tvst

Article

Relationship Between Changes in the Choroidal Structure and Blood Flow of the Macula After Trabeculectomy

Yuki Takamatsu[1,2,*], Tomomi Higashide[1,*], Satoshi Takeshima[3], Makoto Sasaki[1], Yoshimi Manbo[4], Sachiko Udagawa[1], Shinji Ohkubo[1,5], Shozo Sonoda[6], Taiji Sakamoto[6], and Kazuhisa Sugiyama[1]

[1] Department of Ophthalmology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan
[2] Department of Ophthalmology, Toyama City Hospital, Toyama, Japan
[3] Department of Ophthalmology, Toyama Red Cross Hospital, Toyama, Japan
[4] Department of Ophthalmology, Koseiren Takaoka Hospital, Takaoka, Japan
[5] Ohkubo Eye Clinic, Kanazawa, Japan
[6] Department of Ophthalmology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Correspondence: Tomomi Higashide, Department of Ophthalmology, Kanazawa University Graduate School of Medical Sciences, 13-1 Takara-machi, Kanazawa 920-8641, Japan. e-mail: eyetomo@med.kanazawa-u.ac.jp

Received: June 23, 2021
Accepted: November 24, 2021
Published: December 29, 2021

Keywords: trabeculectomy; choroidal structure; choroidal blood flow; optical coherence tomography; laser speckle flowgraphy

Citation: Takamatsu Y, Higashide T, Takeshima S, Sasaki M, Manbo Y, Udagawa S, Ohkubo S, Sonoda S, Sakamoto T, Sugiyama K. Relationship between changes in the choroidal structure and blood flow of the macula after trabeculectomy. Transl Vis Sci Technol. 2021;10(14):30, https://doi.org/10.1167/tvst.10.14.30

Purpose: To elucidate the relationship between changes in the choroidal structure and blood flow of the macula after trabeculectomy.

Methods: A prospective study of 30 eyes of 30 patients with glaucoma who underwent trabeculectomy. Enhanced depth imaging optical coherence tomography with choroidal image binarization and laser speckle flowgraphy of the macula were performed at baseline and 1, 3, and 6 months postoperatively. Mixed-effects models with adjustment for confounders were used to analyze longitudinal changes in the mean choroidal thickness (mCT), mean choroidal vascular thickness (mCVT), mean choroidal interstitial thickness (mCIT), and mean blur rate (MBR).

Results: The decrease in the intraocular pressure (IOP; 45%–51%) and axial length (0.5%–0.8%) and the increase in ocular perfusion pressure (OPP; 34%–38%), mCT (16%–19%), mCVT (16%–20%), mCIT (17%–20%), and MBR (22%–25%) were significant at each postoperative time point (all $P < 0.001$). In the multivariate analysis, the mCVT changes were positively correlated with the OPP and MBR changes ($P = 0.04$ and $P < 0.001$, respectively), whereas the mCIT changes were negatively correlated with IOP changes ($P = 0.005$). The MBR changes correlated significantly with changes in mCVT but not mCIT ($P < 0.001$ and $P = 0.39$, respectively).

Conclusions: Thickness changes in the intraluminal and extraluminal parts of the choroid were closely associated with changes in blood flow and IOP, respectively, although both parts thickened comparably after IOP reduction by trabeculectomy.

Translational Relevance: The choroid reacts to IOP reduction differently between the intraluminal and extraluminal areas, blood flow dependence in the vascular area, and IOP dependence in the stromal area.

Introduction

Although the primary site of pathology in glaucoma is the optic nerve head, choroidal circulation in the macula has gained some attention. A histological study showed a decrease in choroidal vessel density in eyes with end-stage glaucoma.[1] Clinical studies examining subfoveal choroidal blood flow using laser Doppler flowmetry reported greater diurnal fluctuation and lower blood flow in eyes with early glaucoma.[2,3] More recently, a study on macular blood flow using optical coherence tomography (OCT) angiography revealed lower perfusion density in the choriocapillaris in

the eyes of patients with normal tension glaucoma.[4] Accordingly, the macular choroidal circulation may be impaired in eyes with glaucoma.

Trabeculectomy is a standard glaucoma surgery, and its intraocular pressure (IOP) lowering effect has been proven to suppress glaucoma progression in many studies, including landmark randomized controlled trials.[5] However, overfiltration by trabeculectomy may cause significant vision loss owing to hypotony maculopathy, which is characterized by chorioretinal folds in the macula. Even without hypotony, IOP reduction causes structural changes in the macula. Multiple studies[6–11] have reported an increase in choroidal thickness (CT) after trabeculectomy, which correlated with a decrease in IOP[6,8–11] or axial length (AL)[6,8,10,11] and with an increase in ocular perfusion pressure (OPP).[6] Furthermore, recent studies have examined postoperative CT changes in the intraluminal and extraluminal areas of the choroid separately by using image binarization methods for choroidal images derived from enhanced depth imaging OCT (EDI-OCT).[12,13] These studies indicate that changes in the choroidal structure may differ with time after trabeculectomy.

CT changes may be associated with alterations in the choroidal blood flow. Berisha et al.[14] reported that pulsatile choroidal blood flow in the macula, which was assessed by fundus pulsation amplitude, increased with an increase in OPP, 10 weeks after trabeculectomy. However, the exact relationship between CT changes and choroidal blood flow changes in the macula after trabeculectomy remains unknown.

Laser speckle flowgraphy (LSFG) has been used to study blood flow changes in the macula owing to various ocular diseases or pathophysiological conditions. Among them, the mean blur rate (MBR) in the macula decreased concomitantly with regression of central serous chorioretinopathy.[15] The MBR is an index of the LSFG for blood flow velocity. Hirooka et al.[16] examined the relationship between MBR in the macula and CT in patients with Vogt–Koyanagi–Harada disease and found that an increase in MBR correlated significantly with a decrease in CT after systemic corticosteroid therapy. Moreover, Akahori et al.[17] studied the relationship between MBR and choroidal structural changes in the macula after an acute artificial increase in IOP in healthy subjects. The results showed that there was no correlation between a decrease in the MBR and a decrease in the choroidal luminal area.[17]

In this study, we compared choroidal structural changes and MBR changes in the macula after trabeculectomy. We aimed to elucidate the relationship between structural and blood flow changes in the macular choroid as a result of IOP reduction after trabeculectomy.

Methods

Study Subjects

This was a prospective cohort study. Patients with open-angle glaucoma who underwent trabeculectomy conducted at Kanazawa University Hospital were included. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the ethical committee of Kanazawa University Hospital. Written informed consent was obtained from all patients.

The glaucoma types included primary open-angle glaucoma and exfoliation glaucoma. Cases with EX-PRESS glaucoma filtration devices were included in the study. Exclusion criteria were as follows: previous intraocular surgery except for uncomplicated cataract surgery and trabeculectomy, postoperative antiglaucoma medication, and intraocular surgeries including bleb revision or cataract surgery during the study period; fundus abnormalities other than glaucomatous optic neuropathy, including chorioretinal atrophy and retinal photocoagulation, long AL (≥27.0 mm), and postoperative follow-up period of less than 6 months. Eyes with hypotony maculopathy or choroidal detachment at any of the 1-, 3-, or 6-month study visits were also excluded. If both eyes were eligible, the first eye was included in the study. Standard trabeculectomy with mitomycin C was performed with a fornix-based conjunctival flap and a half-thickness scleral flap. The EX-PRESS devices were used at the discretion of the surgeon. The postoperative use of topical levofloxacin hydrate and betamethasone sodium phosphate was tapered as needed.

