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What ocular and systemic variables affect choroidal circulation in healthy eyes

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Abstract

The aim of the study was to investigate the relationship between choroidal blood flow and systemic and ocular variables in patients with healthy eyes.

In this prospective cross-sectional study, we examined 241 eyes of 241 healthy Japanese subjects (92 males and 149 females; mean age, 37.8±17.0 years). The mean blur rate, a measure of the relative blood flow of the choroid, was determined using laser speckle flowgraphy. The total cross-sectional choroidal, luminal, and stromal areas of the choroid were determined by the binarization method. We investigated the correlation between choroidal MBR and systemic and ocular variables.

Choroidal mean blur rate correlated with age ($r=-0.385$, $P<0.001$) and choroidal thickness ($r=0.264$, $P<0.001$). The choroidal area correlated with choroidal mean blur rate ($r=0.374$, $P<0.001$), age ($r=-0.184$, $P=0.004$), axial length ($r=-0.251$, $P<0.001$), and choroidal thickness ($r=0.468$, $P<0.001$). The luminal area correlated with choroidal mean blur rate ($r=0.403$, $P<0.001$), age ($r=-0.244$, $P<0.001$), axial length ($r=-0.218$, $P=0.001$), and choroidal thickness ($r=0.435$, $P<0.001$). On multiple stepwise regression analyses, age ($\beta=-0.321$, $P<0.001$) and luminal area ($\beta=0.320$, $P<0.001$), heart rate ($\beta=0.136$, $P=0.018$), and mean ocular perfusion pressure ($\beta=0.126$, $P=0.045$) were independent factors indicating the choroidal mean blur rate. Furthermore, axial length ($\beta=-0.352$, $P<0.001$), choroidal mean blur rate ($\beta=0.273$, $P<0.001$), age ($\beta=-0.247$, $P<0.001$), gender ($\beta=-0.226$, $P<0.001$), and mean ocular perfusion pressure ($\beta=0.193$, $P=0.002$) were independent factors indicating the luminal area.

The choroidal blood flow positively correlated with the luminal area and negatively correlating with age. In addition, the luminal area was negative correlated with age. It is suggested that aging causes a reduction in choroidal blood flow and luminal area, and as a result of aging effect, decreased choroidal blood flow would correlate with decreased luminal area.

Abbreviations: COV = coefficient of variation, DBP = diastolic blood pressure, EDI = enhanced depth-imaging, IOP = intraocular pressure, IVB = intravitreal bevacizumab, LSFG = laser speckle flowgraphy, MAP = mean arterial blood pressure, MBR = mean blur rate, MOPP = mean ocular perfusion pressure, ONH = optic nerve head, RPE = retinal pigment epithelium, SBP = systolic blood pressure, SD-OCT = spectral-domain OCT, SFCT = subfoveal choroidal thickness.

Keywords: aging, binarization, choroidal circulation, laser speckle flowgraphy, mean blur rate, normal eye

1. Introduction

Choroidal blood flow is the major source of oxygen and nutrients for the choroid and outer retina. Accordingly, assessment of choroidal blood flow is essential to understanding the pathology and to treat chorioretinal diseases.[1] Choroidal blood flow decreased in various disorders, for example, "myopic retinopathy,[1,2] choroidal neovascularization,[1] retinitis pigmentosa,[3,4] and glaucoma [5,6]" and increased in other disorders such as "central serous chorioretinopathy[7] and pachychoroid.[8]"

However, measuring choroidal blood flow is particularly challenging because the choroidal vessels are hidden from view by the retinal pigment epithelium (RPE). Various techniques for measuring choroidal blood flow have been developed, including laserferometry,[9] computerized pneumotonometry,[10] indocyanine green angiography,[11] and near-infrared Doppler flowmetry[12]; however, it is difficult to capture the same place and reproduce the same image for the choroid, resulting in low reproducibility. A more sophisticated approach involves blood velocity measurements using laser Doppler velocimetry.[13–16] However, clinical use of this technique is hampered by the time-consuming nature of the procedure, which renders it unsuitable for use in large-scale trials. Sogawa et al measured total choroidal blood flow in only 25 healthy subjects using laser Doppler velocimetry, thereby reporting no correlation between choroidal thickness and choroidal blood flow.

