Intra-Subject Variability and Diurnal Cycle of Ocular Perfusion Pressure as Characterized by Continuous Telemetry in Nonhuman Primates

Katherine I. Wilson,[1] Pooja Godara,[1] Jessica V. Jasien,[2] Emma Zohner,[3] Jeffrey S. Morris,[4] Christopher A. Girkin,[1] Brian C. Samuels,[1] and J. Crawford Downs[1]

[1]Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States
[2]Vision Science Graduate Program, School of Optometry, University of Alabama at Birmingham, Birmingham, Alabama, United States
[3]Rice University, Houston, Texas, United States
[4]University of Pennsylvania, Perelman School of Medicine, Pittsburgh, Pennsylvania, United States

Correspondence: J. Crawford Downs, Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham School of Medicine, 1670 University Boulevard, VH 390A, Birmingham, AL 35294, USA; cdowns@uab.edu.

Received: January 18, 2020
Accepted: April 7, 2020
Published: June 3, 2020

Citation: Wilson KI, Godara P, Jasien JV, et al. Intra-subject variability and diurnal cycle of ocular perfusion pressure as characterized by continuous telemetry in nonhuman primates. *Invest Ophthalmol Vis Sci.* 2020;61(6):7. https://doi.org/10.1167/iovs.61.6.7

Purpose. To characterize ocular perfusion pressure (OPP) fluctuations with continuous telemetry over 24-hour periods across multiple days in nonhuman primates (NHPs) to test the hypotheses that OPP differs among NHPs and that the diurnal cycle of OPP is characterized by low OPP during sleep.

Methods. We have developed and validated two implantable radiotelemetry systems that allow continuous measurement of intraocular pressure (IOP), arterial blood pressure (BP), and OPP up to 500 Hz. OPP was measured unilaterally in 12 male NHPs for periods of 38 to 412 days. IOP transducers were calibrated directly via anterior chamber manometry, and OPP was calculated continuously as central retinal artery BP minus IOP. OPP data were corrected for signal drift between calibrations and averaged hourly.

Results. OPP varied widely among animals, with daily averages ranging from ~47 to 65 mm Hg. In eight of 12 NHPs, OPP was significantly lower during sleep compared to waking hours. In three animals, the diurnal cycle was reversed and OPP was significantly higher during sleep ($P < 0.05$), and one NHP showed no diurnal cycle. Day-to-day OPP variability within NHPs was the largest source of overall OPP variability, even larger than the differences between NHPs. Average daily OPP showed an unexplained ~32-day cyclic pattern in most NHPs.

Conclusions. Average OPP varied widely and exhibited differing diurnal cycles in NHPs, a finding that matches those of prior patient studies and indicates that OPP studies in the NHP model are appropriate. Infrequent snapshot measurements of either IOP or BP are insufficient to capture true IOP, BP, and OPP and their fluctuations.

Keywords: ocular perfusion pressure, telemetry, nonhuman primate, diurnal rhythm

Age, intraocular pressure (IOP), increased optic disc cupping, central corneal thickness, and African ancestry are the most frequently identified independent risk factors for the development and progression of glaucoma,[1–7] the second leading cause of blindness worldwide.[8] Lowering IOP is the only effective clinical treatment that has been shown to retard glaucoma onset or progression. However, patients may exhibit visual field deterioration despite IOP reduction,[9,10] and patients with epidemiologically defined normal IOP of <21 mm Hg can also develop the disease.[11] Hence, factors other than IOP are thought to contribute to individual susceptibility to glaucoma.

A large body of evidence suggests that disturbance in vascular perfusion plays a crucial role in glaucoma.[11–14] Ocular perfusion pressure (OPP) is defined as the ophthalmic artery blood pressure (BP) minus IOP; it is clear from this relationship that IOP and vascular perfusion are inseparably intertwined.[15–18] Large population-based studies have linked vascular factors to an increased prevalence of primary open-angle glaucoma and have determined that BP, OPP, and their variability or fluctuations are part of the complex etiology of this disease.[1,10,19–25] Specifically, low diastolic BP, low mean OPP, and low diastolic perfusion pressure (calculated by diastolic BP – IOP) are independent risk factors for glaucoma prevalence[1,20,21] and visual field progression.[26–29] BP and OPP fluctuations, such as the magnitude of the nocturnal OPP dip or the magnitude of daily OPP fluctuations, have also been shown to increase the risk of glaucoma and visual field progression in the disease.[24–36] Although most recent studies have found a link between BP and OPP and glaucoma, several have not found an association,[37,38] whereas others have found that vascular hypertension rather than hypotension is associated with glaucoma.[30,32] Some of this confusion may arise from vari-

Table. Animal Demographics

NHP	Eye	Age, y	Arterial BP Transducer Location	Monitoring Period		Number of Days of Data	Number of Days Excluded	Telemetry System
				Start	End			
9028	OS	5	Aorta	3/1/2014	12/12/2014	228	7	Konigsberg
9140	OS	4.5	Aorta	11/27/2013	6/17/2014	173	3	Konigsberg
9160	OS	4.5	Aorta	11/27/2013	3/23/2015	399	1	Konigsberg
0804025	OS	5.5	Aorta	11/27/2013	4/7/2015	412	2	Konigsberg
150171	OS	6.5	Femoral	9/8/2017	7/2/2018	62	0	Stellar
150152	OS	7	Carotid	8/24/2018	5/7/2019	189	1	Stellar
150110	OD	5.5	Femoral	4/20/2018	2/12/2019	205	1	Stellar
150069	OD	6	Femoral	6/16/2018	1/31/2019	119	10	Stellar
12.38	OD	6.5	Carotid	10/5/2018	3/5/2019	94	2	Stellar
13.171	OS	5.5	Carotid	12/22/2018	4/9/2019	59	16	Stellar
13.86	OD	5.5	Carotid	1/24/2019	4/29/2019	62	2	Stellar
13.106	OS	5.5	Carotid	12/14/2018	1/30/2019	38	0	Stellar

ability in the methods used to calculate and measure BP or OPP, the times and frequency of measurement, the methods used to determine glaucoma prevalence and progression, and the length of patient follow-up. Although it has been well established in most studies that low BP and/or OPP are associated with glaucoma onset and progression, few studies have been done to characterize OPP fluctuations themselves using accurate ocular BP and IOP measurements.