Study Examinations

Preoperatively, each patient underwent a comprehensive ophthalmic examination including best-corrected visual acuity, autorefractometry, slit-lamp examination, IOP measurement using Goldman applanation tonometry, gonioscopy, dilated fundus examination, AL measurement using time-domain OCT (OA-1000; Tomey Corporation, Nagoya, Japan), visual field testing using a Humphrey field analyzer (Carl Zeiss Meditec, Dublin, CA) with the 24-2 Swedish Interactive Threshold Algorithm.

EDI-OCT of the macula was performed using the RS 3000 Advance Retina Scan (Nidek Inc., Gamagori, Aichi, Japan). Choroidal images were

derived from 6 mm horizontal line scans through the fovea and averaged 50 times. Postoperative scans were performed at the same retinal location as the baseline, using a follow-up image capture function. The choroidal structure was analyzed semiautomatically using the EyeGroud software, which showed highly reproducible measurement results of choroidal structure intra-rater and inter-rater agreement of 0.998 to 0.999 and 0.974 to 0.997 for the total, luminal, or stromal areas of the choroid, respectively).[18] Using the software, the manual procedures were selection of three representative vascular lumens in Haller's layer of the OCT image that are most darkly seen, and segmentation of the chorioscleral border. Then, the entire choroidal area, luminal area, and stromal area within a 1500-µm-wide area centered on the fovea were calculated separately by the software after automatic binarization using the Niblack method. The mean CT (mCT), mean choroidal vascular thickness (mCVT), and mean choroidal interstitial thickness (mCIT) were determined by dividing the entire choroidal area, luminal area, and stromal area by 1500 µm. OCT scans with low signal strength index (<6) or low-quality choroidal images (i.e., chorioscleral border is not distinctly seen)[19] were excluded from the analysis.

Details of the principles and procedures of LSFG (LSFG-NAVI, Softcare Ltd, Kyushu, Japan) have been described previously.[20–22] MBRs are determined by the blurring of the speckle pattern generated by the interference of reflected light from the movement of blood cells in the ocular fundus, representing the relative velocity of blood flow. Continuous MBR images acquired at a rate of 30 frames per second over 4 seconds were averaged over cardiac cycles to produce a composite color map of MBR using the LSFG analyzer software (version 3.1.68.2; Softcare Ltd, Kyushu, Japan). After mydriasis with 0.4% tropicamide, LSFG images of the macula were consecutively captured three times. To exclude large retinal vessels from the analytical area of the LSFG when measuring macular choroidal blood flow, the mean MBR value in the square area centered on the fovea (300 × 300 pixels corresponding with 7.1° × 7.1°) was used for the subsequent analysis. The same square analytical area was set at the same retinal location as the baseline in postoperative MBR images using the LSFG analyzer software. MBR images with artifacts owing to suboptimal focusing were excluded from the analysis.

Blood pressure (BP) was measured using an automated sphygmomanometer. OPP was calculated as follows: OPP = 2/3 mean arterial pressure (MAP) – IOP, where MAP = diastolic BP + 1/3 (systolic BP – diastolic BP). The pulse rate was extracted from the LSFG analysis.

The IOP, AL, BP, EDI-OCT, and LSFG measurements were performed within 1 hour at baseline and 1, 3, and 6 months postoperatively. Baseline measurements were usually scheduled in the evening on the first day of hospitalization for trabeculectomy. Postoperative measurements were usually performed from morning to noon in an outpatient setting. Given that variables such as IOP, MAP, CT,[23–26] and choroidal blood flow[2,27] may show diurnal variation, the difference in the timing of the OCT measurement from baseline was adopted as a possible confounding variable for the statistical analyses.

Statistical Analysis

Longitudinal changes in IOP, MAP, OPP, pulse rate, AL, mCT, mCVT, mCIT, and MBR were examined using mixed-effects models. Patient-specific random intercepts and slopes accounting for repeated measurements were incorporated into mixed-effects models. To compare postoperative changes between different parameters, percent changes were used. Postoperative changes (%) in IOP, MAP, OPP, pulse rate, AL, mCT, mCVT, mCIT, and MBR were compared in scatter plots. The significance of the correlation between the two variables was determined using mixed-effects models. Various factors including sex, glaucoma type, age, systemic hypertension, baseline AL, AL changes, baseline pulse rate, changes in pulse rate, preoperative medication scores, and preoperative use of brimonidine or bunazosin were tested as potential determinants of postoperative changes (%) of mCT, mCVT, mCIT, and MBR in the univariate mixed-effects models. Categorical variables with infrequent occurrences ($n < 5$) were not evaluated. Multivariate mixed-effects models were created with variables with a P value of less than 0.2 in the univariate analysis and selected variables. The final model was created using backward selection by successively removing variables with least-significant effects until only those variables with significant effects remained,[28] selected variables were retained in the models regardless of the P values. The difference in the OCT measurement time of the day from baseline was entered into the models as a possible confounder. The Akaike information criterion and Bayesian information criterion were used to determine the model with an optimal balance of the fit of the model and model complexity. Statistical analyses were performed using STATA 16.0, for Windows (StataCorp, College Station, TX).

Results

Among the 46 eyes of 46 patients enrolled in this study, 16 eyes with no eligible OCT or LSFG images at baseline or postoperative visits owing to image exclusion criteria were omitted (OCT, 3 eyes; LSFG, 11 eyes; both, 2 eyes). Consequently, data from 30 eyes of 30 patients were analyzed. The baseline patient characteristics are shown in Table 1. OCT and LSFG images taken at baseline and at 1, 3, and 6 months from a representative case are shown in Figure 1. The longitudinal changes in IOP, MAP, OPP, pulse rate, AL, MBR, mCT, mCVT, and mCIT are shown in Figure 2. The postoperative decrease in the IOP (45%–51%) and AL (0.5%–0.8%) and increases in the OPP (34%–38%), mCT (16%–19%), mCVT (16%–20%), mCIT (17%–20%), and MBR (22%–25%) were significant at each postoperative time point (all $P < 0.001$) (Table 2). The MAP and pulse rate remained stable during the study period. Differences in the timing of the OCT measurement from baseline were significant at each postoperative time point (all $P < 0.001$).