Laser speckle flowgraphy (LSFG) is a noninvasive, real-time method used to measure the relative blood flow in the choroid and optic nerve head (ONH) for 4s without the use of contrast agents.[17–19] LSFG can detect the speckle contrast pattern produced by the interference of illuminating laser light that is scattered by the movement of erythrocytes in the blood vessels

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and enables measurement of the relative blood flow, expressed as the mean blur rate (MBR), in the vessels.[17–19] LSFG values correlated well with the actual blood flow determined with use of hydrogen gas clearance and microsphere methods,[20,21] which implies that the variables determined with LSFG would be comparable between individuals. Aizawa et al[22] reported that the coefficient of variation (COV) for MBR was 4.7 for the choroid and 3.4 for the ONH. Therefore, LSFG is considered suitable for the measurement of choroidal blood flow in large-scale trials.

The choroid is mainly composed of vessels and stroma (extravascular tissue) and much of the choroidal space is taken by vessels differentiated in 3 vascular layers, that is, choriocapillaris, Sattler's layer, and Haller's layer. The choriocapillaris forms a continuous network with the small-sized vessels bordering Bruch's membrane; the Sattler's layer has medium-sized vessels, and the Haller's layer is the outermost layer with large-sized vessels.[23] These layers are supplied by short ciliary arteries.[1]

It is important to understand the variations in the choroidal structure in normal eyes and to compare choroidal blood flow with the vessel area. However, it is difficult to differentiate the luminal area from the stromal area in the choroid in vivo. Recently, Sonoda et al[24,25] reported the use of a binarization method involving OCT images, which can differentiate the choroidal luminal area from the stromal area and quantify the areas using a software Image-J with a high repeatability. They reported that the area of all of the components, including the luminal and stromal areas, decrease with increasing age.[25]

Thus, this study aimed to determine choroidal blood flow using LSFG and to investigate the correlation of choroidal blood flow with variables, including the luminal and stromal areas, after differentiating and quantifying the choroidal luminal area from the stromal area by the binarization method in healthy eyes.

2. Methods

2.1. Ethics statement

In this prospective cross-sectional study, the procedures used were approved by the Ethics Committee of the Nagoya University Hospital and were conducted at the Nagoya University Hospital, and the study conformed to the tenets of the Declaration of Helsinki. An informed consent was obtained from all subjects after explaining the nature and possible consequences of the study.

2.2. Subjects

Visual acuity, blood pressure, and axial length were assessed to rule out any ocular disease (Fig. 1). The axial length was measured on partial optical coherence interferometry (IOL-Master; Carl Zeiss Meditec, La Jolla, CA). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the left brachial artery at the height of the heart in a sitting position with an automatic sphygmomanometer (CH-483C; Citizen, Tokyo, Japan). All subjects had a best-corrected visual acuity of ≥20/20 and were examined to determine if any ocular disease was present. Slit-lamp examination and indirect ophthalmoscopy were used to examine the anterior and posterior segments of the eye, respectively. The exclusion criteria included the presence of any macular abnormalities such as choroidal neovascularization or asymptomatic pigment epithelial detachment, history of ophthalmic or general disorders, ocular laser or incisional surgery in the experimental eye, cataract formation to avoid an underestimation of MBR,[26] topical or systemic medications, including hormonal medications, SBP > 150 mm Hg, DBP > 90 mm Hg, and axial length >27.0 mm.[27] Furthermore, subjects were screened for any medical condition that might influence the hemodynamics of the eye, such as diabetes, hypertension, arrhythmia, and vascular diseases. As a result, a total of 241 eyes of 241 healthy volunteers with no ophthalmic or systemic diseases were enrolled in this study.

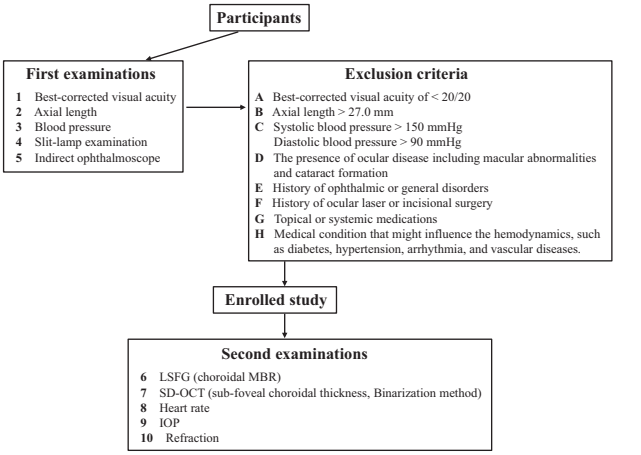

Figure 1. Flowchart showing examinations and selection of eligible enrolls in this study.