Low OPP, which likely leads to poor ocular tissue perfusion, can be the result of low arterial BP and/or high IOP. Previous studies have relied upon snapshot measurements of both IOP and arterial BP, and the BP measurement is typically acquired in the brachial artery of the upper arm. Because these measurements do not track the fluctuations of BP and IOP continuously, OPP is interpolated between measurement time points, largely ignoring short-term fluctuations in IOP or BP over time. IOP can fluctuate as much as 10 mm Hg per minute in glaucoma patients,[39] so snapshot measurements of IOP and BP do not accurately capture the dynamic nature of IOP, BP, or OPP. Also, the studies cited above that report "OPP" do not accurately measure true OPP because they assume the arterial BP measured in a peripheral artery to be equal to the BP in the ophthalmic or central retinal artery. Although the error associated with this approach has not been characterized, it is reasonable to assume that the BP in the ocular arteries will be somewhat lower than the BP in the brachial artery due to both smaller vessel lumen size and total perfused tissue mass.

To better understand the differences in OPP dynamics among individuals and how OPP dynamics change with time, we used a fully implantable wireless telemetry system to continuously record IOP and BP up to 500 times per second, 24 hours a day, in nonhuman primates (NHPs). NHPs have ocular features, vascular biology, and upright posture analogous to humans, making them the ideal choice to model ocular physiology similar to that of human eyes.[40,41] Using a novel calibration approach, we can accurately scale continuous BP measurements taken in the carotid artery, femoral artery, or aortic arch to the BP in the central retinal artery (CRA), which can then be combined with continuous IOP measurements to calculate accurate measurements of continuous OPP. The NHP model of glaucoma, coupled with continuous pressure telemetry should improve our understanding of ocular physiology, as well as glaucoma pathogenesis and progression, through accurate characterization of natural IOP, BP, and OPP patterns and changes. In this study, we characterize OPP fluctuations over 24-hour time scales across multiple days in 12 bilaterally normal NHPs to test the hypotheses that OPP differs between animals and that the diurnal cycle of OPP in NHPs is characterized by low OPP during sleep and higher OPP during waking periods.

Methods

Animals

Twelve eyes of 12 young adult male rhesus macaques, 4 to 7 years old and having no ocular abnormality, were used for the study. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research under a protocol approved and monitored by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. There are three primary reasons why we used only male primates. First, the hormonal cycles of female primates have been shown to affect physiological processes, including those related to the variables of interest in our study. Second, male primates are easier to procure than females, as males are not in as high demand for breeding. Finally, National Institutes of Health (NIH) officially classifies primates as a "scarce research resource," and thus they are exempt from the NIH requirement that sex be considered. After implantation of the telemetric IOP and BP monitoring system, the animals were allowed to heal for at least 6 weeks prior to data collection. NHPs were kept on a 6 AM-to-6 PM light/dark cycle and fed at approximately 6 AM and 2 PM daily. Water was provided ad libitum via continuous feed. Food and water intake were not measured. The demographics of the animals are listed in the Table.

IOP/BP Telemetry Monitoring System

We have developed and validated two fully implantable IOP/BP radiotelemetry systems—a Konigsberg fully implanted wireless telemetry system (Konigsberg Instruments, Pasadena, CA, USA) (Fig. 1) and the Stellar Implantable Transmitter (TSE Systems, Chesterfield, MO, USA) (Fig. 2)—which allow continuous measurement of IOP and arterial BP up to 500 times per second for approximately 24 months. These telemetry systems measured IOP and arterial BP either continuously (Konigsberg; 500 Hz)[39,42–46] or on a 10% duty cycle of 15 seconds out of every 150-second period (TSE Systems Stellar; 200 Hz).[47] All animals underwent surgical implantation of the telemetry

