observer reliability, the intra-class correlation coefficient was determined to be between 0.98 and 0.99 with incredibly low confidence bounds. Similarly, for intra-observer reliability, it was determined to be  $>0.99$ . Therefore, this method of measuring retinal vessel dimensions was found to be free of observer variability.

**Comparison of dimensions with image J**

A single observer measured vessel caliber for randomly selected 40 eyes by using Image J after 1 week and compared with the normative database. The Bland-Altman plot showed that the difference between measurements using two methods lies within the limits of agreement approximately 95% of the time. Therefore, both these methods of imaging were found to be highly comparable.

**Discussion**

Though there is a vast literature on retinal vascular measurement from population-based studies, the availability of a normative database is lacking, specifically in an objective study environment with accurate imaging.<sup>[8]</sup> Thus, the limitation in using retinal vasculature as an astute clinical marker remains an inability to quantitatively define the normal range. One of the challenges has been to control the confounding effect of systemic diseases in the sample.

This study proposes a unique non-invasive method for measuring the caliber of the retinal blood vessels by using the confocal scanning laser ophthalmoscopy system. We were able to measure lumen diameter and vessel diameter of four prominent vessel pairs along with wall thickness, wall thickness-to-lumen ratio, and arteriolar-to-venular ratio for both lumen and vessel separately.

**Measurements**

In theory, the retinal vessel wall cannot be visualized by the fundus picture because it is transparent.<sup>[11]</sup> Pakter *et al.*<sup>[12]</sup> had proven the same by utilizing fundus angiography for vessel lumen measurement and comparing the results of vessel diameter measurement in the fundus picture. It was also described by Rassam *et al.*<sup>[13]</sup> This warranted the need for the methodology to measure both lumen and wall thickness.

Previously, Doppler optical coherence tomography and spectral-domain optical coherence tomography have been used to measure retinal blood column and vessel diameter in retinal and optic nerve head diseases.<sup>[14,15]</sup> Unlike Wang *et al.*<sup>[15]</sup> who reported on the diameters of all veins around the optic disc that were scanned within two fixed diameter circular scans, we measured the retinal vessel diameter at a known constant distance from the optic disc center regardless of the disc diameter. This is particularly advantageous to avoid inter-observer variations.

**Table 3: Comparison with previous studies (All values are in  $\mu\text{m}$ )**

Studies	Hogan <sup>[16]</sup>	Lee <sup>[19]</sup>	García-Ortiz <sup>[17]</sup>	Goldenberg <sup>[14]</sup>	Zhu <sup>[18]</sup>	Rim <sup>[20]</sup>	Present study
Year	1963	1998	2012	2013	2014	2016	2017
Methodology	Histological Dissection	Optic Disc Photographs	Retinal Photographs	Spectralis domain-optical coherence tomography with Image J At 960 $\mu\text{m}$	Volume scan in Spectralis domain-optical coherence tomography Between half and one-disc distance	Spectralis domain-optical coherence tomography with intensity graph	confocal scanning laser ophthalmoscopy
Distance of measurement	Peri-papillary border	Peri-papillary border	-	-	-	-	1800 $\mu\text{m}$ from the center of the disc
<b>Parameters</b>							
<b>Arteriolar</b>							
Internal dimension- lumen	100	-	-	-	80.94 $\pm$ 11.78	95.1 $\pm$ 16.1	85.10 $\pm$ 12.40
External dimension	130	-	-	-	109.33 $\pm$ 13.18	-	105.04 $\pm$ 12.05
Mean arteriolar calibre	-	102 $\pm$ 16 (ST) 93 $\pm$ 12 (IT)	106.00	127.81 $\pm$ 13.42	-	-	-
Wall thickness	15	-	-	-	14.19 $\pm$ 1.10	23.9 $\pm$ 4.9 (inner sides) 21.2 $\pm$ 3.5 (outer sides)	19.71 $\pm$ 8.04
Wall thickness-to-lumen ratio	-	-	-	-	-	0.218 $\pm$ 0.045	0.26 $\pm$ 0.12
<b>Venular</b>							
Internal dimension- lumen	200	-	-	-	115.46 $\pm$ 0.74	132.6 $\pm$ 17.8	133.84 $\pm$ 16.62
External dimension	-	-	-	-	139.90 $\pm$ 12.65	-	145.42 $\pm$ 16.13
Mean arteriolar calibre	-	130 $\pm$ 0.2 (ST) 117 $\pm$ 17 (IT)	138.88	145.33 $\pm$ 14.97	-	-	-
Wall thickness	-	-	-	-	12.22 $\pm$ 0.27	20.7 $\pm$ 4.2 (inner sides) 16.3 $\pm$ 4.3 (outer sides)	11.02 $\pm$ 5.65
Wall thickness-to-lumen ratio	-	-	-	-	0.36 $\pm$ 0.04	0.117 $\pm$ 0.027	0.10 $\pm$ 0.05
<b>Arteriolar-to-Venular Ratio (AVR)</b>							
AVR- Lumen*	-	-	-	-	-	-	0.66 $\pm$ 0.1
AVR- External dimension*	-	-	-	-	-	-	0.74 $\pm$ 0.09
AVR- Mean*	0.65	-	-	0.9	0.70 $\pm$ 0.09	-	-

