

Changes in Pulse Waveforms in Response to Intraocular Pressure Elevation Determined by Laser Speckle Flowgraphy in Healthy Subjects

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Abstract

Background: To determine whether autoregulation of the blood flow is present in the blood flow on the optic nerve head (ONH) by intraocular pressure (IOP) elevations using pulse waveform parameters determined by laser speckle flowgraphy (LSFG) in normal subjects.

Methods: We conducted this prospective cross sectional study at the Nagoya University Hospital. An ophthalmodynamometer was pressed on the sclera to increase the IOP by 20 mmHg or 30 mmHg for 1 min (Experiment 1, 16 subjects) and by 30 mmHg for 10 min (Experiment 2, 10 subjects). The mean blur rate (MBR) and the eight pulse waveform parameters determined by LSFG were measured before, immediately after and during an IOP elevation, and after the IOP returned to the baseline pressure.

Results: A significant elevation of the IOP and a significant reduction in the ocular perfusion pressure (OPP) were found after applying the ophthalmodynamometer (both, $P < 0.001$). The blowout score (BOS) ($P < 0.001$) was significantly reduced, and the flow acceleration index (FAI; $P < 0.01$) and resistivity index (RI; $P < 0.001$) was significantly increased immediately after increasing the IOP by 20 or 30 mmHg (Experiment 1). During the IOP elevation throughout the 10 min, the BOS and the RI significantly recovered to the baseline at time 10 compared to time 0, ($P < 0.001$ and $P = 0.008$, respectively) (Experiment 2).

Conclusions: Our results indicate that the blood flow on the ONH is autoregulated by several mechanisms for changes in the OPP induced by an elevation of the IOP in normal subjects.

Introduction

Autoregulation plays an important role in controlling the blood flow in various organs and a fully functioning autoregulatory system can adjust the blood flow to supply a constant amount of oxygen and nutrients to maintain organ function [1]. A dysfunction of autoregulation has been reported to be the cause of various ocular diseases including glaucoma [1–3]. Thus, it is very important to investigate the autoregulation of ocular blood flow under different situations and different ocular diseases.

One method to study autoregulation has been to elevate the intraocular pressure (IOP) artificially and thereby reduce the ocular perfusion pressure (OPP). This reduced OPP should then result in a reduction of the ocular blood flow. However, earlier studies have shown that slight change in the OPP did not alter the blood flow significantly [4]. This was then good evidence for an autoregulatory mechanism in the ocular circulatory system.

The effect of changes in the IOP on the ocular blood flow has been investigated by various methods mainly in laboratory experiments on different animal species [1, 5–7], and the results have shown that the blood supply to the optic nerve head (ONH) was well autoregulated to maintain a constant blood flow in spite of changes in the IOP and OPP [8–13]. However, a good model to study the effect of changes in the IOP on the ocular blood flow has not been established for humans although the use of a suction cup and laser Doppler flowmetry has been used [8, 14]. This method involves attaching a suction cup to the sclera

and inducing an elevation of the IOP by applying a negative pressure. This technique has been used to study autoregulation in normal humans, but there are several limitations for this technique including a difficulty of applying continuous stable pressure.

Earlier, we established a testing protocol that was used to examine the response of blood flow on the ONH induced by changes in the OPP induced by an artificial elevation of the IOP [15, 16]. This method elevated the IOP continuously by applying pressure on the eye with an ophthalmodynamometer, and we found that it was a stable and reproducible.

Laser speckle flowgraphy (LSFG, Softcare, Fukutsu, Japan) can be used to evaluate the ocular blood flow non-invasively and quickly, and it is a reliable and reproducible technique [17–20]. An update of the software embedded in the most recent LSFG analyzer (LSFG Analyzer, v. 3.1.6;) has allowed us to record synchronized images from each cardiac cycle and determine various pulse waveform parameters which can be a new biomarker for detecting and evaluating vascular diseases. The results of earlier studies have shown that the relationship between these waveform parameters and other variables e.g., age [21–24], mean intima–media thickness [25], and normal-tension glaucoma [21]. Accordingly, these pulse waveform parameters should be able to provide new information about the ONH autoregulation in response to OPP changes induced by artificial IOP elevation.

Thus, the purpose of this study was to determine whether the blood flow on the ONH is autoregulated in normal subjects. To accomplish this, we measured different parameters of the pulse waveform determined by LSFG before, immediately after an elevation of the IOP, and after the IOP was returned to the baseline level.

Methods

All volunteers were asked to abstain from alcoholic and caffeinated beverages on the morning of the examination. The pupil was dilated 30 minutes before the examinations, and the subjects rested in a quiet dark room for 10 to 15 min before the measurements to achieve stable hemodynamics conditions. All examinations were performed in the sitting position at approximately 12:00 h to avoid diurnal variations [26, 27]. The axial lengths were measured by partial optical coherence interferometry (IOLMaster; Carl Zeiss Meditec, La Jolla, CA), and the IOP was measured with a handheld tonometer (Icare; TiolatOy, Helsinki, Finland). The SBP and DBP were measured with an automatic sphygmomanometer (CH-483C; Citizen, Tokyo, Japan). The mean arterial blood pressure (MAP) and OPP were calculated as:

$$\text{MAP} = \text{DBP} + 1/3(\text{SBP}-\text{DBP});$$

$$\text{OPP} = 2/3\text{MAP} - \text{IOP}.$$

Exclusion criteria

Eyes were excluded if the best-corrected visual acuity was less than 20/20, history of ophthalmic or systemic disorders including glaucoma, diabetes, hypertension, and arrhythmia, history of treatment, e.g., ocular laser or incisional surgery in the experimental eye, systolic blood pressure (SBP) more than 150 mmHg, diastolic blood pressure (DBP) more than 90 mmHg, axial length more than 27.0 mm, and medical conditions that could influence the hemodynamics of the eye, e.g., vascular diseases, and a regular smoking habit.