As the second examinations, the relative blood flow was determined by LSFG-NAVI instrument (Softcare, Fukuoka, Japan), as described below. Choroidal images were obtained by spectral-domain OCT (SD-OCT; Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany), as described below. Heart rate, intraocular pressure (IOP), and refraction were also measured. IOP was measured with a handheld tonometer (Icare; Tiolat Oy, Helsinki, Finland), and the refractive error (spherical equivalent) was measured with an autorefractometer (KR8900; Topcon, Tokyo, Japan). The mean arterial blood pressure (MAP) and mean ocular perfusion pressure (MOPP) were calculated as follows: MAP = DBP + 1/3(SBP − DBP) and MOPP = 2/3MAP − IOP, respectively.[28]

Because alcohol[29] and caffeine[30] intake can influence IOP, all subjects were asked to abstain from alcoholic and caffeinated beverages from the evening before the day of the study. In addition, all subjects were asked to abstain from consuming any food 2 hours prior to each experiment to minimize the potential effect of food on blood pressure. All examinations were performed in the sitting position on the same day between 13:00 and 15:00 hours to preclude any effect of diurnal variations.[31,32] Each subject rested for 10 to 15 minutes in a quiet room immediately prior to the test; each experimental session was completed within 15 minutes.

2.3. Laser speckle flowgraphy

LSFG-NAVI was used to determine the relative ocular blood flow. The principles of LSFG have been described in detail elsewhere.[33–35] Briefly, this instrument comprises of a fundus camera equipped with an 830-nm diode laser and a charge-coupled camera (750 width × 360 height pixels). The laser speckle phenomenon is an interference event when coherent light sources such as lasers are scattered by a diffusing surface, for

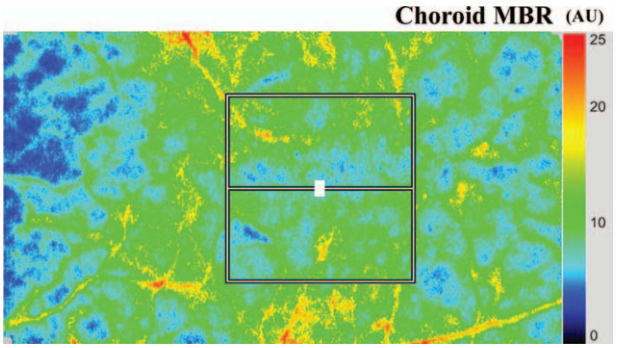

Figure 2. Representative composite color maps using the mean blur rate (MBR) as measured by laser speckle flowgraphy (LSFG). The red color indicates a high MBR, and the blue color indicates a low MBR. To measure the MBR of choroidal blood flow, the center of a square was set at the fovea (250 × 250 pixels, degree: 6.31° × 6.31°). LSFG = laser speckle flowgraphy, MBR = mean blur rate, MOPP = mean ocular perfusion pressure.

example, the ocular fundus. The speckle pattern, which appears under the illuminati on of laser irradiation, can be described statistically. The structure of the speckle pattern varies rapidly depending on the blood flow velocity. MBR is determined by examining the pattern of the speckle contrast produced by the interference of the laser light that is scattered by the movement of the blood cells in the ocular blood vessels and is a measure of the relative blood flow. MBR images are acquired at a rate of 30 frames/s over a 4-seconds period. The embedded analysis software synchronizes all of MBR images with each cardiac cycle, and the averaged MBR of a heartbeat is displayed as a composition map.

To evaluate the changes in choroidal blood flow, a square (250 × 250 pixels) was placed around the macula (Fig. 2). The software in the instrument was able to track the eye movements during the measurement period. None of the subjects enrolled in this study had any retinal/arteriolar vessels in the square area. LSFG was measured twice for each time point in all of the eyes. Average MBR values were calculated for each square using the LSFG Analyzer software (V.3.1.59).

2.4. Measurement of choroidal thickness

The choroidal thickness was measured by SD-OCT using the enhanced depth-imaging (EDI) technique, in which SD-OCT was placed close enough to the eye to obtain inverted images as previously described.[36] The choroidal thickness at the fovea was measured as the distance from the hyper-reflective RPE line to the choroid–sclera border with the caliper tool on SD-OCT by 2 experienced clinicians who were blinded to other study parameters.