Figure 3 illustrates the scatter plots showing the relationship of postoperative change between the two variables. The IOP change correlated significantly with AL change, mCT change, mCVT change, and mCIT change, but not with MBR change ($P = 0.04$, $P = 0.005$, $P = 0.009$, $P = 0.03$, and $P = 0.10$, respectively). The OPP change correlated significantly with mCT change, mCVT change, and MBR change, but not with AL change and mCIT change ($P = 0.01$, $P = 0.001$, $P = 0.005$, $P = 0.87$, and $P = 0.39$, respectively). The AL change correlated significantly with mCT change and mCVT change, but not with mCIT change and MBR change ($P = 0.03$, $P = 0.02$, $P = 0.30$,

Table 1. Baseline Patient Characteristics

Age (Years)	68.6 ± 8.8 (61 to 73)
Sex (male/female, cases)	11/19
Diagnosis (POAG/XFG, eyes)	24/6
Systemic hypertension, cases (%)	15 (50.0)
Diabetes mellitus, cases (%)	1 (3.3)
Hyperlipidemia (%)	4 (13.3)
Smokers (%)	3 (10.0)
AL (mm)	24.1 ± 1.2 (23.3 to 25.2)
Spherical equivalent refractive error (diopters)	−2.0 ± 2.3 (−2.8 to −0.4)
IOP (mm Hg)	18.0 ± 4.6 (15 to 20)
Mean deviation (dB)	−19.9 ± 5.7 (−24.3 to −14.2)
BP (mm Hg)	
Systolic BP	128.7 ± 24.4 (114 to 139)
Diastolic BP	70.6 ± 12.5 (63 to 80)
MAP	90.0 ± 15.7 (80 to 102)
OPP (mm Hg)	42.0 ± 10.7 (34 to 49)
Pulse rate (beats/min)	72.5 ± 11.5 (63 to 82)
No. of preoperative antiglaucoma medications (range)	4.2 ± 0.8 (4 to 6)
Prostaglandin analogues (%)	30 (100)
β-Antagonists (%)	28 (93.3)
Carbonic anhydrase inhibitors (%)	27 (90.0)
α-1 Antagonist (bunazosin, %)	10 (33.3)
α-2 Agonist (brimonidine, %)	24 (80.0)
Rho kinase inhibitor (ripasudil, %)	3 (10.0)
Previous intraocular surgeries, eyes (%)	
Cataract surgery	8 (26.7)
Trabeculotomy	2 (6.7)
Laser trabeculoplasty	3 (10.0)

Unless otherwise indicated, data are expressed as the mean ± standard deviation (interquartile range). POAG, primary open-angle glaucoma; XFG, exfoliation glaucoma.

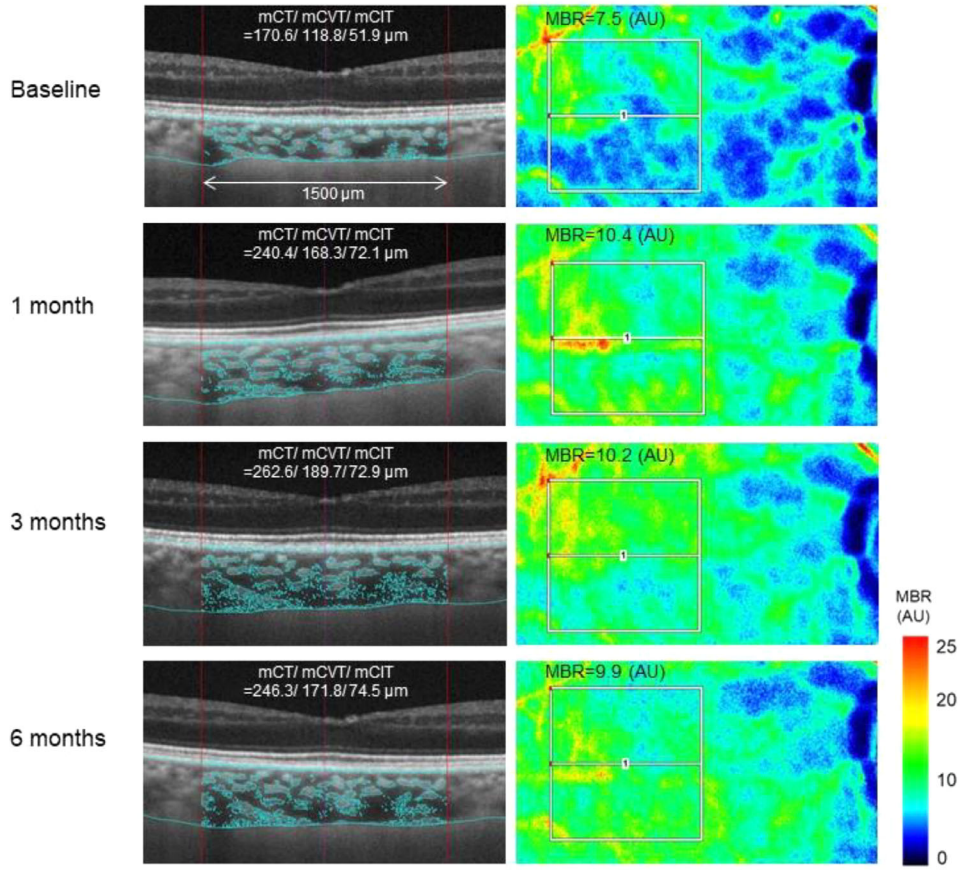

Figure 1. Representative OCT and LSFG images in the macula of the same eye at different time points. (*Left*) Horizontal OCT b scan images showing the area of CT measurements (width of 1500 µm centered on the fovea). The demarcated regions represent the choroidal stromal area, and the area outside the demarcated regions represents the choroidal vascular area. (*Right*) Color-coded MBR maps of LSFG. Square measurement regions (300 × 300 pixels) were set in the central macula. Measurements in postoperative OCT and LSFG images were performed at the same retinal location as the baseline. The mCT in both mCVT and mCIT and MBR increased at all postoperative time points.

and $P = 0.98$, respectively). MBR changes correlated significantly with mCT changes and mCVT changes, but not with mCIT changes ($P < 0.001$, $P < 0.001$, and $P = 0.11$, respectively).

The factors associated with postoperative changes in the mCT, mCVT, mCIT, and MBR were also explored. In the univariate analysis (Table 3), sex and preoperative brimonidine use were significant factors for all CT parameters and MBR. Female sex and preoperative brimonidine use were associated with less increase in CT and MBR. Larger AL shortening and higher baseline IOP were significantly associated with increased mCT and mCVT. A higher preoperative medication score was significantly associated with a lower increase in mCT and mCIT.

To determine which of the two models (i.e., with IOP changes and MAP changes or OPP changes as independent variables) had superior goodness of fit for changes in CT parameters and MBR, Akaike information criterion, and Bayesian information criterion were compared between models with either IOP changes and MAP changes or OPP changes as independent variables (Supplementary Tables S1, S2). IOP changes and MAP changes were better for mCT and mCIT changes, whereas OPP changes were better for mCVT and MBR. The variables in the multivariate analysis were selected accordingly.