Experimental Elevation of IOP

An ophthalmodynamometer (Inami, Tokyo, Japan) was used to apply pressure on the eye to increase IOP after topical anesthesia (0.4% Benoxil ophthalmic solution; Santen pharmaceutical co. ltd, Osaka, Japan). The device was pressed perpendicularly to the globe to make a fixed external pressure on the sclera [15]. Only the right eye was used for the experiment. The scale on ophthalmodynamometer showed the force applied to the eye for increasing IOP. The IOP was measured during the application of the pressure with the Icare tonometer (Icare®; Tiolat Oy, Helsinki, Finland) before, during, and after the application of the pressure.

Experiment 1

One experimenter placed the ophthalmodynamometer on the temporal sclera and another experimenter recorded the LSFSG images (Fig. 1). The IOP was increased by 20 mm Hg or 30 mmHg from the baseline for 1 minute. The LSFSG images were recorded at 1 minute before the IOP elevation, 1 min after the elevation of the IOP, and at 20 minutes after the release of the pressure (Fig. 2).

Experiment 2

The IOP was elevated by 30 mmHg from the baseline for 10 minutes using completely different subjects. We recorded the LSFSG images before, immediately after the IOP elevation (time 0), and at 1 min (time 1), 3 min (time 3), 5 min (time 5), 7 min (time 7), and 10 min (time 10) while the IOP was elevated. In addition, we recorded the LSFSG images at 1 min (time 11), 3 min (time 13), and 5 min (time 15) after the release of the pressure on the eye (Fig. 2).

Laser speckle flowgraphy (LSFG)

The principles of LSFG have been described in detail [28–31]. The LSFG analyzer software separates the vascular and the tissue area. We analyzed only the tissue area of the ONH for the pulse waveform analysis. In addition, the LSFG Analyzer software enables the recordings of synchronized images from each cardiac cycle (Fig. 1B, C) and determines the values of various heartbeat waveform parameters. We evaluated eight pulse waveform parameters (Fig. 3). The calculation of the pulse waveform parameters has been described in detail [20]. The LSFG was measured 2 times at each time point in all of the eyes. The average of values was used for the statistical analyses.

Statistical analyses

We evaluated the pulse waveforms using a linear mixed model to incorporate possible correlations between repeated measured values of the parameters for each eye over time within a subject, which was the same statistical method as previously reported [15][16]. Briefly, we assumed the following model,

$$y_{ij} = a_i + f(tj:b) + \varepsilon_{ij}$$

i (subject) = 1, ..., 10, j (time) = before, 0, 1, 3, 5, 7, 10, 11, 13, 15 (min) where y_{ij} is the pulse waveform parameters at time j of subject i and a_i is a subject-specific random effect. The function $f(tj:b)$, which represents a fixed effect of time on the refraction, was specified as a polynomial function. The b parameters represent the fixed time effects and interaction between the time and group effects, respectively. The order of polynomials in $f(tj:b)$ was selected on the basis of the Akaike information criteria.

The level for statistical significance was 0.05. The statistical analyses were performed with SAS9.3 MIXED procedure (SAS Inc, Cary).

Results

Subject Dispositions, Demographics, and Baseline Characteristics

Thirty healthy Japanese individuals were recruited for the two experiments, and the demographics of the participating volunteers are shown in Table 1 for Experiment 1 and Table 2 for Experiment 2. Two subjects could not complete and dropped out of Experiment 1 because they could not tolerate the application of the ophthalmodynamometer on the sclera. In addition, two subjects were excluded from Experiment 2 because their SBP was > 150 mmHg. In the end, sixteen volunteers with the average age of 33.1 ± 8.6 -years completed all phases of the examinations in Experiment 1. Ten volunteers with the average age of 28.6 ± 1.0 -years completed all phases of Experiment 2. No adverse events were observed during and after the measurements in any of the volunteers.

Table 1
Baseline Characteristics of Subject

Characteristics (n = 16)	mean ± SD
Age (years)	33.1 ± 8.6
IOP (mmHg)	15.2 ± 3.0
Axial length (mm)	25.7 ± 0.96
Refractive error (diopter)	-4.19 ± 2.60
Systolic blood pressure (mmHg)	116.9 ± 14.3
Diastolic blood pressure (mmHg)	72.6 ± 11.0
Heart rate (BPM)	70.1 ± 6.9

Table 2
Baseline Characteristics of Subject

Characteristics (n = 10)	mean ± SD
Age (years)	28.6 ± 1.0
IOP (mmHg)	12.7 ± 2.5
Axial length (mm)	25.8 ± 1.16
Refractive error (diopters)	-4.84 ± 2.74
Systolic blood pressure (mmHg)	110.6 ± 7.9
Diastolic blood pressure (mmHg)	67.7 ± 6.6
Heart rate (BPM)	72.6 ± 10.4

Changes in pulse waveform parameters on ONH in Experiment 1

The mean MBR was significantly reduced from 11.9 ± 1.9 AU to 9.8 ± 2.3 AU (-18.3%) after increasing the IOP by 20 mmHg and to 9.1 ± 2.5 AU (-25.0%) by 30 mmHg (both, $P < 0.001$). The IOP returned to the baseline after the release of the pressure.

Of the eight waveform parameters, three parameters as mentioned below were significantly changed immediately after the elevation of the IOP and those recovered to their baseline after the release of the pressure (Fig. 4). The blowout score (BOS) ($P < 0.001$) decreased significantly, and the flow acceleration index (FAI; $P < 0.01$) and resistivity index (RI; $P < 0.001$) increased significantly. The Skew, blowout time (BOT), rising rate (RR), falling rate (FR), and the acceleration time index (ATI) did not significantly change immediately after the elevation of the IOP by 20 mmHg and also by 30 mmHg. The percentage increase

in the RI was + 35.0% at an IOP elevation of 20 mmHg and + 50.4% ($P < 0.001$) at an IOP elevation of 30 mm Hg.

Changes in IOP and OPP in Experiment 2

A stable and significant increase in the IOP by 30 mmHg was caused by the application of the ophthalmodynamometer for 10 min ($P < 0.001$; Fig. 5a), and the mean arterial pressures did not change significantly (Fig. 5b). As a result, the OPP was significantly decreased during the IOP elevation (Fig. 5c). After the release of the pressure, the IOP and OPP returned to pressures that did not differ significantly from the baseline pressures.