\*AVR: Arteriolar-to-Venular Ratio

### Vessel and lumen dimensions

Table 3 compares and summarizes the findings of this study with those of the comparable available literature.<sup>[14,16-19]</sup> In their postmortem study, Hogan *et al.*<sup>[16]</sup> reported that the mean diameter of the principal retinal arterial branches was 130.0  $\mu\text{m}$ . Our results of mean arteriolar luminal and outer vessel diameter were different from their findings. As the measurements of the arterioles and venules in our study were performed more peripherally comparatively, the diameter was expected to be smaller. Further, ours is an in-vivo study, while their study was in postmortem eyes with obvious possibility of inaccuracies related to cause of fatality and changes in circulation after death.

### Arteriolar-to-venular ratio

The mean arteriolar-to-venular ratio has been previously reported to be 0.65,<sup>[16]</sup> which was comparable with our findings of luminal arteriolar-to-venular ratio. The compatibility of the results in both studies emphasizes the potential for accuracy in retinal vessel measurement by using a non-invasive technique. In addition, from the present study, it can be well deduced that the luminal arteriolar-to-venular ratio was 2:3, which corresponds well to the clinical arteriolar-to-venular ratio on fundoscopy, whereas the actual outer vessel arteriolar-to-venular ratio was 3:4. The reason for this difference is that the clinical arteriolar-to-venular ratio depends on the normal light reflex from the retinal vessels, which in turn is formed by the reflection from the interface of the blood column and the vessel wall. Thin-walled veins appear dark due to the strong absorbance of green light by hemoglobin.<sup>[5,21]</sup> Thus, the arteriolar-to-venular ratio based on fundus evaluation does not include the outer diameter of the vessel. There has been limited success with the previously studied clinical arteriolar-to-venular ratio as a marker for systemic and ocular disease.<sup>[22]</sup> Herein, we can conclude that the quantitative arteriolar-to-venular ratio of the retinal outer vessel is different from the clinical arteriolar-to-venular ratio, which emphasizes the need for a future study to compare both and their changes in different systemic diseases.

### Wall thickness

The arteriolar walls were thicker than venular walls (19.7 and 11.0  $\mu\text{m}$ , respectively), which was consistent with previous studies.<sup>[16,18]</sup> Chui *et al.*<sup>[23]</sup> also demonstrated that the venular wall was relatively thinner compared with arterioles with similar lumen diameters due to their differing structure.