The results of multivariate analysis for factors associated with changes in CT parameters and MBR are shown in Tables 4 and 5. Sex was a significant factor associated with mCT and mCVT changes. Moreover, males had greater increases in mCT and mCVT. Greater AL shortening correlated with increased mCVT, whereas greater preoperative medication scores were significantly associated with a lesser mCIT increase. A greater MBR increase correlated with a greater increase in the mCT and mCVT, but not in the mCIT. For the MBR change, earlier timing of the

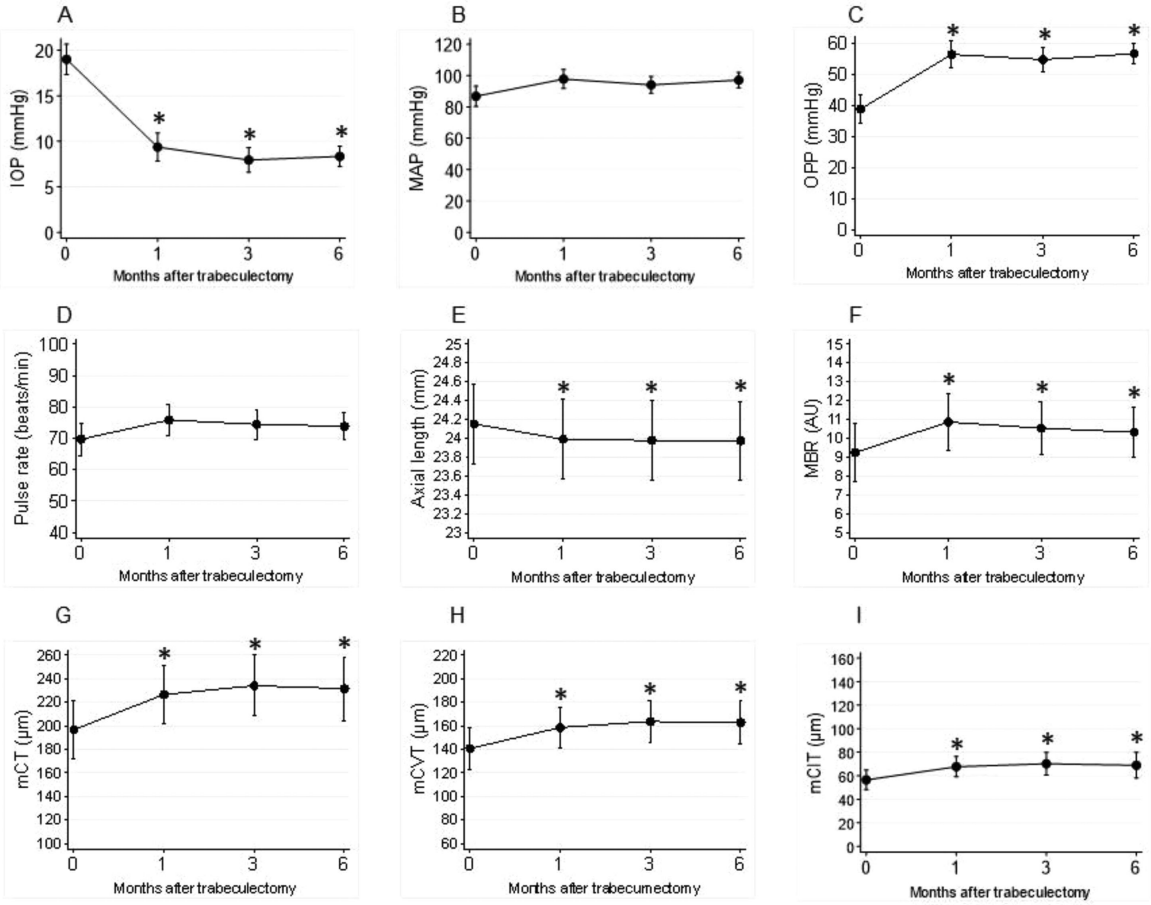

Figure 2. Longitudinal changes in the various parameters. Measurements were performed at baseline and 1, 3, and 6 months postoperatively. (A) IOP (mm Hg). (B) MAP (mm Hg). (C) OPP (mm Hg). (D) Pulse rate (beats/min). (E) AL (mm). (F) MBR (AU). G: mCT (μm). (H) mCVT(μm). (I) mCIT (μm). The estimated marginal means ± standard error were derived from mixed-effects models with patient-specific random intercepts and slopes, accounting for repeated measurements. Differences in the timing of the OCT measurement from baseline were entered into the models as a possible confounder. *Bonferroni-corrected $P < .05$ compared with baseline values.

OCT measurement compared with baseline or greater baseline mCT were associated with an increase in the MBR, regardless of the selection of CT-change parameters as an independent variable. Preoperative brimonidine use was significantly associated with a lower increase in MBR.

Discussion

Although an increase in macular CT along with a decrease in IOP after trabeculectomy seemed to be consistent in many studies,[6,8–12] few studies have addressed choroidal blood flow changes in the macula.[14] Berisha et al.[14] showed that a postoperative increase in the pulsatile choroidal blood flow significantly correlated with changes in the OPP, but not the IOP, which is consistent with MBR changes in our study. Given that the OPP is determined by the difference between the MAP and the IOP, the effects of the IOP change on choroidal blood flow may be offset by changes in BP, especially when the magnitude of the IOP decrease is small.

It is plausible that an increase in choroidal blood flow may cause choroidal thickening after trabeculectomy. To date, the exact relationship between the changes in CT and blood flow has not been demonstrated. In this study, we compared choroidal structural changes quantified by EDI-OCT with image binarization and blood flow changes as indicated by MBR from LSFG in the macula after trabeculectomy. The novel finding was that changes in the thickness of the intraluminal and extraluminal parts of the choroid were closely associated with changes in blood flow parameters (i.e., OPP and MBR) and the IOP, although both parts thickened comparably after the decrease in the IOP by trabeculectomy. Our results indicate that

Table 2. Baseline Values and Postoperative Changes of Various Parameters After Trabeculectomy

	Baseline	1 Month	3 Months	6 Months	*P* Value (1, 3, 6 Months vs. Baseline)
IOP, mm Hg	18.0 ± 4.6 (15 − 20)	−8.5 ± 0.9 (−45.0 ± 3.9%)	−9.7 ± 0.9 (−51.3± 3.6%)	-9.5 ± 0.9 (−50.3 ± 3.4%)	<0.001, <0.001, <0.001
MAP, mm Hg	90.0 ± 15.7 (80–102)	+5.9 ± 2.4 (+7.4 ± 2.8%)	+3.1 ± 2.6 (+5.1 ± 2.8%)	+5.2 ± 3.3 (+8.0 ± 3.7%)	0.08, 1.0, 0.69
OPP, mm Hg	42.0 ± 10.7 (34–49)	+12.6 ± 1.9 (+34.2 ± 6.0%)	+12.0 ± 2.0 (+34.8 ± 5.6%)	+12.8 ± 2.3 (+37.5 ± 6.5%)	<0.001, <0.001, <0.001
Pulse rate, beats/min	72.5 ± 11.5 (63–82)	+2.2 ± 1.9 (+3.9 ± 2.8%)	+0.7 ± 2.0 (+1.9 ± 2.8%)	0.002 ± 2.0 (+0.8 ± 2.8%)	1.0, 1.0, 1.0
AL, mm	24.1 ± 1.2 (23.3–25.2)	−0.12 ± 0.02 (−0.5 ± 0.1%)	−0.15 ± 0.02 (−0.6 ± 0.1%)	−0.19 ± 0.02 (−0.8 ± 0.1%)	<0.001, <0.001, <0.001
mCT, µm	196.3 ± 63.5 (147.3–235.4)	+31.1 ± 5.5 (+16.1 ± 3.0%)	+37.4 ± 5.4 (+19.2 ± 2.9%)	+35.4 ± 5.3 (+18.9 ± 2.8%)	<0.001, <0.001, <0.001
mCVT, µm	138.5 ± 44.8 (107.0–169.5)	+21.4 ± 4.2 (+15.6 ± 3.2%)	+26.2 ± 4.1 (+19.4 ± 3.1%)	+25.2 ± 4.1 (+19.9 ± 3.1%)	<0.001, <0.001, <0.001
mCIT, µm	57.7 ± 21.8 (40.3–68.7)	+9.9 ± 2.0 (+18.2 ± 3.7%)	+11.2 ± 2.0 (+19.7 ± 3.4%)	+10.1 ± 2.0 (+17.3 ± 2.9%)	<0.001, <0.001, <0.001
MBR, AU	9.0 ± 3.9 (6.4–11.1)	+1.9 ± 0.3 (+22.1 ± 5.5%)	+1.6 ± 0.3 (+24.9 ± 5.4%)	+1.4 ± 0.4 (+21.6 ± 5.3%)	<0.001, <0.001, <0.001
Differences in OCT measurement time of the day from baseline (h)	NA	−5.0 ± 0.5	−4.7 ± 0.5	−3.9 ± 0.5	<0.001, <0.001, <0.001