2.5. Differentiation of luminal and stromal areas

Binarization of the choroidal area in the EDI-OCT image was performed by the modified Niblack's method as previously reported.[24] The EDI-OCT image was analyzed by the ImageJ software (ImageJ version 1.47, NIH, Bethesda, MD). The examined area was 1500 μm wide in the subfoveal choroid, extending vertically from the RPE to the choriosclera border (Fig. 3). This choroidal area was selected with the ImageJ ROI Manager. Then, the image was converted to 8 bits. The vitreous cavity in front of the macular area was selected by the Oval Selection Tool on the ImageJ tool bar, and the maximum reflectivity of these areas was determined. The maximum brightness was set at the minimum value to minimize noise in the OCT image. After adjusting by the Niblack Auto Local Threshold, the luminal area was determined using the threshold tool. The light pixels were defined as the interstitial areas, and the dark pixels were defined as the luminal areas. After adding the data of the distance of each pixel, the luminal and interstitial areas were automatically calculated. Two clinicians who were masked to the other findings measured the area.

2.6. Statistical analyses

The value of each parameter was presented as the mean ± standard deviation. Independent *t*-tests were used to compare normally distributed data. Spearman and Pearson correlation coefficient test was used to determine the correlation coefficients between the variables. Multiple stepwise regression analysis was used to determine the association between choroidal MBR or the luminal area and variables. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23 (IBM Corp., Armonk, NY). The significance level was set at a probability (P) value of <0.05.

3. Results

Demographic data on all study subjects are shown in Table 1. A total of 241 eyes of 241 healthy Japanese subjects (92 males and 149 females; mean age, 37.8 ± 17.0 years) were examined. Mean IOP was 13.8 ± 2.4 mm Hg, mean refraction was –2.74 ± 2.68 diopter, mean axial length was 24.71 ± 1.23 mm, mean foveal thickness was 215.2 ± 19.6 μm, mean subfoveal choroidal thickness (SFCT) was 240.9 ± 60.9 μm, mean SBP was 118.5 ± 14.8 mm Hg, mean DBP was 72.2 ± 10.2 mm Hg, MOPP was 42.8 ± 7.5 mm Hg, mean heart rate was 74.3 ± 10.5 bpm.

Table 2 shows choroid MBR and the area determined by the binarization method. Mean choroidal MBR was 11.5 ± 3.7, mean choroidal area was 0.412 ± 0.120 mm^2, mean luminal area was 0.277 ± 0.085 mm^2, and mean stromal area was 0.135 ± 0.044 mm^2 across all study subjects.

Table 3 and supplemental content 1 show the linear single regression results, and Fig. 4 displays the correlation of choroidal MBR with various ocular and systemic variables. Choroidal MBR correlated with age ($r=-0.383$, $P<0.001$) and SFCT ($r=0.276$, $P<0.001$). Figure 5 illustrates the correlation between choroid MBR and the area determined by the binarization method. The choroidal area correlated with choroidal MBR ($r=0.359$, $P<0.001$); age ($r=-0.266$, $P=0.004$); axial length ($r=-0.249$, $P<0.001$); and SFCT ($r=0.459$, $P<0.001$). The luminal area correlated with choroidal MBR ($r=0.390$, $P<0.001$); age ($r=-0.316$, $P<0.001$); axial length ($r=-0.224$, $P=0.001$); SFCT ($r=0.444$, $P<0.001$); and MOPP ($r=0.206$, $P=0.003$).

On multiple stepwise regression analysis, age ($\beta=-0.321$, $P<0.001$) and luminal area ($\beta=0.320$, $P<0.001$), heart rate ($\beta=0.136$, $P=0.018$), and MOPP ($\beta=0.126$, $P=0.045$) were independent factors indicating the choroidal MBR (Table 4). Furthermore, SFCT ($\beta=-0.352$, $P<0.001$), choroidal MBR ($\beta=0.273$, $P<0.001$), age ($\beta=-0.247$, $P<0.001$), gender ($\beta=-0.226$, $P<0.001$), and MOPP ($\beta=0.193$, $P=0.002$) were independent factors indicating the luminal area (Table 5).