Changes in pulse waveform parameters on ONH in Experiment 2

The MBR was significantly reduced from 10.4 ± 2.0 AU to 7.4 ± 2.3 AU immediately after the IOP was elevated by 30 mmHg and remained significantly reduced until 7 min after the IOP elevation ($P < 0.001$, Fig. 5d). During the 30 mmHg IOP elevation, the MBR was significantly increased from time 1 ($P < 0.05$) through time 10 ($P < 0.01$) compared to time 0. After the release of the pressure, the MBR returned to the baseline level immediately.

The changes in the eight waveform parameters on the ONH are shown in Fig. 6. The BOS was significantly reduced during the IOP elevation throughout the 10 min ($P < 0.001$), and the FAI ($P < 0.001$) and RI ($P < 0.001$) were significantly increased during the same time period. All three parameters returned to the baseline after the pressure was released. The skew, the blowout time, the rising rate, the falling rate, and the ATI did not significantly change during the IOP elevation.

Of the three parameters with significant changes during the IOP elevation, the BOS and the RI significantly recovered to the baseline at time 10 compared to time 0, ($P < 0.001$ and $P = 0.008$, respectively).

Discussion

We investigated the presence of autoregulation on the ONH using different parameters of the pulse waveform determined by LSFG. It is very important to investigate it, because dysfunction of autoregulation is related to the cause of various ocular diseases including glaucoma [1–3]. Our results showed that the BOS, FAI, and RI of the pulse waveforms changed significantly in response to changes in the OPP induced by an elevation of the IOP in normal subjects.

The BOS demonstrated the constancy of the blood flow during a beat, and the RI indicated the resistance to flow in the arterial vessels. Considering the formula for calculating the BOS and RI, these parameters should have a strong inverse relationship [20, 32]. Takeshima et al reported that there was a significant increase in the BOS and a significant decrease in the RI in glaucoma patients, and a decrease in the IOP after trabeculectomy. In addition, the reduction in the IOP was significantly associated with the changes

in BOS and RI [32]. We evaluated the inverse changes by an IOP elevation, and our results showed that the opposite changes in those waveforms, e.g. a significant BOS reduction and a significant RI increase were observed after IOP elevation by 20 or 30 mmHg. In addition, the degree of the BOS decrease and the RI increase was greater with the higher increase of the IOP. Kiyota et al. reported similar changes in these parameters on the ONH and the choroid after artificial experimental IOP elevation [33]. Our results corroborate those previous reports [32, 33].

Representative MBR pulse waveforms of the ONH before (a), immediately after (b), and 10 min after IOP elevation are shown in Fig. 7. These waveforms show that the blood flow is reduced and becomes unstable during the IOP elevation. These results are in good agreement with earlier color Doppler imaging-derived findings that the retrobulbar central retinal artery blood flow velocity decreased and the RI increased during the IOP elevation [34]. The FAI was increased during the IOP elevation, and it was calculated from the maximum change of all frames (1/30 s) in a rising curve [20, 35]. The degree of the maximum MBR was not severely reduced compared with the minimum MBR, and the large difference between the maximum and minimum MBRs caused the lower BOS and higher RI and FAI. A situation in which there is not a totally decreased MBR during the IOP elevation, should be associated autoregulation on the ONH.

During the 10 minutes of IOP elevation by 30 mmHg in Experiment 2, the BOS and RI of the pulse waveform and the MBR recovered to the baseline values during the 10 min IOP elevation. However, the OPP was remained reduced. Interestingly, both BOS and RI significant recovered at 10 min after IOP elevation, but the MBR significant recovered as soon as 1 min after the IOP elevation. The result of MBR confirmed previous findings that blood flow on the ONH recovers just after an experimental increase in IOP in rabbits [36] and cats [37]. These results indicate that the blood flow on the ONH is autoregulated. The blood flow on the ONH originates from the short posterior ciliary artery which suggests that a local autoregulatory system might exist in the deep region of the ONH.

Autoregulation is caused by changes in the blood vessel tone and in the resistance to blood flow, and it is related to dilatations of the blood vessels in spite of changes in the OPP. Myogenic and metabolic mechanisms control the blood flow on the ONH when the OPP changes [4]. The myogenic responses are activated after changes in the diameter of the blood vessel lumens and the intraluminal pressure. Our results showed an increase in the RI immediately after the IOP elevation which probably occurs because the pressure on the ocular vessels causes a decrease in the vascular capacity [38]. There was a large difference in the percentage change in the RI (+ 50.4%) and the MBR (-25.0%) at an IOP elevation of 30 mmHg. The myogenic responses should be activated when the IOP is decreased by the IOP elevation. Accordingly, the myogenic responses would develop to regulate blood flow on the ONH immediately after an increase of the IOP.

Both the BOS and RI recovered at 10 min during the IOP elevation, Furthermore, the percentage reduction of BOS and RI were significant correlated with that of the OPP, but then was not significantly during the 10 min IOP elevation. These results imply the presence of another autoregulatory process. A long

duration of IOP elevation results in hypoxia of the tissues which causes accumulation of metabolites [39] and the release of nitrous oxide (NO) [5]. These changes induce a vasodilatation of the retinal vessels. Our study showed that the decreasing BOS and the increasing RI immediately after IOP elevation significantly recovered during the 10 min IOP elevation indicating that the activation of some metabolic mechanisms during a longer period of IOP elevation on the ONH even though the OPP remained reduced.

The blood flow recovered even though the IOP was elevated because of decreasing resistance to blood flow, but it did not fully return to baseline level during the 10 minutes measuring period. A longer period of IOP elevation measurements is needed to determine whether the ONH blood flow fully recovers. However, this it is difficult to do because of ethical issues. Eyes with poor autoregulation are at much greater risk of developing ONH ischemic damage than those with efficient autoregulation. Thus, under the abnormal conditions in which autoregulation of the blood flow is lost, such as in eyes with diabetic retinopathy [40] and with glaucoma [41], it is important to pay more attention to the increase in the IOP in such diseases.