Investigative Ophthalmology & Visual Science

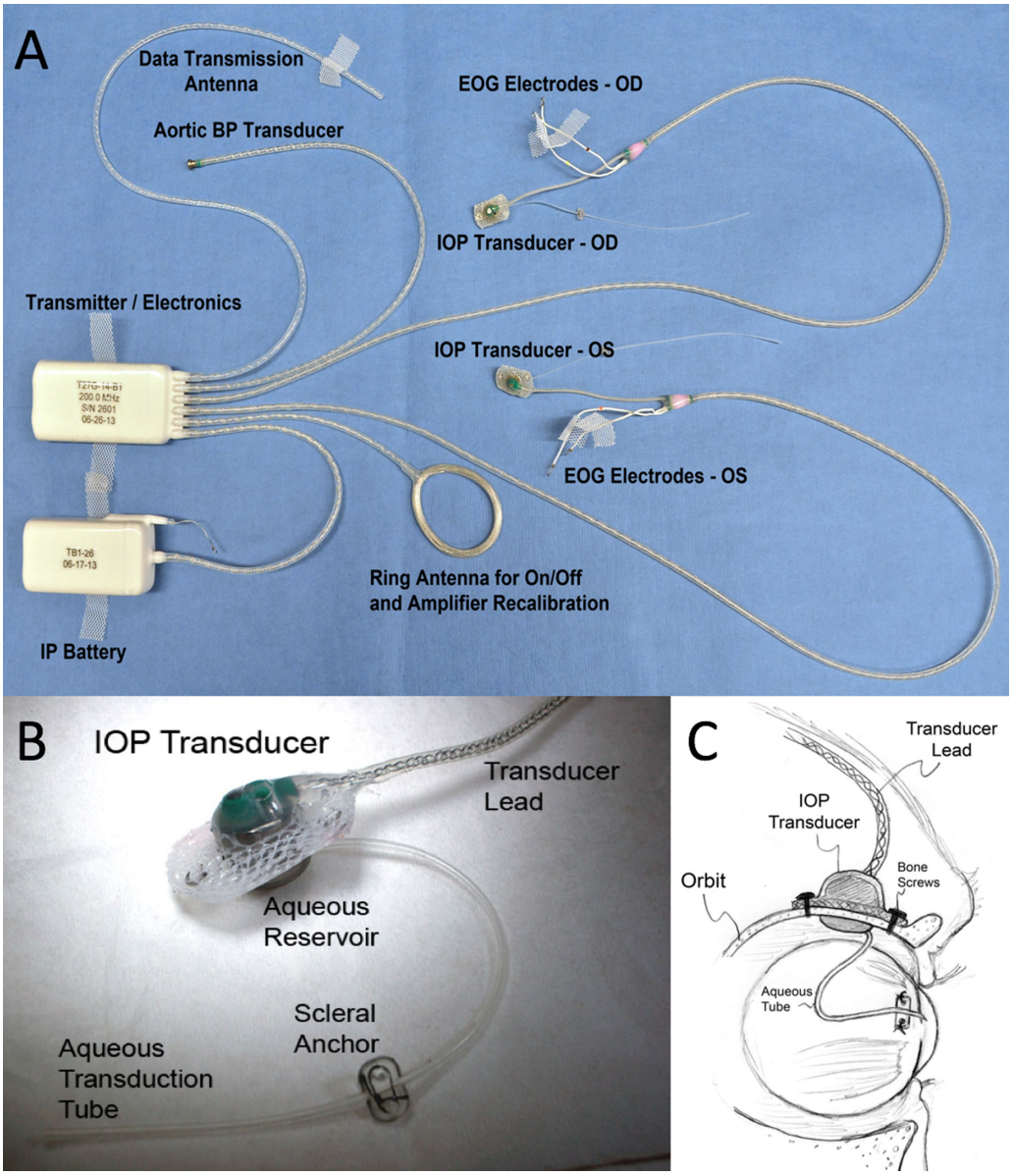

Figure 1. (**A**) Konigsberg bilateral IOP/BP total implant system, (**B**) close-up view of the IOP transducer and aqueous transduction tube, and (**C**) surgical placement of the IOP transducer in the orbital wall and the aqueous transduction tube in the NHP eye. Reprinted with permission from Markert JE, Jasien JV, Turner DC, Huisingh C, Girkin CA, Downs JC. IOP, IOP transient impulse, ocular perfusion pressure, and mean arterial pressure relationships in nonhuman primates instrumented with telemetry. *Invest Ophthalmol Vis Sci*. 2018;59:4496–4505.

system, including bilateral IOP sensors and an arterial BP sensor placed in the lumen of the femoral, carotid, or aortic artery, as described previously.[42,47] IOP and OPP calibrations occurred every 2 weeks after an initial 6-week heal-in period as described in subsequent sections and our previous publications.[42,43] All data and associated time-synced video for calibration were captured using commercial data acquisition software (NOTOCORD-hem; Instem, Paris, France). Data were continuously collected 24 hours a day, 7 days a week, for the duration of the study, except on calibration days due to anterior chamber calibration effects on IOP.[48] OPP was measured and recorded unilaterally in all 12 NHPs for periods of 38 to 412 days depending on the animal (Table).

Telemetric IOP Transducer Calibration

We have previously described the telemetric IOP transducer calibration procedure in detail[39,42,43] and briefly summarize it here. To ensure that the data acquired were corrected for drift, transducer calibration was performed every 2 weeks for the duration of the study. Each animal was placed under 1% to 3% isoflurane anesthesia after initial induction with an intramuscular injection of ketamine/dexmedetomidine, and the implanted eyes were cannulated with a 27-gauge needle placed into the anterior chamber through the cornea at the limbus. The needle was connected to a bottle of sterile isotonic saline solution via a sterile infusion set, and the connecting tube was fitted with an in-line, digital pressure gauge (XPi; Crystal Engineering, San Luis Obispo, CA, USA)

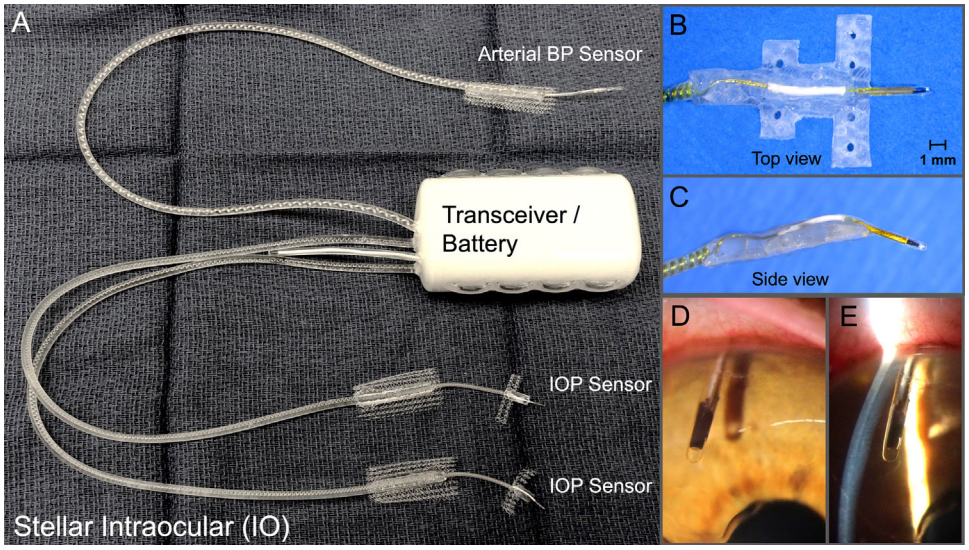

Figure 2. (**A**) Stellar bilateral IOP/BP total implant system, (**B**) top view of the IOP transducer and integrated scleral baseplate, (**C**) side view of the IOP transducer and integrated scleral baseplate, (**D**) en face photograph of the piezoelectric IOP transducer in the anterior chamber, and (**E**) slit-lamp photograph of the intraocular placement of the piezoelectric IOP transducer in the anterior chamber relative to the cornea and iris. Adapted with permission from Jasien et al.[47]

placed at the level of the needle insertion into the eye. The manometer-controlled IOP was set to 5 mm Hg and allowed to stabilize, and it was then raised in increments of 5 mm Hg from 5 to 30 mm Hg while telemetric and gauge IOP values were recorded and compared to quantify IOP transducer signal accuracy and drift at each stable pressure step. Raw IOP telemetry data were drift corrected via software postprocessing on a continuous basis using the 15-mm Hg calibration offset, assuming a linear, monotonic transducer drift between biweekly calibrations. Note that the transducers were linear; therefore, offset (transducer error) was similar at all calibration pressure steps.