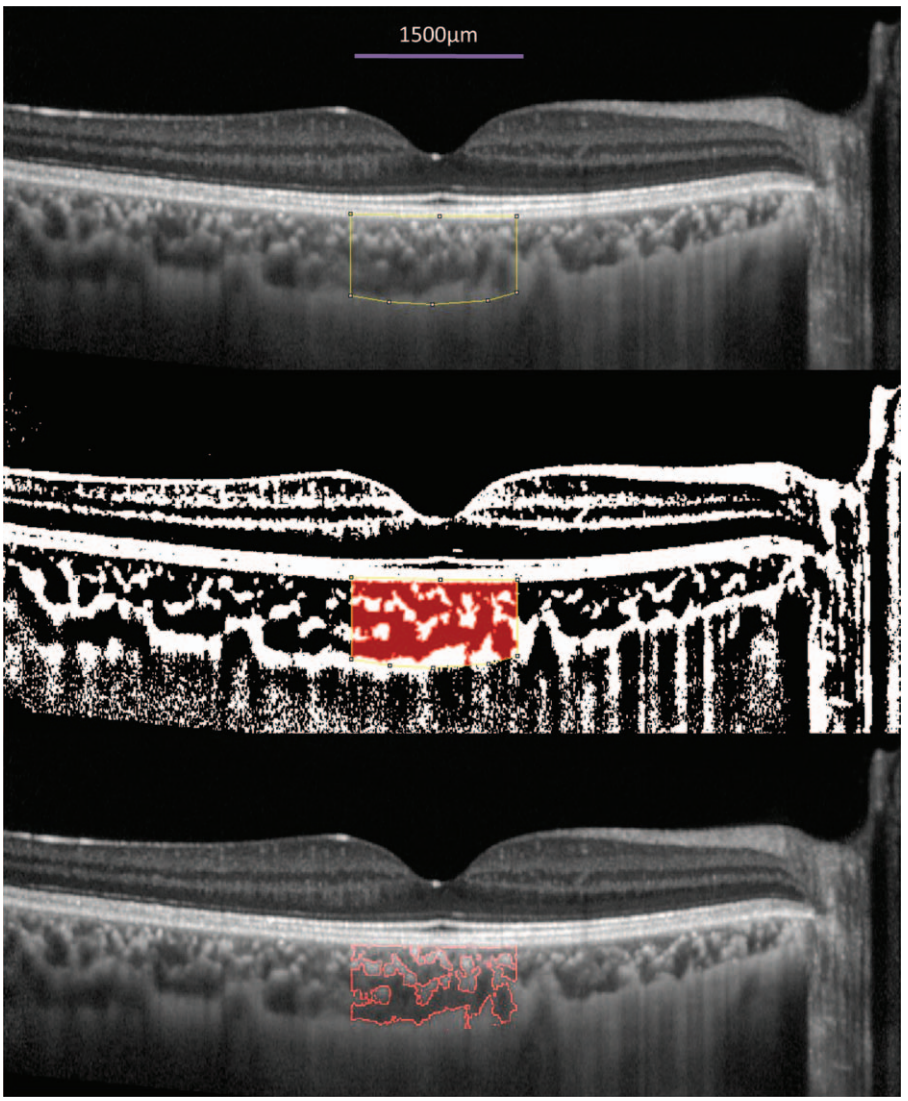

Figure 3. Representative binarization image of a choroidal area in an enhanced depth imaging (EDI) optical coherence tomography (OCT) image. The area of interest of the choroid is demarcated (top). The EDI-OCT image is converted to a binary image using the ImageJ software. The rectangle surrounded by the red line was excised, and the dark areas were traced by the modified Niblack method (middle). The binarized image and the margin of the traced area are merged, which demonstrates that the traced area is consistent with the dark areas of the choroidal areas of the OCT image (bottom). EDI = enhanced depth imaging, OCT = optical coherence tomography.

4. Discussion

We measured choroidal blood flow in 241 healthy eyes using LSFG. On linear single regression analysis, choroidal MBR positively correlated with SFCT and the luminal area, as determined by the binarization method, and negatively correlated with age. Furthermore, the luminal area negatively correlated

Table 1

Clinical characteristics of subjects.

Characteristic	Mean ± SD
Age, y	37.8 ± 17.0
Gender, male/female	92/149
Intraocular pressure, mm Hg	13.8 ± 2.4
Refraction, D	–2.74 ± 2.68
Axial length, mm	24.71 ± 1.23
Foveal thickness, μm	215.2 ± 19.6
Subfoveal choroidal thickness, μm	240.9 ± 60.9
Systolic blood pressure, mm Hg	118.5 ± 14.8
Diastolic blood pressure, mm Hg	72.2 ± 10.2
Mean ocular perfusion pressure, mm Hg	42.8 ± 7.5
Heart rate, bpm	74.3 ± 10.5

SD = standard deviation.

Table 2

Choroid MBR and the area determined by the binarization method.

Parameters	Mean ± SD
Choroid MBR, AU	11.5 ± 3.7
Choroidal area, mm^2	0.412 ± 0.120
Luminal area, mm^2	0.277 ± 0.085
Stromal area, mm^2	0.135 ± 0.044

AU = arbitrary unit, MBR = mean blur rate, SD = standard deviation.