There are limitations in our study. First, the variables were measured only with 20 and 30 mmHg elevations. Many studies reported on the effects of stepwise elevations of the IOP [13, 14, 42–45]. Second, the ONH blood flow largely recovered due to autoregulation after 10 minutes of IOP elevation, but it did not fully return to the baseline levels. However, times longer than 10 min were painful, and it could not be extended for ethical reasons. Third, our study had many myopic eyes. The morphological features of the optic disc of myopic and non-myopic eyes are different [46] which might affect the results. Fourth, only relatively young subjects were studied, and the results cannot be extrapolated to elderly subjects. Fifth, our sample size was relatively small. Further studies with a larger number of subjects including non-myopic subjects, a wider range of ages, and IOP elevations in a step-by-step manner are needed.

Conclusion

In conclusion, the BOS, FAI, and RI of the pulse waveforms were significantly changed by an acute elevation of the IOP. The percentage increase in the RI was much higher than that in the MBR. The BOS, RI, and the MBR recovered to the baseline after 10 min of continuous IOP elevation. These results indicate that the ONH is autoregulated by several mechanisms for changes in the OPP induced by an elevation of the IOP in normal subjects.

Abbreviations

ONH: optic nerve head; IOP: intraocular pressure; LSFG: laser speckle flowgraphy; MBR: mean blur rate; OPP: ocular perfusion pressure; BOS: blowout score; FAI: flow acceleration index; RI: resistivity index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial blood pressure; BOT: blowout time; RR: rising rate; FR: falling rate; ATI: acceleration time index

Declarations

Ethics approval and consent to participate

The procedures used were approved by the Institutional Review Board and the Ethics Committee of the Nagoya University Graduate School of Medicine and registered with the University Hospital Medical Network (UMIN)-clinical trials registry (UMIN000024980: November 24th 2016). The procedures conformed to the tenets of the Declaration of Helsinki, and a signed informed consent was obtained from all subjects after a full explanation of the procedures to be used and the possible complications.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and analyzed during the current study are not publicly available due to privacy and ethical concerns but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The study received no external funding.

Authors' contributions

CI, TI, TA and ER designed the study and performed data acquisition. RT and

KY performed statistical analysis. TI and HT helped with data interpretations.

CI and TI drafted the manuscript. All authors read and approved the final manuscript.

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Figures

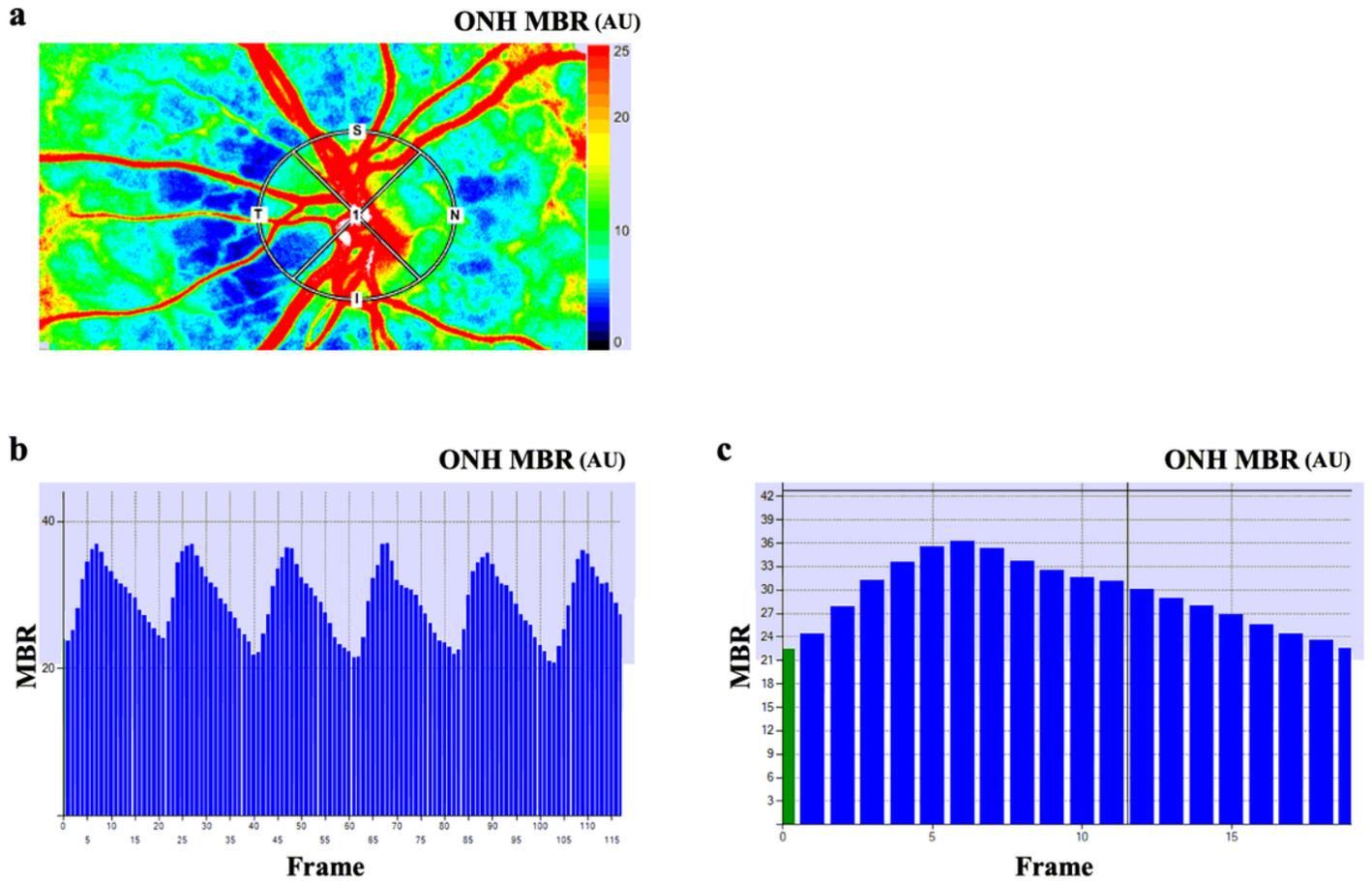


Figure 1

Analysis of the pulse waveform at the optic nerve head (ONH) using laser speckle flowgraphy (LSFG). Representative color-coded composite map (a). The ONH, the mean blur rate (MBR), and other waveform parameters can be measured within the marked circle. Pulse waves showing changes in the MBR due to the cardiac cycle for 4 s. The total number of frames is 118 in one scan (b). The change of the MBR in one heartbeat (c).