OPP Calibration

Ocular perfusion pressure is defined as the arterial blood pressure within the eye relative to IOP (BP − IOP) and calculated directly from the continuous IOP and arterial BP telemetry data as described in our previous study.[42] Briefly, calibrated OPP was calculated from the calibrated IOP telemetry data and the uncalibrated arterial BP telemetry data by relating CRA diastolic and systolic BP to continuous telemetric measurement of both IOP and remote arterial systolic and diastolic BP. By raising IOP via manometry and measuring the IOP levels at which momentary and full CRA collapse first occurred, CRA diastolic and systolic BPs were obtained relative to the telemetric BP measured at the same time (Fig. 3). Continuous, calibrated OPP was then calculated directly from the telemetric arterial BP and IOP data streams between OPP calibration exams as

$$OPP = \frac{(CRA\,systolic\,BP - CRA\,diastolic\,BP)}{(Systolic\,BP - Diastolic\,BP)} \times (BP - Systolic\,BP) + CRA\,Systolic\,BP - IOP \quad (1)$$

where BP and IOP denote the continuous arterial BP and IOP telemetric data, respectively. The arterial systolic and diastolic BP values in Equation 1 were captured along with the CRA systolic and diastolic BP at the time of OPP calibration. This estimate of OPP was calculated directly from the IOP and arterial BP telemetry signals up to 500 times per second using arterial and CRA systolic and diastolic BP values as shown.[14] Note that Equation 1 scales the arterial pulse pressure amplitude taken via telemetry in a remote large artery (aorta, carotid, or femoral) to the smaller pulse pressure in the CRA, accounts for the higher overall BP in the remote artery relative to the smaller CRA, and corrects for any drift or error in the telemetry BP transducer reading itself.

Data Filtering

Wireless telemetry occasionally experiences periods of signal loss or excessive noise. As in previous studies,[39,42,47–49] these artifacts were continuously quantified and eliminated from the data using fully automated post hoc filtering, and only hours in which at least 75% of the data were deemed reliable after noise and dropout filtering were used in the analysis. After obtaining all OPP data, averages of four 6-hour time blocks (0:00–6:00, 6:00–12:00, 12:00–18:00, and 18:00–24:00) were calculated for each day and each eye. Additionally, overall averages of the same four 6-hour time blocks were calculated across all days within each eye. The average OPP from each 6-hour time block on each day was subtracted from the average OPP of the corresponding 6-hour time block across all days to determine outliers. In each eye, OPP data from all individual days that exhibited differences greater than ±20 mm Hg in any 6-hour time from the overall mean OPP for that same 6-hour period were excluded.

Statistical Analysis

Nested, linear mixed-effects models were used to assess the sources of variance in the OPP data as follows.

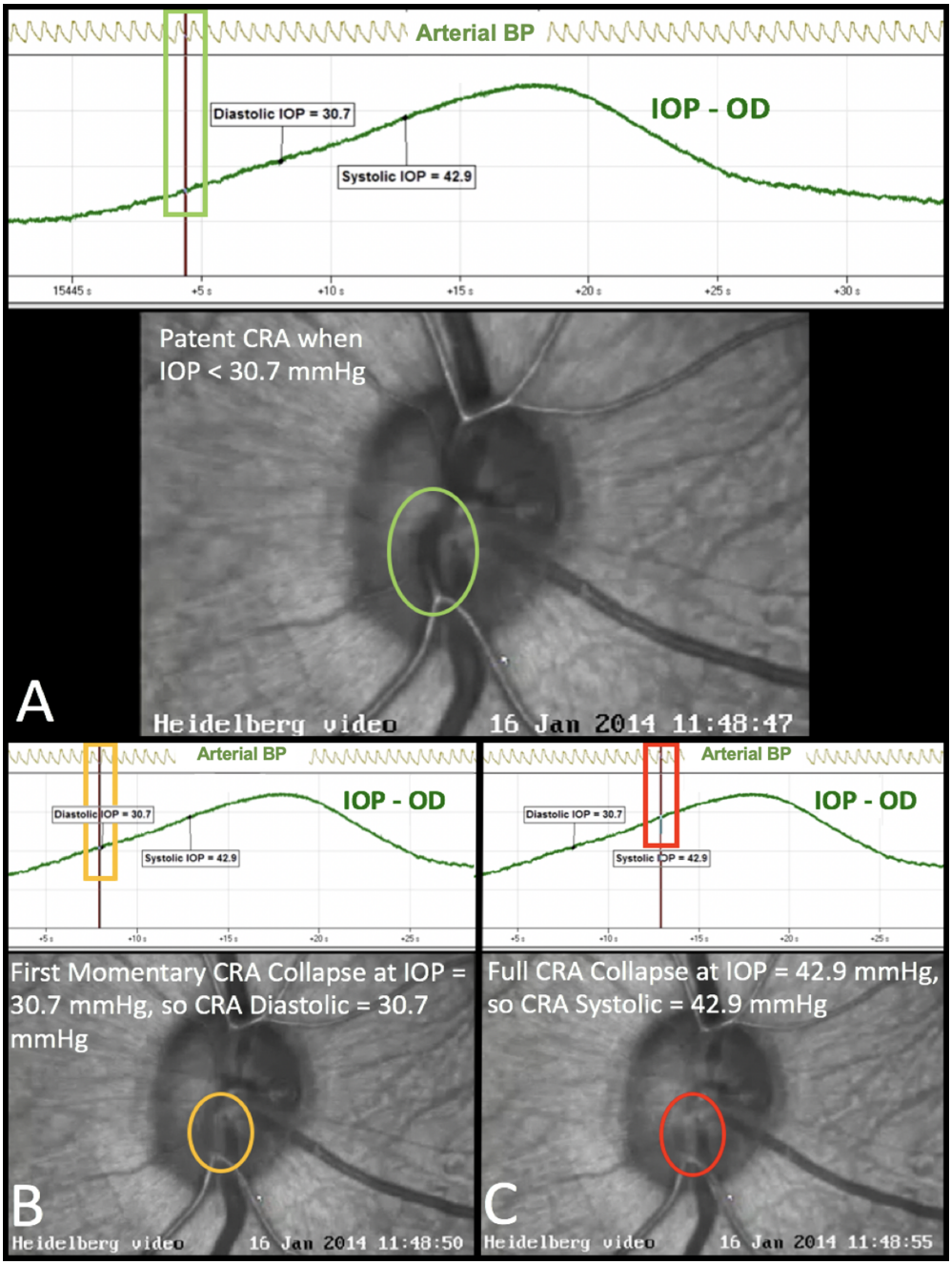

Figure 3. (**A**) IOP is manually elevated while IOP and aortic BP are monitored via telemetry, and the optic nerve hypoplasia (ONH) is visualized in real time using the infrared image from the Spectralis OCT device. The ONH video image is slaved into the NOTOCORD-hem data acquisition system along with the telemetry data stream, and CRA patency is monitored. (**B**) The CRA first collapses momentarily at 30.7 mm Hg when IOP equals CRA BP, so CRA diastolic BP is 30.7 mm Hg. (**C**) The CRA fully collapses at 42.9 mm Hg when IOP equals CRA BP, so CRA systolic BP is 42.9 mm Hg. Adapted with permission from Markert et al.[42]

The first model assessed NHPs (only unilateral data were used in this study) by day within eyes and by hour within eyes, within both the waking and sleeping periods. A second nested linear mixed-effects model assessed the difference in OPP between the Stellar and Konigsberg implant systems. Finally, a third nested linear mixed-effects model assessed the differences in OPP between the waking and sleeping periods within NHPs and overall across animals.