Baseline values are shown as the mean ± standard deviation (interquartile range). Postoperative changes were examined with mixed-effects models with patient-specific random intercepts and slopes accounting for repeated measurements, and are shown as estimated marginal means ± standard error. Differences in OCT measurement time of the day from baseline was entered into the models as a possible confounder. *P* values were adjusted for multiple comparisons by the Bonferroni correction.

AU, arbitrary unit; NA, not applicable.

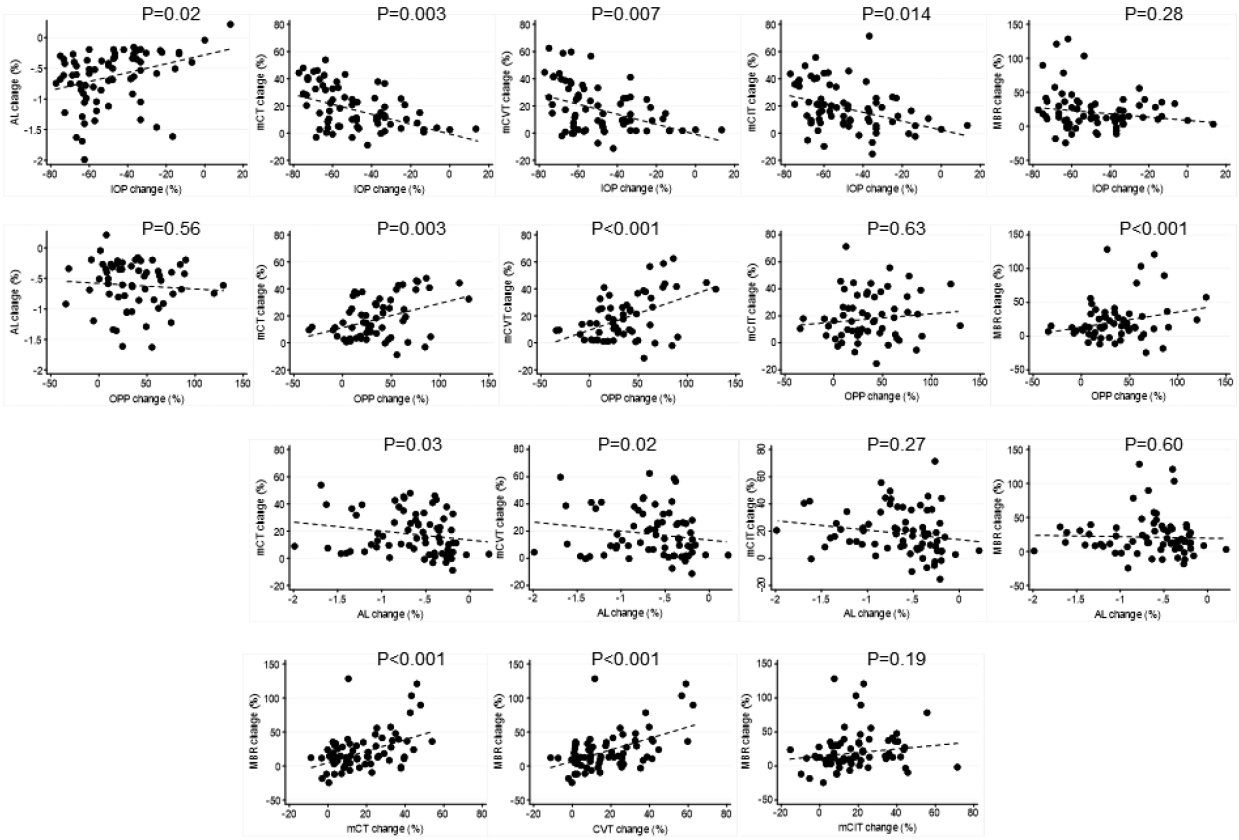

Figure 3. Scatter plots showing the relationship between two variables of postoperative changes (%). All data at 1, 3, and 6 months are combined. (A–E) AL, mCT, mCVT, mCIT, and MBR changes (%) versus IOP changes (%), respectively. (F–J) AL, mCT, mCVT, mCIT, and MBR changes (%) versus OPP changes (%), respectively. (K–N) mCT, mCVT, mCIT, and MBR changes (%) versus AL changes (%), respectively. (O–Q) mCT, mCVT, and mCIT changes (%) versus MBR changes (%), respectively. *P* values are derived from univariate mixed-effects models with patient-specific random intercepts and slopes accounting for repeated measurements. Differences in the timing of the OCT measurement from baseline were entered into the models as a possible confounder. The dotted line indicates a regression line in each graph.

the choroid reacts to decreases in the IOP differently between the intraluminal and extraluminal areas, blood flow dependence in the vascular area, and IOP dependence in the stromal area.

As shown in Supplementary Tables S1 and S2, mCVT changes were linked more closely with changes in the OPP and MBR than IOP changes. The results are conceivable, given that thickness changes in the intraluminal part (i.e., mCVT) should reflect volume changes in the large choroidal blood vessels as per the hemodynamic status. In contrast, the primary determinant of the mCIT change, namely, the thickness change of the extraluminal part corresponding with the stromal area, seemed to be the direct effect of the IOP, but not the dynamic changes in choroidal blood flow. The mechanism of stromal expansion by the decrease in the IOP may be fluid accumulation from the choroidal capillaries owing to decreased hydrostatic pressure in the stromal area, which is in common with choroidal effusion. Although eyes with hypotony maculopathy or choroidal detachment were excluded from this study, subclinical stromal changes may emerge in the macular choroid after IOP reduction.

The difference in thickness changes between the vascular and stromal areas of the choroid has been addressed by two groups. The first report by Zhang et al.[12] showed that the increase in the thickness of the vessel and stromal areas was comparable up to 6 months after trabeculectomy. In contrast, Kojima et al.[13] reported that the thickness in the vessel area increased at 2 weeks, but returned to the preoperative level at 1 year, whereas the thickness increase in the stromal area persisted until 1 year. Our results did not contradict previous reports. The percent increase in thickness from baseline was comparable between the mCVT and mCIT, which remained stable from 1 month to 6 months postoperatively.