Experiment 1

Scheduled time (minutes)	-20	-1	0	1	2	19	20	21	22	39
Rest	■				■				■	
Measurement of IOP		●	●			●	●			●
Measurement of BP	■	■	■			■	■			■
Recording of LSG		◆	◆			◆	◆			◆
IOP increase by 20 mmHg			→							
IOP elevation by 30 mmHg							→			

Experiment 2

Scheduled time (minutes)	-20	-1	0	1	3	5	7	10	11	13	15	17
Rest	■								■			
Measurement of IOP		●	●	●	●	●	●	●	●	●	●	●
Measurement of BP	■	■	■	■	■	■	■	■	■	■	■	■
Recording of LSG		◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
IOP elevation by 30 mmHg			→									

Figure 2

Illustration of the time course of the experiments

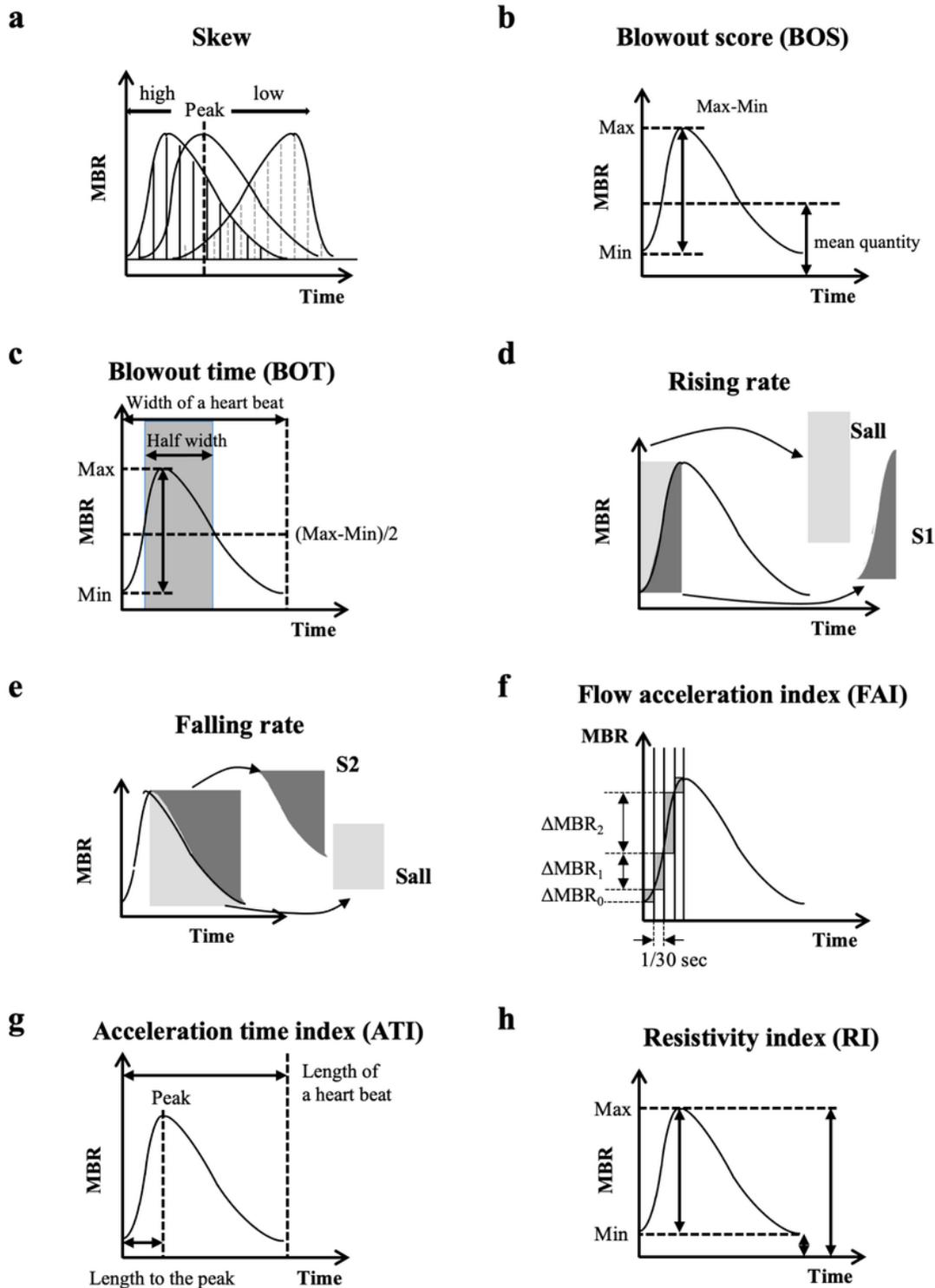


Figure 3

Pulse waveform analysis of the eight waveform parameters. The skewed shape shows the asymmetry of the waveform (a). If the shape of the waveform is symmetrical, the skew is 0 and if the peak comes earlier than that, the degree of skewness increases and the peak slower, and the degree of skewness decreases. The blowout score (BOS) indicates the amount of the blood flow volume in one heartbeat (width of a heartbeat) (b). The blowout time (BOT) represents the length of time that the wave maintained

more than half of the mean of the maximum and minimum MBR in a heartbeat (c). The half-width is the duration of the time that the MBR is higher than $(\text{MBR max} - \text{MBR min})/2$. The rising rate (RR) is the relationship of the area of the S1 to that of S all (d). S all is the square area before the peak and the S1 is the increasing area (d). The falling rate (FR) is the proportion of the area of the S2 to the S all (e). S all is the area of the square after the peak and the S2 is the decreasing area (e). The flow acceleration index (FAI) is the maximum change of the increasing MBR in 1/30 seconds (f). The acceleration time index (ATI) is the ratio of the duration of the time to reach peak (width to reach peak) in one heartbeat (width of a heartbeat) (g). The resistivity index (RI) is calculated by dividing the difference of MBR max and the MBR min by the MBR max (h).