Results

Mean OPP varied from ~47 to 65 mm Hg among the NHPs, representing an 18-mm Hg (38%) difference between animals. Overall, OPP was 4.28 ± 0.07 mm Hg higher during waking hours than during sleeping hours ($P < 0.0001$). However, the diurnal cycle of OPP differed between animals, as seen in Figures 4 and 5, which show the average hourly OPP across days by NHP and the average OPP for the

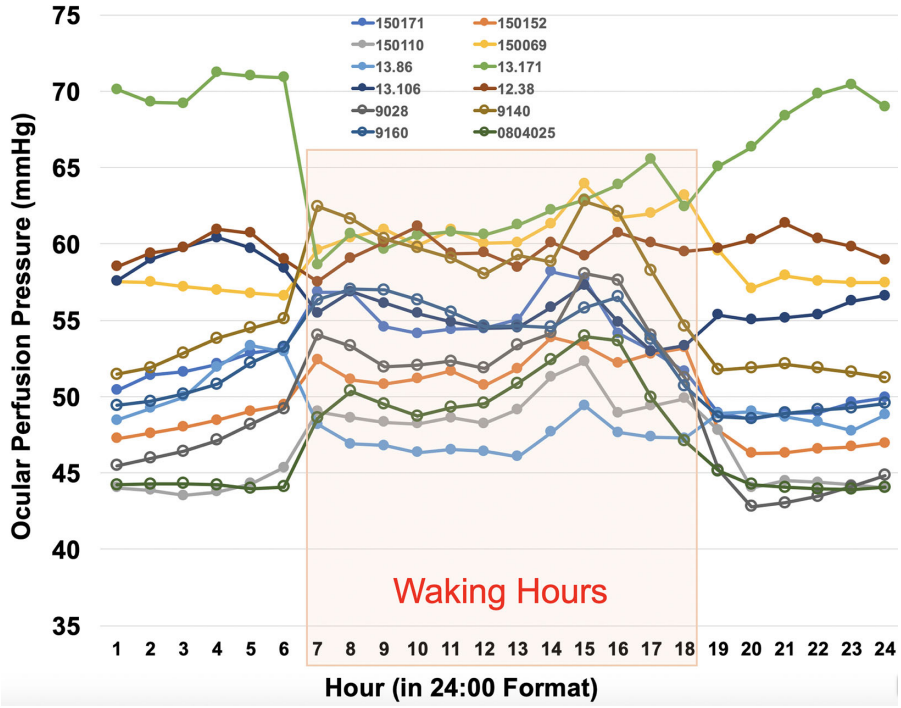

Figure 4. Hourly averages of OPP for 12 eyes of 12 NHPs over a 24-hour time period calculated via continuous IOP and BP telemetry. Waking hours for the NHPs were 6 AM to 6 PM and are reflected as hours 7 to 18 above; *open circles* denote data acquired with the Konigsberg system, and *filled circles* denote data from the Stellar system. There was no significant difference in OPP between data collected with the Konigsberg system and data collected with the Stellar system ($P = 0.10$).

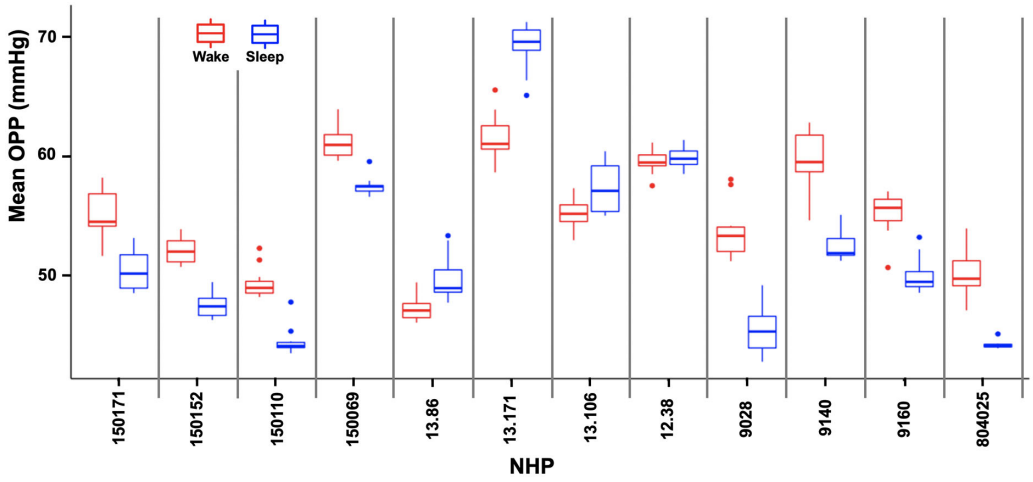

Figure 5. Boxplots comparing the distributions of mean OPP in the sleeping and waking periods by NHP. The central line in the box represents the mean OPP in each period, the box extents represent the central 50% of the data, bars represent the 95% confidence interval, and dots represent outliers. OPP was significantly different in the sleeping and waking periods in all NHPs ($P < 0.05$) except 12.38, for which no sleep/wake difference was found.

sleeping and waking periods averaged across all days for each NHP, respectively. Note that OPP is higher during waking hours in eight of 12 NHPs but lower during waking hours in three NHPs; no difference was found between the sleeping and waking periods in one animal (NHP 12.38).

Figure 6 shows the daily sleeping and waking period OPP averages within each NHP for each day in which data were collected and accepted. There is an obvious cyclic pattern in OPP in most animals, especially those with longer monitoring periods. The OPP distribution density plots for each NHP for the sleeping and waking periods are shown in Figure 7. Interestingly, some NHPs show a much wider range of OPP than others, indicating that their physiological OPP fluctuations are larger across time.