Table 3

Result of Spearman's rank correlation coefficient between the choroid MBR and clinical parameters in all subjects.

Parameters	Choroid MBR	Age	Gender	IOP	AL	FT	SFCT	MOPP	HR
Choroid MBR, AU	–	−0.383[a]	0.069	0.078	−0.076	0.116	0.276[a]	0.142[c]	0.193[b]
Choroidal area, mm^2	0.359[a]	−0.266[a]	−0.071	−0.011	−0.249[a]	−0.029	0.459[a]	0.156[c]	0.037
Luminal area, mm^2	0.390[a]	−0.316[a]	−0.08	0.007	−0.224[a]	0.007	0.444[a]	0.206[b]	0.063
Stromal area, mm^2	0.127	−0.142	−0.049	−0.042	−0.246[a]	−0.092	0.395[a]	0.028	−0.020

AL = axial length, FT = foveal thickness, HR = heart rate, IOP = intraocular pressure, MBR = mean blur rate, MOPP = mean ocular perfusion pressure, ONH = optic nerve head, SFCT = subfoveal choroidal thickness.

[a] $P < 0.001$.

[b] $P < 0.01$.

[c] $P < 0.05$.

with age. On multiple stepwise regression analysis, age and the luminal area were the independent factors indicating choroidal MBR, and age and choroid MBR were the independent factor indicating the luminal area.

We evaluated choroidal MBR within the macular area of 250×250 pixels (degree: $6.31° \times 6.31°$). The width is close to that 1500 μm determined by the binarization method in eye with normal axial length, such as 24 mm. MBR calculations mainly reflect choroidal blood flow because of the lack of large retinal vessels found near the fovea. In addition, it has been reported that after the induction of a branch retinal artery occlusion in monkey eyes there is a little change in the post-induction panoramic map compared with the vascular patterns observed prior to the occlusion.[37]

Until the development of the EDI-OCT technique,[38] precise choroidal thickness assessment was challenging. Consequently, only a few reports are available that describes the relationship between choroidal blood flow and choroidal thickness in healthy subjects. Sogawa et al[39] first reported no correlation between choroidal blood flow as determined by laser Doppler velocimetry and SFCT in healthy young subjects. However, we found that choroidal blood flow determined by LSFG was significantly positively correlated with SFCT in a larger number of normal eyes. The wavelength used in the laser Doppler velocimetry is 670 nm,[39,40] which is shorter than the 830-nm wavelength used in LSFG, and it measures choroidal blood flow predominantly in the choriocapillaris. In contrast, since LSFG has a longer wavelength, it can measure deeper vascular layers, including Sattler's layer and Haller's layer, that are characterized by large vessels and rich blood flow.[41] The difference in layer measurements between laser Doppler velocimetry and LSFG could explain the difference in blood flow results. OCT angiography also uses a longer wavelength which can reach deeper choroidal layers. However, the algorithm used for measurement in the current commercially available OCTs is from retina to the part of shallow choroidal layer such as choriocapillaris layer, as most chorioretinal lesions are located here. Thus, it does not adequately capture the deeper choroidal layers. The algorithm and the principle in LSFG is different from that of OCT angiography; consequently, the choriocapillaris blood flow does not interfere with the measurement of blood flow in the choroidal vessels (although, RPE somewhat interferes with the measurement).

Okamoto et al reported that intravitreal bevacizumab (IVB) injection significantly reduced SFCT and choroidal blood flow.