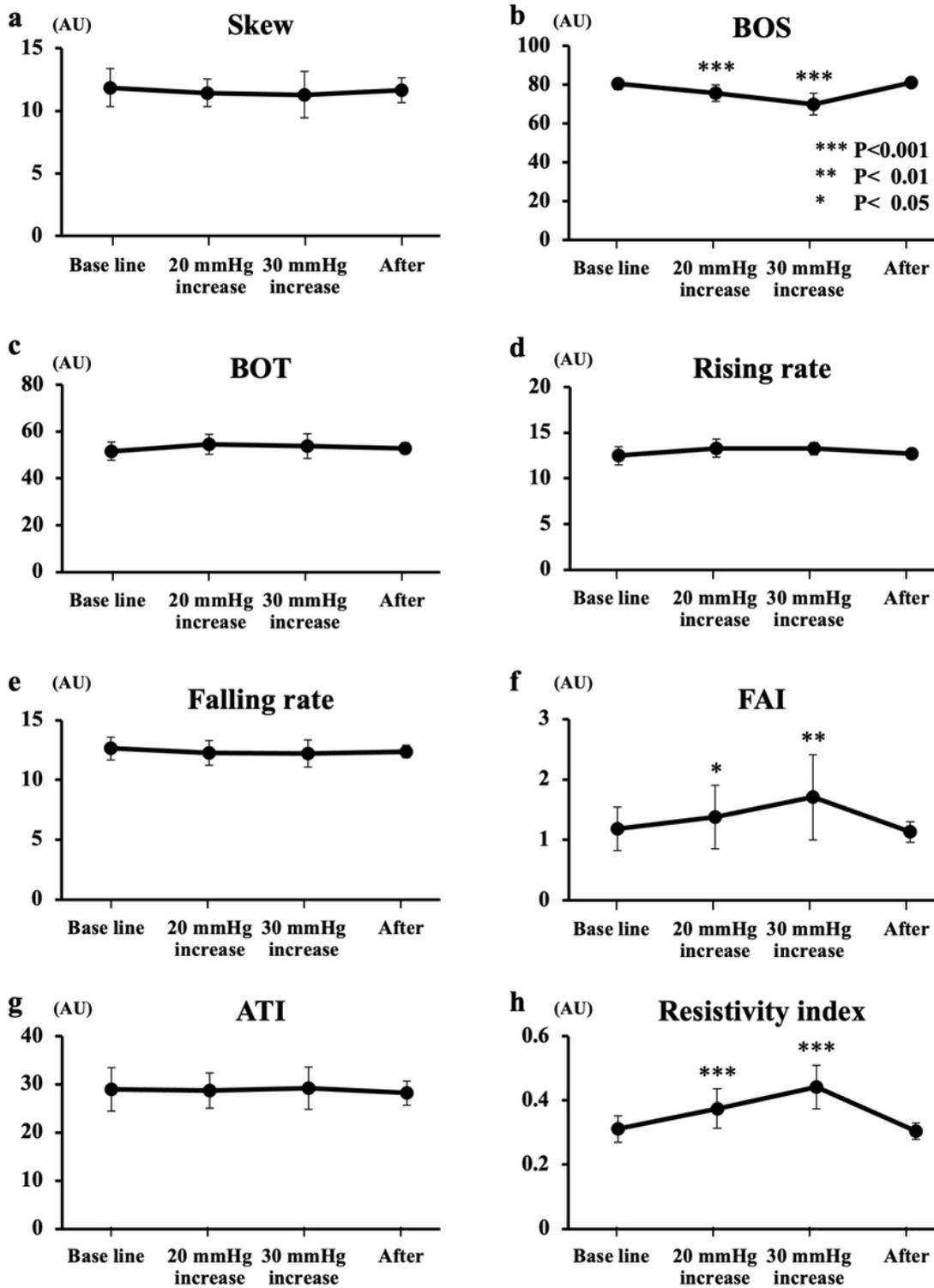


Figure 4

Changes in the eight waveform parameters of the blood flow on the ONH are shown (a-h). Of the eight pulse waveform parameters, the BOS (b) ($P < 0.001$) was significantly increased during IOP elevation, and falling rate (e, $P < 0.001$), FAI (f, $P < 0.001$) and the resistivity index (h, $P < 0.001$) were significantly reduced after the elevation of the IOP by 30 mmHg. The values of four parameters returned to the baseline after release of the IOP. Skew (a), BOT (c), rising rate (d), and ATI (g) did not significantly change after the IOP

elevation. Of the four pulse waveform parameters that changed significantly, the BOS and the resistivity index significantly recovered to the baseline ($P < 0.001$ and $P = 0.008$, respectively) from 58.6 and 0.55 AU immediately after the beginning of the IOP elevation to 67.7 and 0.46 at 10 min during IOP elevation, respectively. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