Table 3. Univariate Analysis of Various Factors for Postoperative Changes of mCT, mCVT, mCIT, and MBR

Independent Variables	mCT Change (%) Coefficient (SE), *P* Value	mCVT Change (%) Coefficient (SE), *P* value	mCIT Change (%) Coefficient (SE), *P* Value	MBR Change (%) Coefficient (SE), *P* Value
Baseline values of dependent variable, μm or AU	−0.02 (0.04), 0.61	−0.07 (0.06), 0.30	0.06 (0.14), 0.68	−3.2 (1.0), 0.002
Sex, male vs. female	14.3 (4.9), 0.004	14.2 (5.4), 0.009	13.2 (5.6), 0.02	18.4 (8.7), 0.03
Glaucoma type, POAG vs. XFG	−6.9 (6.5), 0.28	−9.6 (7.0), 0.17	0.9 (7.2), 0.90	−13.1 (10.7), 0.22
Age, years	0.40 (0.29), 0.17	0.42 (0.32), 0.196	0.32 (0.32), 0.31	0.12, (0.51), 0.81
Systemic hypertension	−6.6 (5.2), 0.202	−6.0 (5.7), 0.29	−5.9 (5.7), 0.31	7.9 (8.8), 0.37
Baseline AL, mm	1.1 (2.3), 0.63	0.33 (2.5), 0.89	1.9 (2.4), 0.44	−7.0 (3.6), 0.051
AL change, (%)	−5.6 (2.6), 0.03	−6.8 (2.9), 0.02	−3.5 (3.4), 0.30	−0.16 (6.7), 0.98
Baseline PR, beats/min	−0.23 (0.23), 0.31	−0.25 (0.25), 0.31	−0.18 (0.25), 0.47	0.26 (0.38), 0.49
PR change (%)	0.11 (0.09), 0.22	0.11 (0.09), 0.22	0.14 (0.11), 0.18	−0.06 (0.21), 0.78
Baseline mean deviation, dB	−0.42 (0.47), 0.37	−0.41 (0.51), 0.42	−0.32 (0.51), 0.52	−0.70 (0.78), 0.37
Preoperative medication score	−7.4 (3.3), 0.02	−6.3 (3.7), 0.09	−9.4 (3.5), 0.007	−5.5 (5.9), 0.35
Preoperative brimonidine	−16.8 (5.9), 0.004	−18.1 (6.4), 0.005	−16.1 (6.9), 0.02	−28.3 (9.7), 0.003
Preoperative bunazosin	6.2 (5.5), 0.26	6.3 (6.0), 0.30	4.5 (6.1), 0.46	3.2 (9.3), 0.74
Pseudophakia	3.0 (6.0), 0.61	4.2 (6.5), 0.52	3.4 (6.5), 0.60	9.9 (9.8), 0.31
Differences in OCT measurement time of the day from baseline (h)	0.64 (0.54), 0.23	0.51 (0.60), 0.40	0.14 (0.85), 0.87	−1.8 (1.3), 0.16
Baseline IOP (mm Hg)	1.2 (0.5), 0.02	1.6 (0.6), 0.005	0.60 (0.63), 0.34	1.6 (0.9), 0.08
IOP change (%)	−0.20 (0.07), 0.005	−0.21 (0.08), 0.009	−0.20 (0.09), 0.03	−0.28 (0.17), 0.10
MAP change (%)	0.12 (0.10), 0.24	0.21 (0.11), 0.045	−0.08 (0.12), 0.48	0.49 (0.23), 0.04
Baseline OPP (mm Hg)	−0.25 (0.25), 0.32	−0.35 (0.27), 0.19	0.11 (0.27), 0.68	−0.58 (0.40), 0.15
OPP change (%)	0.14 (0.06), 0.01	0.21 (0.06), 0.001	0.07 (0.08), 0.39	0.36 (0.13), 0.005
Baseline mCT (μm)	−0.02 (0.04), 0.61	−0.03 (0.05), 0.55	0.01 (0.05), 0.81	0.14 (0.07), 0.052
mCT change (%)	NA	1.05 (0.04), <0.001	0.89 (0.09), <0.001	0.85 (0.22), <0.001
mCVT change (%)	0.86 (0.03), <0.001	NA	0.53 (0.11), <0.001	0.85 (0.19), <0.001
mCIT change (%)	0.62 (0.06), <0.001	0.46 (0.09), <0.001	NA	0.31 (0.21), 0.13
Baseline MBR (AU)	−1.1 (0.7), 0.08	−1.3 (0.7), 0.08	−0.79 (0.74), 0.29	−3.2 (1.0), 0.002
MBR change (%)	0.17 (0.05), <0.001	0.21 (0.05), <0.001	0.10 (0.06), 0.11	NA

The coefficients and *P* value values were derived from univariate mixed-effects models with patient-specific random intercepts and slopes accounting for repeated measurements. Differences in OCT measurement time of the day from baseline was entered into the models as a possible confounder. AU, arbitrary unit; NA, not applicable; POAG, primary open-angle glaucoma; PR, pulse rate; SE, standard error; XFG, exfoliation glaucoma.

We showed that several factors had a significant influence on postoperative changes in the CT and MBR. First, males had greater increases in the mCT and mCVT, adjusting for changes in the IOP, OPP, or MBR, although previous studies reported negative findings for intersex differences.[8,9,12] The CT or volume in healthy subjects was greater in men than that in women after adjusting for age and AL.[29,30] However, the intersex difference in the CT was insignificant in patients with glaucoma in the study by Maul et al.,[19] and the same as the baseline values in the current study (Supplementary Table S3). Thus, the intersex differences in the CT at baseline or its postoperative changes may differ depending on glaucoma status or other factors.

A significant relationship between AL shortening and choroidal thickening has been found in multiple studies,[6,8,10] although negative findings have also been reported.[7,13] In this study, the part of choroidal area that showed thickness changes along with AL shortening appeared to be the intraluminal area (i.e., mCVT), but not the stromal area (i.e., mCIT). According to Saeedi et al.,[8] when the IOP decreases, the AL decreases (owing to scleral relaxation) and the CT increases (because the force of IOP on the choroid is lower). In our study, the mCVT change was more relevant to changes in the OPP or MBR than those of IOP changes, whereas the AL changes correlated with the IOP changes, but not with changes in the OPP or MBR. Therefore, the association between AL shortening and mCVT was not primarily owing to changes in the choroidal blood flow. The measured AL shortening may partly be caused by an inward movement of the inner border of the choroid owing to the increased mCVT.

The difference in the timing of the measurement from baseline was a significant factor for MBR changes in the multivariate analysis. Diurnal variation of the MBR in healthy subjects was reported by Iwase et al.,[27] which showed significant diurnal variations with a trough at 15:00 and a peak at 18:00 in association with BP-related parameters such as systolic BP, MAP, or OPP. Furthermore, Pemp et al.[2] reported that patients with primary open-angle glaucoma had a greater diurnal fluctuation of choroidal blood flow estimated by fundus pulsation amplitude. Therefore, it is plausible and should be noted that the difference in the timing of the measurement may affect the magnitude of MBR changes.