Mixed-effects models were used to assess the sources of variability in hourly OPP statistically. In the waking period, differences between NHPs accounted for 24% of the variability in OPP overall, while day-to-day and hourly variabil-

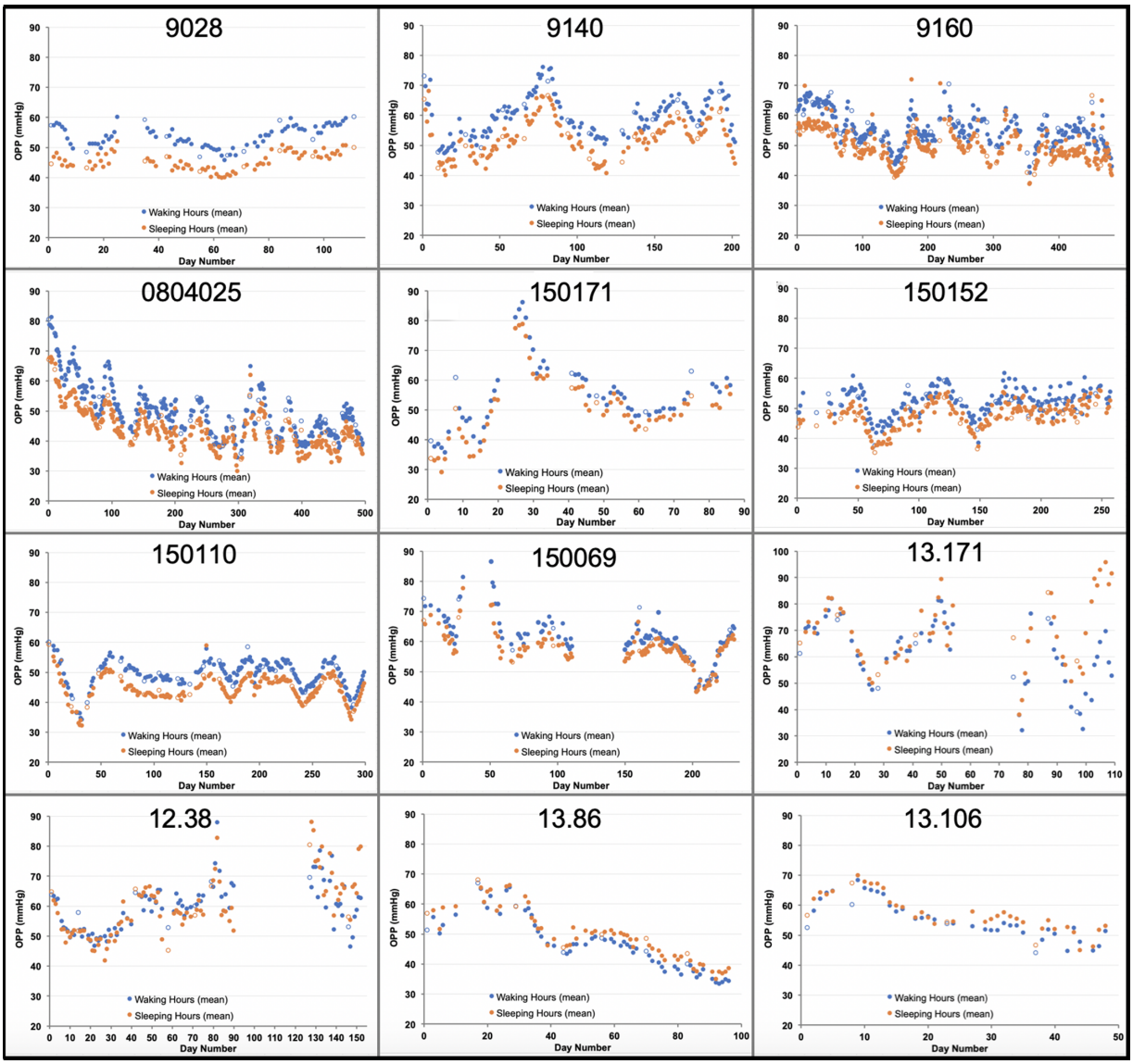

Figure 6. OPP averages for each day within the monitoring period of each NHP. Each data point represents an average of 12 waking hours (6 AM to 6 PM, shown in *blue*) or 12 sleeping hours (6 PM to 6 AM, shown in *orange*). The hollow circles indicate the beginning of a new calibration period and are placed on the first day for which we had data after calibration.

ity within NHPs accounted for 60% and 16% of the variability in OPP, respectively. In the sleeping period, differences between NHPs accounted for 49% of the variability in OPP overall, and day-to-day and hourly variability within NHPs accounted for 44% and 7% of the variability in OPP, respectively. Figure 6 shows the day-to-day variability best; it demonstrates that OPP varied greatly across days and that most of the NHPs exhibited periods of relatively high or low OPP that follow an unexplained ~30-day cyclic pattern.

Discussion

Wireless telemetry allows accurate characterization of rhythms and patterns in true, calibrated OPP over time in awake, behaving, unrestrained NHPs. In this study of 12 NHPs, OPP was significantly different across animals, with daily averages ranging from ~47 to 65 mm Hg, representing a difference of 18 mm Hg (38%) among NHPs. A diurnal cycle characterized by low OPP during sleeping hours and higher OPP during waking hours, with values highest in the late afternoon, was found in eight of the 12 NHPs. Unexpectedly, three of the remaining four NHPs exhibited lower OPP during waking hours and higher OPP values during sleeping hours, and one NHP exhibited a relatively consistent OPP throughout the day, independent of waking or sleeping hours. Day-to-day variability in OPP represented the largest component of total OPP variability within animals, even larger than hourly or diurnal variability, and almost all NHPs exhibited periods of relatively low and high OPP that seemed to follow an unexplained ~30-day cyclic pattern.