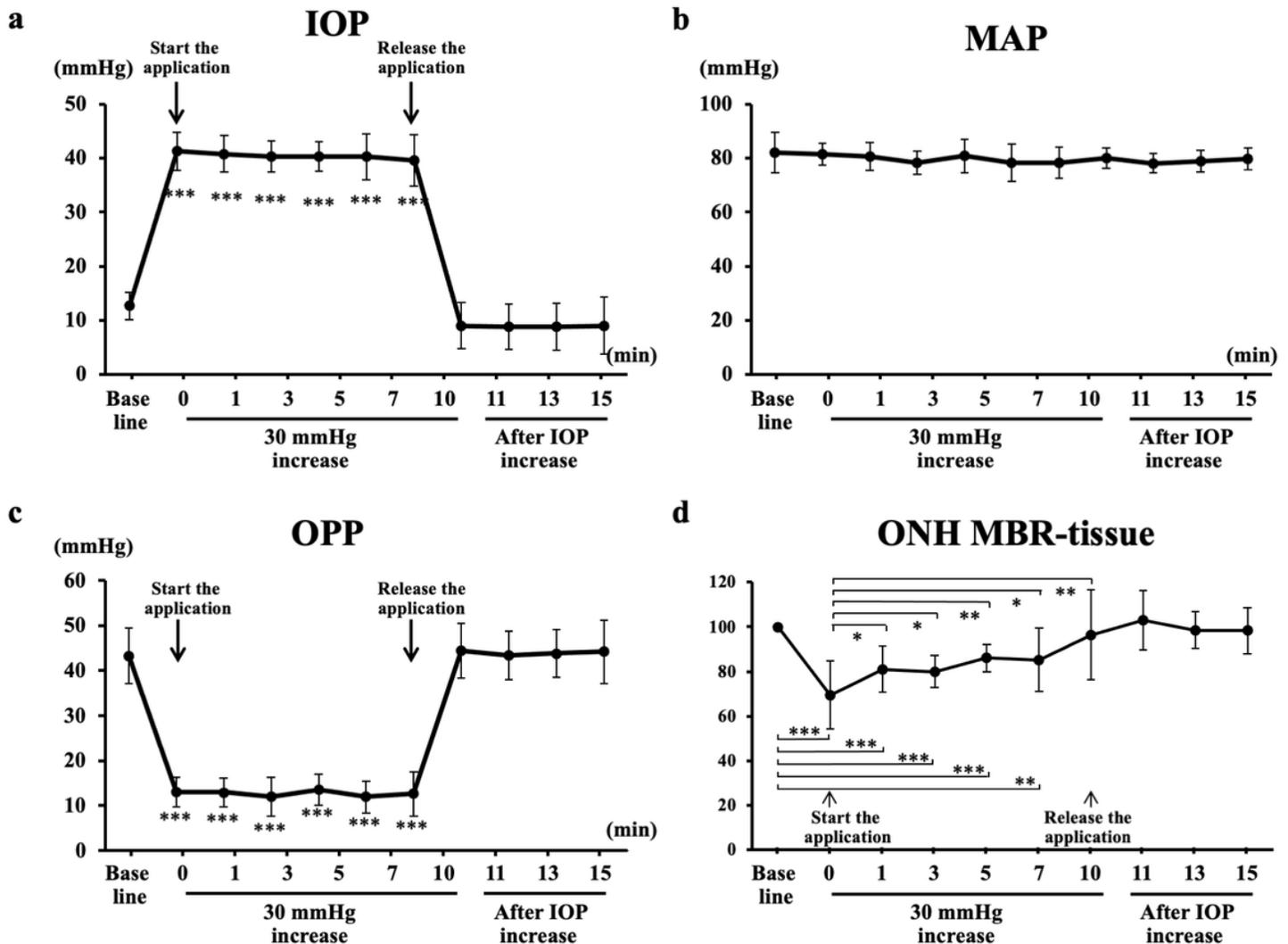


Figure 5

Changes in the systemic and ocular parameters before an increase in the IOP, during 30 mmHg IOP increases for 10 min, and after IOP returned to baseline pressure. The IOP was significantly increased (a), the MAP was not changed (b), and the OPP (c) was significantly decreased during the application of the pressure by the ophthalmodynamometer. The MBR was significantly reduced immediately after the IOP elevation and remained significantly reduced until 7 min after the IOP elevation (d). The MBR was significantly increased from time 1 through time 10 compared to time 0. After the release of the pressure, the MBR returned to the baseline level immediately. *** $P < 0.001$