Although the difference in the timing of the measurement from baseline was not significantly relevant to changes in CT parameters, total CT and

Table 4. Multivariate Analysis of Various Factors for Postoperative Changes of mCT, mCVT, and mCIT

Independent Variables	mCT Change (%) Coefficient (SE), *P* Value	mCVT Change (%) Coefficient (SE), *P* Value	mCIT Change (%) Coefficient (SE), *P* Value
Baseline values of dependent variable, µm		−0.11 (0.04), 0.01	
Sex, male vs. female	10.8 (4.0), 0.007	10.4 (3.8), 0.007	
Glaucoma type, POAG vs. XFG			
Age, years			
Systemic hypertension			
Baseline AL, mm			
AL change, (%)		−7.5 (3.3), 0.02	
Baseline PR, beats/min			
PR change (%)			
Baseline mean deviation, dB			
Preoperative medication score			−7.9 (3.1), 0.01
Preoperative brimonidine			
Preoperative bunazosin			
Pseudophakia			
Differences in OCT measurement time of the day from baseline (hour)	0.85 (0.60), 0.16*	0.70 (0.61), 0.25*	0.84 (0.83), 0.31*
Baseline IOP (mm Hg)			
IOP change (%)	−0.20 (0.08), 0.008*	NA	−0.28 (0.10), 0.005*
MAP change (%)	0.08 (0.09), 0.36*	NA	−0.10 (0.11), 0.38*
Baseline OPP (mm Hg)			
OPP change (%)	NA	0.11 (0.05), 0.035*	NA
Baseline MBR (AU)			
MBR change (%)	0.13 (0.05), 0.01*	0.21 (0.05), <0.001 *	0.05 (0.07), 0.41*

The coefficients and *P* values are derived from multivariate mixed-effects models with patient-specific random intercepts and slopes accounting for repeated measurements Differences in OCT measurement time of the day from baseline was entered into the models as a possible confounder. Independent variables with $P < 0.2$ in the univariate analysis and variables marked with * were entered into the multivariate model. The final model was created by backward elimination. Variables marked with * were retained in the model regardless of the *P* values. AU, arbitrary unit; NA, not applicable; POAG, primary open-angle glaucoma; PR, pulse rate; SE, standard error; XFG, exfoliation glaucoma.

thickness in the luminal area in healthy subjects were reported to show significant diurnal variation, typically thickest at night or early in the morning and thinnest during the day.[23–26] According to Tan et al.,[24] eyes with a thin choroid (≤300 µm) had a small amplitude (10.5 ± 7.4 µm) in diurnal variation. Given that the baseline average mCT in our study was far thinner (<200 µm) than the thin choroid in the study by Tan et al., a relatively flat diurnal variation in the thin choroid in eyes with glaucoma may be the reason for its insignificant influence on CT changes after trabeculectomy.

Preoperative antiglaucoma medication was significantly associated with changes in the CT parameters and MBR in univariate analyses and a few multivariate models. The finding that preoperative brimonidine use correlated with less postoperative increase in MBR may suggest that an increase in MBR owing to brimonidine use might have existed at baseline. However, the baseline MBR was not associated significantly with brimonidine use (Supplementary Table S3). Furthermore, topical brimonidine did not change the choroidal ocular pulse amplitude in patients with primary open-angle glaucoma.[31] Given that no patients had brimonidine monotherapy and brimonidine use correlated with higher medication scores, further studies are needed to reveal the pure effects of preoperative glaucoma medication on MBR changes after trabeculectomy.

Our study has several limitations, including a small sample size and biased patient characteristics, such as our Japanese-only sample. We evaluated CT in a

Table 5. Multivariate Analysis of Various Factors for Postoperative Changes of MBR

Independent Variables	MBR Change (%) Model A Coefficient (SE), *P* Value	MBR Change (%) Model B Coefficient (SE), *P* Value	MBR Change (%) Model C Coefficient (SE), *P* Value
Baseline values of dependent variable, AU	−3.0 (0.8), <0.001	−2.8 (0.8), <0.001	−3.0 (0.7), <0.001
Sex, male vs. female			
Glaucoma type, POAG vs. XFG			
Age, years			
Systemic hypertension			
Baseline AL, mm			
AL change, (%)			
Baseline PR, beats/min			
PR change (%)			
Baseline mean deviation, dB			
Preoperative medication score			
Preoperative brimonidine			−20.5 (7.9), 0.01
Preoperative bunazosin			
Pseudophakia			
Differences in OCT measurement time of the day from baseline (h)	−3.5 (1.3), 0.006*	−3.4 (1.2), 0.006*	−3.2 (1.3), 0.014*
Baseline IOP (mm Hg)			
Baseline OPP (mm Hg)			
OPP change (%)	0.31 (0.10), 0.002*	0.25 (0.10), 0.01*	0.39 (0.09), <0.001*
Baseline mCT (μm)	0.18 (0.05), <0.001	0.18 (0.05), <0.001	0.17 (0.05), 0.001
mCT change (%)	0.66 (0.21), 0.002*	NA	NA
mCVT change (%)	NA	0.73 (0.20), <0.001*	NA
mCIT change (%)	NA	NA	0.15 (0.17), 0.39*
AIC/BIC	524.9/ 545.5	521.9/ 542.5	527.4/ 550.1

The coefficients and *P* values are derived from multivariate mixed-effects models with patient-specific random intercepts and slopes accounting for repeated measurements. Differences in OCT measurement time of day from baseline was entered into the models as a possible confounder. mCT, mCVT or mCIT were entered individually into models A, B, or C, respectively. Independent variables with $P < 0.2$ in the univariate analysis and variables marked with * were entered into the multivariate model. Final model was created by backward elimination. Variables marked with * were retained in the model regardless of the *P* values. AIC and BIC are shown to indicate the model with optimal balance of model fit and model complexity.

AIC, Akaike information criterion; AU, arbitrary unit; BIC, Bayesian information criterion; NA, not applicable; POAG, primary open angle glaucoma; PR, pulse rate; SE, standard error; XFG, exfoliation glaucoma.

horizontal OCT B scan in the range of 1500 μm centered on the fovea. In contrast, the imaging area of 300 × 300 pixels in LSFG corresponds with 2.1 × 2.1 mm^2 in the Gullstrand model eye with an AL of 24.38 mm. Ideally, the choroidal structure should be evaluated using three-dimensional OCT scans in the area corresponding exactly with the measurement area of the LSFG, which is currently technically unavailable. Although the choroid in the central region is thicker and more relevant to central vision than that in the peripheral region, measurements in a wider area are needed to explore regional differences in postoperative changes. The image binarization is useful to evaluate the choroidal structure. However, it may have image artifacts. The punctate findings in the choroidal B scan images after image binarization corresponded to speckle noise, which may increase in OCT images with a low signal-to-noise ratio (Fig. 1, postoperative scans). Given that the sum of areas of punctate findings was relatively small compared with the total analytical area, the presence of punctate artifacts should not have affected the results significantly. In future studies, the effects of choroidal blood flow changes on visual function and the correlation of blood flow changes between the choroid and the optic nerve head also need to be addressed.

In summary, we compared choroidal structural changes quantified by EDI-OCT with image binarization and blood flow changes indicated by the MBR from LSFG in the macula after trabeculectomy. Thickness changes in the intraluminal and extraluminal parts of the choroid were closely associated with changes in the blood flow parameters (i.e., OPP and MBR) and the IOP, although both parts thickened comparably after IOP reduction by trabeculectomy. The novel findings indicate that the choroid reacts to IOP reduction differently between the intraluminal and extraluminal areas, blood flow dependence in the vascular area, and IOP dependence in the stromal area.

Acknowledgments

Disclosure: **Y. Takamatsu**, None; **T. Higashide,** None; **S. Takeshima,** None; **M. Sasaki,** None; **Y. Manbo,** None; **S. Udagawa,** None; **S. Ohkubo,** None; **S. Sonoda,** None; **T. Sakamoto,** None; **K. Sugiyama,** None

* YT and TH contributed equally to the work presented here and should therefore be regarded as equivalent authors.