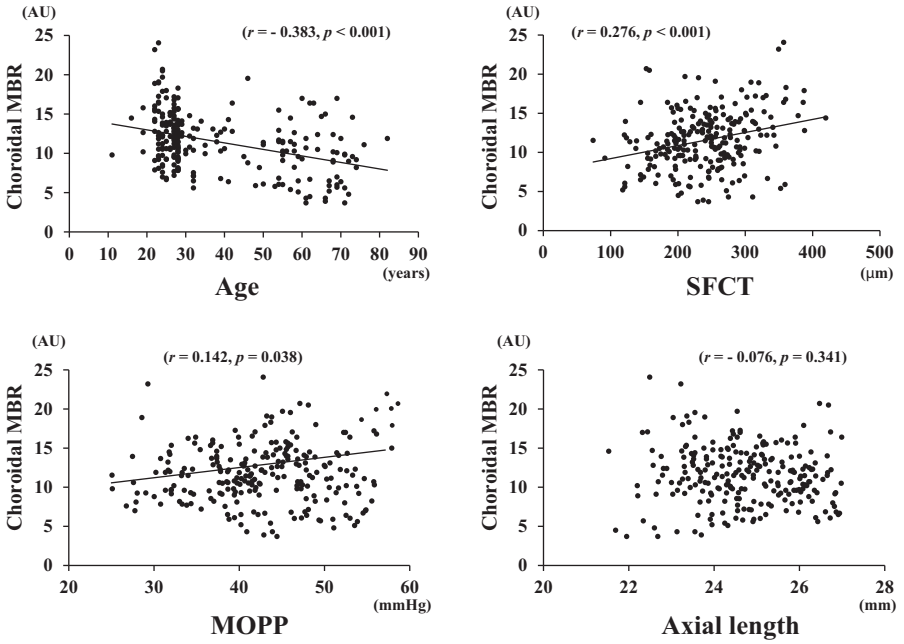

Figure 4. Relationship between choroidal MBR and ocular and systemic parameters on univariate analysis. Choroidal MBR negatively correlated with age ($r = -0.383$, $P < 0.001$) (top left), and positively correlated with subfoveal choroidal thickness ($r = 0.276$, $P < 0.001$) (top right) and MOPP ($r = 0.142$, $P = 0.038$) (bottom left), whereas it did not correlate with axial length (bottom right). AU = arbitrary units, MBR = mean blur rate, MOPP = mean ocular perfusion pressure, SFCT = subfoveal choroidal thickness.

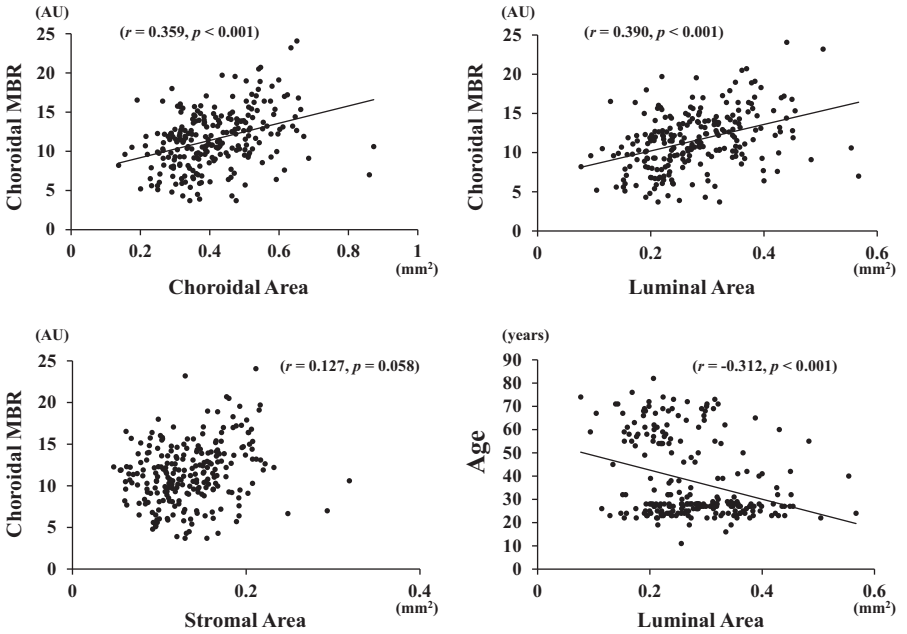

Figure 5. Relationship between choroidal MBR and components of the choroid by univariate analysis using the binarization method. Choroidal MBR positively correlated with choroidal area ($r=0.359$, $P<0.001$) (top left) and the luminal area ($r=0.390$, $P<0.001$) (top right), whereas it did not correlate with the stromal area (bottom left). The luminal area negatively correlated with age ($r=-0.312$, $P<0.001$). AU = arbitrary units, MBR = mean blur rate.

They concluded that the reduction in SFCT after IVB was likely to be caused by a reduction in subfoveal choroidal blood flow.[42] Sildenafil citrate increases choroidal thickness because of its vasodilatory effect on the choroidal circulation, indicating that increased choroidal circulation may be associated with increased choroidal thickness.[43,44] Additionally, longitudinal analysis revealed a significant increase in SFCT during altitude exposure because of increased choroidal blood flow.[45] In the present study, choroidal blood flow and the luminal area showed a slight positive correlation with MOPP. There have been reports stating that choroidal vessels were poorly autoregulated, and changes in the perfusion pressure directly affect the blood flow.[46–48] Altogether, it is most likely that choroidal blood flow is positively correlated with choroidal thickness.