### Wall-to-lumen ratio

In 2009, Ritt *et al.*<sup>[24]</sup> reported that arteriolar wall thickness-to-lumen ratio changes may reflect vascular structure remodeling, and in the same year, Baleanu *et al.*<sup>[25]</sup> showed that wall thickness-to-lumen ratio is a more sensitive indicator than clinical arteriolar-to-venular ratio in the assessment of hypertensive cerebrovascular damage. Moreover, Cuspidi *et al.*<sup>[26]</sup> suggested that arteriolar wall thickness-to-lumen ratio can be a potential marker of endothelial dysfunction in both the retinal and systemic vasculatures.

The mean arteriolar and venular wall thickness-to-lumen ratio in this study was 0.3 and 0.1, respectively. In 2014, Zhu *et al.*<sup>[18]</sup> using spectral-domain optical coherence tomography, reported the mean arteriolar wall thickness-to-lumen ratio as 0.36. This difference may be due to the different devices and methodologies used for measuring the retinal vascular caliber.

Therefore, a study directly comparing both methods may be necessary to understand the reasons for this discrepancy.

### Observer variation

The Atherosclerosis Risk in Communities study reported inter- and intra-observer correlation coefficients of 0.69 and 0.74 for arteriole caliber, and 0.89 and 0.77 for venule caliber, respectively.<sup>[9]</sup> Garcia-Arumí *et al.*<sup>[27]</sup> reported an interobserver intra-class correlation coefficient ranging from 0.96 to 0.98 for veins and arterioles; their intra-observer intra-class correlation coefficient was also very high, ranging from 0.97 to 0.99. Sherry *et al.*<sup>[28]</sup> used a method based on the ARIC study and reported improved interobserver correlations (range: 0.78–0.90), and intra-observer correlations (range: 0.79–0.92), although the values achieved in our study were not reached. Muraoka *et al.*<sup>[29]</sup> using spectral domain-optical coherence tomography, reported inter-visit, inter-examiner, and inter-evaluator intra-class correlation coefficients ranging from 0.944 to 0.982 by using an optic disc centered circle scan method, similar to the results of our study.

### Comparison of dimensions with image J

Direct measurements of vessel diameters by using image J software in previous studies have reported high repeatability and reproducibility,<sup>[29]</sup> similar to our results. Herein, we can conclude that both methods can be used to measure vessel caliber interchangeably.

### Limitations

In this study, all the measurements were done in a circular zone between half-a-disc diameter and one-disc diameter from the optic disc margin, and we came across juxtaposed vessels and intertwining of vessels due to normal retinal vascular architecture variations in the measurement zone in some subjects, which made it difficult to differentiate between vessels, especially in autofluorescence images. It was overcome by taking infrared images as reference and measurements were done in a magnified view. As has been highlighted in previous studies, errors can arise from other factors such as the phase-in cardiac cycle when the image has been captured. It has been shown that retinal caliber may vary up to 15% depending on the moment in the cardiac cycle when the image was taken.<sup>[30]</sup> Any static vessel analysis does not allow assessment of the vascular function.<sup>[12,20]</sup> This limitation was negated to a certain extent by enrolling a large healthy cohort.

### Conclusion

To summarize our study, we discuss a new non-invasive methodology for measuring retinal vascular caliber by using inbuilt calipers on the confocal scanning laser ophthalmoscopy system. This new methodology showed high observer reliability. Most importantly, in an era of developing artificial intelligence and teleophthalmology, we provide a normative database for the retinal vascular dimensions, which has hitherto been unavailable in published literature.<sup>[8]</sup> Future studies using this normative database as a baseline in varied systemic diseases, particularly cardiometabolic disorders, would help in a better understanding of their relationship with retinal vascular dimensions. This normative database can also be used to ascertain if retinal vascular measurements can be used as a biomarker or proxy for risk stratification and disease control in patients with diseases such as hypertension and diabetes.



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## Conflicts of interest

There are no conflicts of interest.

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