References

1. Spraul CW, Lang GE, Lang GK, Grossniklaus HE. Morphometric changes of the choriocapillaris and the choroidal vasculature in eyes with advanced glaucomatous changes. *Vision Res.* 2002;42(7):923–932.
2. Pemp B, Georgopoulos M, Vass C, et al. Diurnal fluctuation of ocular blood flow parameters in patients with primary open-angle glaucoma and healthy subjects. *Br J Ophthalmol*. 2009;93(4):486–491.
3. Marangoni D, Falsini B, Colotto A, et al. Subfoveal choroidal blood flow and central retinal function in early glaucoma. *Acta Ophthalmol*. 2012;90(4):e288–294.
4. Tepelus TC, Song S, Borrelli E, et al. Quantitative analysis of retinal and choroidal vascular parameters in patients with low tension glaucoma. *J Glaucoma.* 2019;28(6):557–562.
5. Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. *Am J Ophthalmol*. 1998;126(4):487–497.
6. Kara N, Baz O, Altan C, Satana B, Kurt T, Demirok A. Changes in choroidal thickness, axial length, and ocular perfusion pressure accompanying successful glaucoma filtration surgery. *Eye (Lond)*. 2013;27(8):940–945.
7. Usui S, Ikuno Y, Uematsu S, Morimoto Y, Yasuno Y, Otori Y. Changes in axial length and choroidal thickness after intraocular pressure reduction resulting from trabeculectomy. *Clin Ophthalmol*. 2013;7:1155–1161.
8. Saeedi O, Pillar A, Jefferys J, Arora K, Friedman D, Quigley H. Change in choroidal thickness and axial length with change in intraocular pressure after trabeculectomy. *Br J Ophthalmol*. 2014;98(7):976–979.
9. Yoshikawa M, Akagi T, Nakanishi H, et al. Longitudinal change in choroidal thickness after trabeculectomy in primary open-angle glaucoma patients. *Jpn J Ophthalmol*. 2017;61(1):105–112.
10. Kadziauskiene A, Kuoliene K, Asoklis R, Lesinskas E, Schmetterer L. Changes in choroidal thickness after intraocular pressure reduction following trabeculectomy. *Acta Ophthalmol*. 2016;94(6):586–591.
11. Bouillot A, Pierru A, Blumen-Ohana E, Brasnu E, Baudouin C, Labbé A. Changes in choroidal thickness and optic nerve head morphology after filtering surgery: nonpenetrating deep sclerectomy versus trabeculectomy. *BMC Ophthalmol*. 2019;19(1):24.
12. Zhang X, Cole E, Pillar A, et al. The effect of change in intraocular pressure on choroidal structure in glaucomatous eyes. *Invest Ophthalmol Vis Sci.* 2017;58(7):3278–3285.
13. Kojima H, Hirooka K, Nitta E, Ukegawa K, Sonoda S, Sakamoto T. Changes in choroidal area after intraocular pressure reduction following trabeculectomy. *PLoS One.* 2018;13(8):e0201973.
14. Berisha F, Schmetterer K, Vass C, et al. Effect of trabeculectomy on ocular blood flow. *Br J Ophthalmol*. 2005;89(2):185–188.
15. Saito M, Saito W, Hashimoto Y, et al. Macular choroidal blood flow velocity decreases with regression of acute central serous chorioretinopathy. *Br J Ophthalmol*. 2013;97(6):775–780.
16. Hirooka K, Saito W, Namba K, et al. Relationship between choroidal blood flow velocity and choroidal thickness during systemic corticosteroid therapy for Vogt-Koyanagi- Harada disease. *Graefes Arch Clin Exp Ophthalmol*. 2015;253(4):609–617.

17. Akahori T, Iwase T, Yamamoto K, Ra E, Terasaki H. Changes in choroidal blood flow and morphology in response to increase in intraocular pressure. *Invest Ophthalmol Vis Sci.* 2017;58(12):5076–5085.
18. Sonoda S, Sakamoto T, Kakiuchi N, et al. Semi-automated software to measure luminal and stromal areas of choroid in optical coherence tomographic images. *Jpn J Ophthalmol*. 2018;62(2):179–185.
19. Maul EA, Friedman DS, Chang DS, et al. Choroidal thickness measured by spectral domain optical coherence tomography: factors affecting thickness in glaucoma patients. *Ophthalmology.* 2011;118(8):1571–1579.
20. Tamaki Y, Araie M, Kawamoto E, Eguchi S, Fujii H. Non-contact, two- dimensional measurement of tissue circulation in choroid and optic nerve head using laser speckle phenomenon. *Exp Eye Res.* 1995;60(4):373–383.
21. Isono H, Kishi S, Kimura Y, Hagiwara N, Konishi N, Fujii H. Observation of choroidal circulation using index of erythrocytic velocity. *Arch Ophthalmol*. 2003;121(2):225–231.
22. Takeshima S, Higashide T, Kimura M, et al. Effects of trabeculectomy on waveform changes of laser speckle flowgraphy in open angle glaucoma. *Invest Ophthalmol Vis Sci.* 2019;60(2):677–684.
23. Chakraborty R, Read SA, Collins MJ. Diurnal variations in axial length, choroidal thickness, intraocular pressure, and ocular biometrics. *Invest Ophthalmol Vis Sci.* 2011;52(8):5121–5129.
24. Tan CS, Ouyang Y, Ruiz H, Sadda SR. Diurnal variation of choroidal thickness in normal, healthy subjects measured by spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci.* 2012;53(1):261–266.
25. Usui S, Ikuno Y, Akiba M, et al. Circadian changes in subfoveal choroidal thickness and the relationship with circulatory factors in healthy subjects. *Invest Ophthalmol Vis Sci.* 2012;53(4):2300–2307.
26. Kinoshita T, Mitamura Y, Shinomiya K, et al. Diurnal variations in luminal and stromal areas of choroid in normal eyes. Br J Ophthalmol. 2017;101(3):360–364.
27. Iwase T, Yamamoto K, Ra E, Murotani K, Matsui S, Terasaki H. Diurnal variations in blood flow at optic nerve head and choroid in healthy eyes: diurnal variations in blood flow. *Medicine (Baltimore)*. 2015;94(6):e519.
28. Cheng J, Edwards LJ, Maldonado-Molina MM, Komro KA, Muller KE. Real longitudinal data analysis for real people: building a good enough mixed model. *Stat Med.* 2010; 29: 504–520.
29. Li XQ, Larsen M, Munch IC. Subfoveal choroidal thickness in relation to sex and axial length in 93 Danish university students. *Invest Ophthalmol Vis Sci.* 2011;52(11):8438–8441.
30. Barteselli G, Chhablani J, El-Emam S, et al. Choroidal volume variations with age, axial length, and sex in healthy subjects: a three-dimensional analysis. *Ophthalmology.* 2012;119(12):2572–2578.
31. Schmidt KG, Klingmüller V, Gouveia SM, Osborne NN, Pillunat LE. Short posterior ciliary artery, central retinal artery, and choroidal hemodynamics in brimonidine-treated primary open-angle glaucoma patients. *Am J Ophthalmol.* 2003;136(6):1038–1048.