Histologically, the choroid comprises blood vessels and stroma (extravascular tissue), which contains collagen and elastic fibers, fibroblasts, nonvascular smooth muscle cells, and numerous very large melanocytes.[1] The stromal area is less likely to be correlated with choroidal blood flow because of the extravascular tissue. Therefore, it is more meaningful to investigate the correlation between blood flow and the choroidal vascular area after this area has been differentiated from the stromal area.

Recently, Sonoda et al[24,25] reported the binarization method using SD-OCT images, which can differentiate and quantify the choroidal, luminal, and stromal areas. By the binarization method, we successfully differentiated and calculated these areas on SD-OCT images. We found that choroidal blood flow was more correlated with the luminal area than SFCT and was not correlated with the stromal area. There have been no reports comparing choroidal blood flow with the luminal area component by any technique, including the binarization method. Our results demonstrate that the luminal area is mainly correlated with choroidal blood flow, which appears plausible because the extravascular area of the stromal area is not included in the assessment of the relationship between the choroidal area and blood flow.

Table 4

Results of multiple stepwise regression analysis for independence of factors contributing to choroid MBR.

Variable			
Dependent	**Independent**	β	***P***
Choroid MBR	Age	−0.321	<0.001
	Luminal area	0.320	<0.001
	Heart rate	0.136	0.018
	MOPP	0.126	0.045
	Axial Length	0.110	0.093
	Gender	−0.102	0.120
	SFCT	0.075	0.252

MBR = mean blur rate, MOPP = mean ocular perfusion pressure, SFCT = subfoveal choroidal thickness.

Table 5

Results of multiple stepwise regression analysis for independence of factors contributing to luminal area.

Variable			
Dependent	**Independent**	β	***P***
Luminal area	SFCT	−0.352	<0.001
	Choroid MBR	0.273	<0.001
	Age	−0.247	<0.001
	Gender	−0.226	<0.001
	MOPP	0.193	0.002
	Heart rate	0.060	0.363

MBR = mean blur rate, MOPP = mean ocular perfusion pressure, SFCT = subfoveal choroidal thickness.

Choroidal blood flow and luminal area negatively correlated with age in our study. There have been several reports describing that blood flow is negatively correlated with age.[49,50] The number of choroidal arterioles and the fluorescent intensity in the macular region were observed to decrease with aging using indocyanine green angiography.[51] Dye filling was delayed, and vessels were thickened, running a straight course (instead of tortuous) with reduced branching in older subjects.[51] An increase in the size of patch-like (hypofluorescent) structures was present and persisted longer with an increasing age at the late venous phase.[51] These hypofluorescent regions may result from delayed or irregular dye filling into the choriocapillaries and augmented choroidal interstitial tissue developed from vascular thinning.[51] These findings indicate significant structural changes associated with increasing age. Similarly, an age-related decrease in the foveal choroidal circulation was detected by laser Doppler flowmetry.[52] Reduced choroidal blood flow was mainly attributed to an age-related decrease in choroidal volume as a result of decreased density and lumen diameter of the choriocapillaries.[53] These results are in accordance with this study, thus supporting the theory that choroidal blood flow and the luminal area decrease with increased age. In addition, our study demonstrated that age and luminal area were the independent factors indicating choroidal MBR. These results indicate that aging causes reduced choroidal MBR and luminal area, and as a result of aging, decreased choroidal blood flow was correlated with decreased luminal area.

There are several limitations of this study. First, this study is cross-sectional, that is, parameters may vary among the individual. Accordingly, the investigation of the relationship between changes in choroidal blood flow with aging requires longitudinal study data. Second, we used a square at the fovea to measure MBR of choroidal blood flow, meaning that choroid measurements were conducted only in the center not in the entire choroid. However, we compared choroidal MBR with other variables of choroid, for example, SFCT and vessel and stromal areas in the central area. Third, some of those correlation coefficients were not so high in our results, but were of statistical significance. Further longitudinal studies using a larger number of healthy subjects will be necessary for clarification.

In conclusion, choroidal blood flow, as determined on LSFG, positively correlated with SFCT in this large-scale trial. In addition, the choroidal blood flow positively correlated with the luminal area and negatively correlated with age. The luminal area negatively correlated with age. It is suggested that aging causes a reduction in choroidal blood flow and luminal area, and as a result of the aging effect, decreased choroidal blood flow correlated with decreased luminal area. We believe that our results should be considered when interpreting ocular blood flow data in patients with ocular diseases (e.g., AMD) or systemic diseases, for example, cardiovascular disorders or hypertension.

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