Despite the overarching similarities within the species, each animal exhibited unique OPP patterns of both diurnal cycle and average OPP over time, which is remarkably similar to findings reported in previous patient studies.[34,50,51] Waking hours are characterized by increased ocular activity, specifically focused vision, blinks, and saccades, which

Investigative Ophthalmology & Visual Science

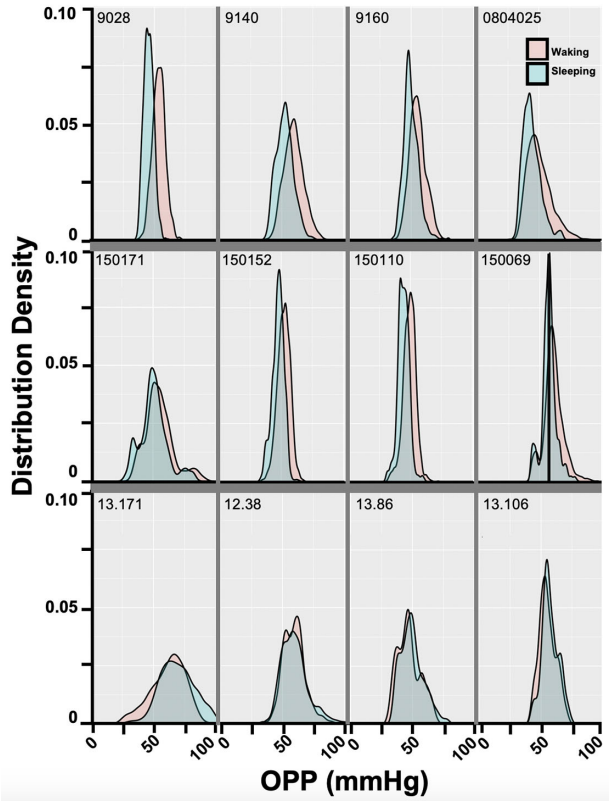

Figure 7. The OPP distribution densities in the sleeping (*blue*) and waking (*red*) periods, plotted for each NHP.

demand greater ocular blood flow. The animals are much more active during waking hours as well, so we would expect higher systemic BPs during that period. Because the variability in arterial BP is much larger than the variability in IOP and therefore drives OPP variability, we expected that OPP would also be higher during waking hours, which it was for eight of the 12 NHPs studied. Three of the NHPs (13.171, 13.86, and 13.106) exhibited the opposite trend, with significantly higher OPP values during sleeping hours and lower OPP values during waking hours; one NHP (12.38) exhibited a fairly consistent OPP of ~59 mm Hg, independent of waking or sleeping hours ($P < 0.05$) (Figs. 4–7). Although there is no obvious explanation for these results, the trends seen in these four NHPs show that OPP is highly variable among animals; thus, even general assumptions about mean OPP levels may not be valid. Two prior patient studies showed similar variability in OPP and roughly similar proportions of patients in which OPP either was higher during the night compared to waking hours or showed no distinct diurnal pattern.[34,51] Hence, in spite of the fact that NHPs sleep upright, the observed variability in NHPs parallels similar differences in the human patient population, so NHP OPP studies can motivate and inform future studies in patients.

Figure 6 shows the daily OPP averages for the sleeping and waking periods by NHP plotted over time, including days for which data were excluded (blank regions). An unexplained cyclic pattern in the OPP data is apparent, especially in those NHPs with long monitoring periods. We reported a cyclic pattern in both baseline and transient IOP fluctuations in a recent study,[52] but these cyclic fluctuations in IOP were not correlated to known environmental or experimental conditions such as experimental schedule, cage cleaning/changing, seasonal pattern, holding room temperature or humidity, or human interactions. The ~25- to 35-day cyclic pattern in OPP that is apparent in some animals is generally similar to the cyclic patterns we recently reported in baseline IOP and transient IOP fluctuations, wherein the strongest cyclic pattern in IOP occurred at an ~30-day interval in most animals.[52] It is important to note that the 10- to 25-mm Hg amplitude of cyclic OPP variation seen in Figure 6 is much larger than the 2- to 6-mm Hg amplitude of cyclic IOP variation we reported previously.[52] Hence, the cyclic pattern in OPP must be driven by arterial BP rather than IOP. It is possible that the cyclic patterns observed in both OPP and IOP may be related to similar physiological processes, which will be explored in future studies.

Also of note, our novel OPP calibration method compensates for differences in BP pulse pressure amplitude, for lower BP in the CRA versus larger remote arteries, and for pressure transducer error and drift over time. As expected, Figures 4 to 7 show that the Konigsberg and Stellar telemetry systems yielded similar results, despite the Konigsberg system using aortic BP sensors and Stellar using either femoral or carotid artery BP sensors. In addition, statistical modeling confirms that variance due to telemetry system type is insignificant ($P = 0.10$), as system type represents only 0.013% and 5.2% of the total OPP variance during the waking and sleeping periods, respectively. Hence, neither system type nor location of arterial BP monitoring is likely to be a source of bias in this study.

This study is limited by the following considerations. First, data from NHPs may not translate directly to human patients, as NHPs are significantly smaller than the average human and sleep sitting up rather than in the supine position, which affects the diurnal cycle of both IOP and BP. Second, although the sample size of 12 animals is relatively large for an NHP study, it is small compared to most patient studies and hence may not adequately represent the population. However, the large differences in average OPP and distinct diurnal cycles reported herein are likely to translate to the wider population of NHPs and are similar to human patient variability,[34,51] so Rhesus macaques can serve as a model to evaluate the effects of OPP variability in ocular diseases such as glaucoma to the extent that they serve as a good model of the disease itself. Third, although the results of this study are distinctive in the continuous nature of IOP, BP, and OPP data collection, the NHPs were not in a completely controlled, natural, or stress-free environment, which may have altered results compared to NHPs in a wild environment; however, the NHPs were housed in the same room and treated similarly throughout the study, so the individual OPP variations reported should be valid. Fourth, the moments of vessel flutter and collapse used in OPP calibration were manually identified by human observers. Observers are trained to identify the two frames within the captured infrared ONH video of momentary and full CRA collapse and to report the exact IOP (to 0.1-mm Hg precision) measured by the telemetry system IOP transducer at that instant. The exact timing of the CRA collapse can be difficult to identify, but the errors in OPP calibration will be minimal due to the slow rate of IOP elevation imposed during the calibration procedure (Fig. 2). Also, calibrations were checked by multiple observers (KIW, JVJ, and JCD), so the likelihood of systemic human error that would bias the reported results is low. Due to the frequency of OPP cali-

bration sessions and the relatively large magnitude of OPP (~47 to 65 mm Hg), accuracy less than 1 mm Hg should not be detrimental to the analysis or bias the results in any way.