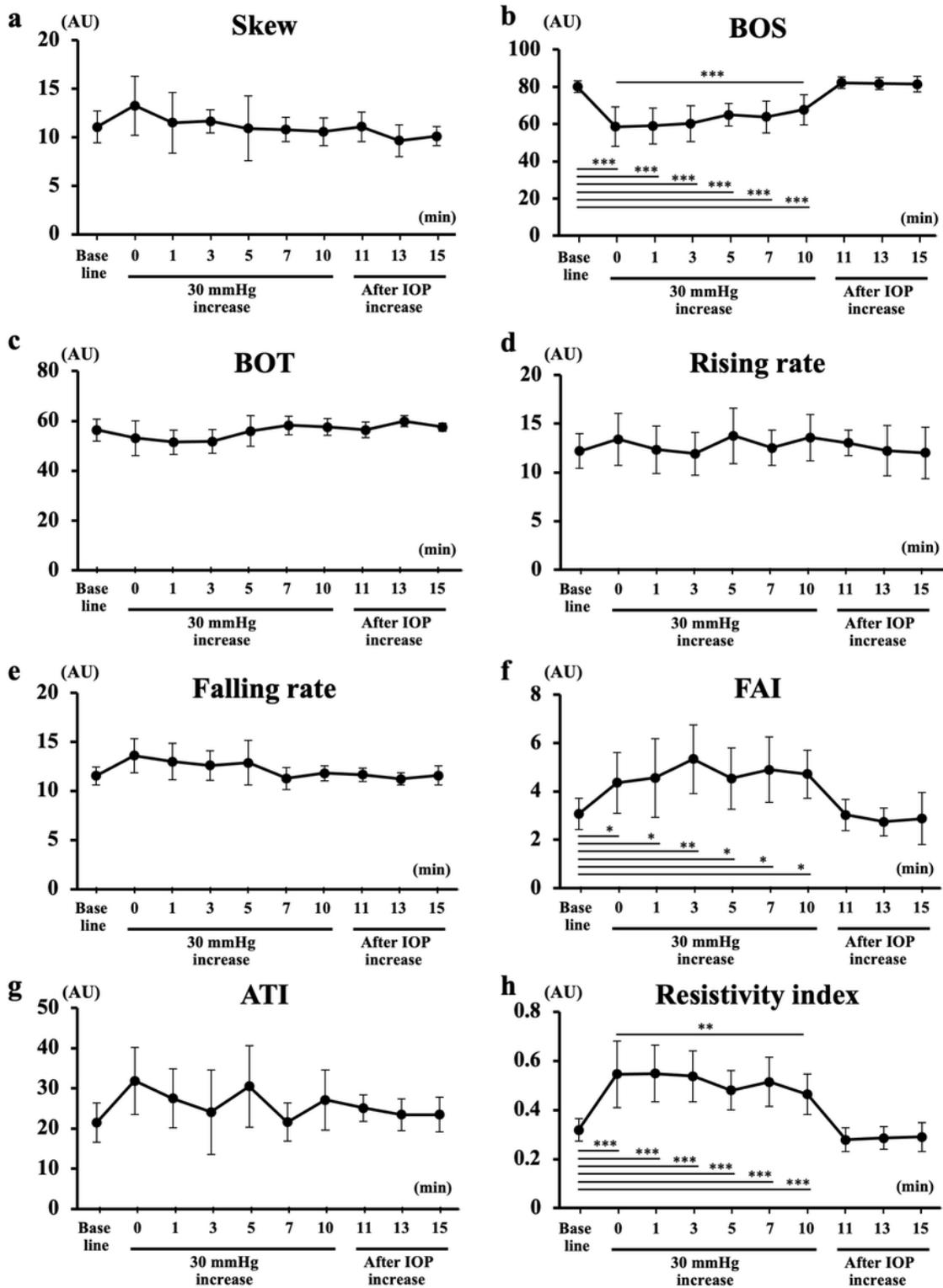


Figure 6

The changes in the eight waveform parameters on the ONH are shown. The BOS was significantly reduced during the IOP elevation throughout the 10 min, and the FAI and RI were significantly increased. Of the three parameters with significant changes during the IOP elevation, the BOS and the RI significantly recovered to the baseline at time 10 compared to time 0.

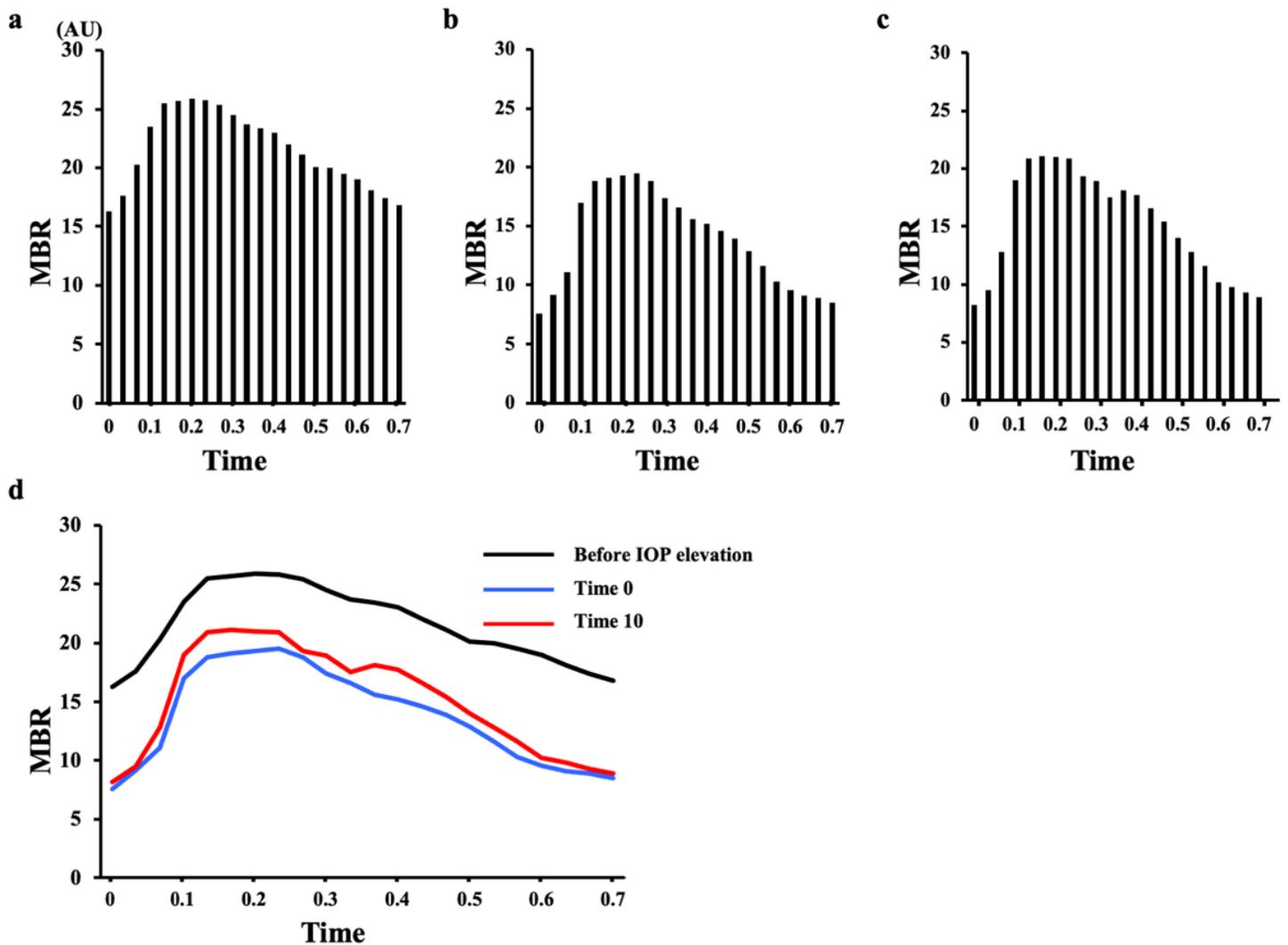


Figure 7

Representative MBR pulse waveforms on the ONH before (a), immediately (time 0) (b), and 10 min after the IOP elevation (time 10) (c). Also, a comparison that highlights the changes that occurred over a heartbeat (d). The observed MBR after IOP elevation was lower throughout the time of a heartbeat than that before IOP elevation, but difference at the peak of the MBR (maximum MBR) between before and after IOP elevation was relatively lower than that in the bottom of the MBR (minimum MBR). In addition, the observed MBR at time 10 was higher throughout frames over a heartbeat than that at time 0.