Finally, it is possible that the method of data filtering for outliers resulted in exclusion of real data. Data were filtered by flagging and removing any days wherein average OPP in at least one of four 6-hour time periods (0:00–6:00, 6:00–12:00, 12:00–18:00, 18:00–24:00) was greater than 20 mm Hg above or below the overall average for that same 6-hour period across all days. This level for exclusion was chosen arbitrarily, although it is reasonable given the variability in the data (Fig. 6) and the possibility that averages greater than 20 mm Hg different from the mean value were affected by outside factors such as cage changes that do not represent normal NHP physiological conditions. Although this was an arbitrary decision, it is highly unlikely that excessive filtering affected the results and analysis, as trends within the OPP data were consistent across calibration periods in all NHPs, and the number of days excluded were generally small (average of 4% exclusion) compared to the number of days of data reported (noted in the Table and as gaps in the data shown in Fig. 6).

In conclusion, OPP differs significantly across NHPs, and a diurnal cycle characterized by significantly lower OPP during sleep and higher OPP during waking was present in eight of the 12 NHPs; surprisingly, the opposite pattern was observed in three NHPs. Further work must be done to understand the presence and prevalence of a diurnal cycle of OPP, how it is intertwined with BP and IOP fluctuations, and how this rhythm ultimately influences both ocular physiology and disease. Results show that OPP differences among animals are both large and change over time, which may put the NHPs with periods of low OPP at risk for ischemic damage at lower IOP levels. Finally, we expected OPP to remain relatively stable over time within individuals, but day-to-day variability was the largest source of OPP variability, even larger than the difference among individuals. This result strongly suggests that infrequent snapshot measurements of either IOP or BP are insufficient to capture true IOP, BP, and OPP and their fluctuations.

Acknowledgments

The authors thank Lisa Hethcox, LVT, and Candice Jackson, LVT, for their invaluable assistance in data acquisition and daily care of the NHPs. We also thank Chester Calvert for his invaluable assistance in data acquisition and filtering.

Supported by grants from the National Institutes of Health (R01-EY026035, JCD; P30-EY003039, BCS); by Research to Prevent Blindness (unrestricted departmental funds); and by the EyeSight Foundation of Alabama (unrestricted departmental funds)

Disclosure: **K.I. Wilson**, None; **P. Godara**, None; **J.V. Jasien**, None; **E. Zohner**, None; **J.S. Morris**, None; **C.A. Girkin**, None; **B.C. Samuels**, None; **J.C. Downs**, None

References

1. Leske MC, Wu SY, Hennis A, Honkanen R, Nemesure B, BESs Study Group. Risk factors for incident open-angle glaucoma: the Barbados Eye Studies. *Ophthalmology*. 2008;115:85–93.
2. Friedman DS, Katz J, Bressler NM, Rahmani B, Tielsch JM. Racial differences in the prevalence of age-related macular degeneration: the Baltimore Eye Survey. *Ophthalmology*. 1999;106:1049–1055.
3. Sommer A. Glaucoma risk factors observed in the Baltimore Eye Survey. *Curr Opin Ophthalmol*. 1996;7:93–98.
4. Coleman AL, Gordon MO, Beiser JA, Kass MA, Ocular Hypertension Treatment Study. Baseline risk factors for the development of primary open-angle glaucoma in the Ocular Hypertension Treatment Study. *Am J Ophthalmol*. 2004;138:684–685.
5. Nouri-Mahdavi K, Hoffman D, Coleman AL, et al. Predictive factors for glaucomatous visual field progression in the Advanced Glaucoma Intervention Study. *Ophthalmology*. 2004;111:1627–1635.
6. European Glaucoma Prevention Study (EGPS) Group,Miglior S, Pfeiffer N, et al. Predictive factors for open-angle glaucoma among patients with ocular hypertension in the European Glaucoma Prevention Study. *Ophthalmology*. 2007;114:3–9.
7. Leske MC, Wu SY, Honkanen R, et al. Nine-year incidence of open-angle glaucoma in the Barbados Eye Studies. *Ophthalmology*. 2007;114:1058–1064.
8. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90:262–267.
9. Musch DC, Gillespie BW, Niziol LM, Lichter PR, Varma R, Group CS. Intraocular pressure control and long-term visual field loss in the Collaborative Initial Glaucoma Treatment Study. *Ophthalmology*. 2011;118:1766–1773.
10. Leske MC, Heijl A, Hyman L, et al. Predictors of long-term progression in the early manifest glaucoma trial. *Ophthalmology*. 2007;114:1965–1972.
11. Goldberg I, Hollows FC, Kass MA, Becker B. Systemic factors in patients with low-tension glaucoma. *Br J Ophthalmol*. 1981;65:56–62.
12. Ahmad SS. Controversies in the vascular theory of glaucomatous optic nerve degeneration. *Taiwan J Ophthalmol*. 2016;6:182–186.
13. Cioffi GA. Ischemic model of optic nerve injury. *Trans Am Ophthalmol Soc*, 2005;103:592–613.
14. Flammer J, Orgul S, Costa VP, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res*. 2002;21:359–393.
15. Downs JC, Roberts MD, Burgoyne CF. Mechanical environment of the optic nerve head in glaucoma. *Optom Vis Sci*. 2008;85:425–435.
16. Downs JC, Girkin CA. Lamina cribrosa in glaucoma. *Curr Opin Ophthalmol*. 2017;28:113–119.
17. Downs JC. Optic nerve head biomechanics in aging and disease. *Exp Eye Res*. 2015;133:19–29.
18. Burgoyne CF, Downs JC, Bellezza AJ, Suh JK, Hart RT. The optic nerve head as a biomechanical structure: a new paradigm for understanding the role of IOP-related stress and strain in the pathophysiology of glaucomatous optic nerve head damage. *Prog Retin Eye Res*. 2005;24:39–73.
19. Leske MC. Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings. *Curr Opin Ophthalmol*. 2009;20:73–78.
20. Tham YC, Lim SH, Gupta P, Aung T, Wong TY, Cheng CY. Inter-relationship between ocular perfusion pressure, blood pressure, intraocular pressure profiles and primary open-angle glaucoma: the Singapore Epidemiology of Eye Diseases study. *Br J Ophthalmol*. 2018;102:1402–1406.
21. Zheng Y, Wong TY, Mitchell P, Friedman DS, He M, Aung T. Distribution of ocular perfusion pressure and its relationship with open-angle glaucoma: the Singapore Malay Eye Study. *Invest Ophthalmol Vis Sci*. 2010;51:3399–3404.

22. Memarzadeh F, Ying-Lai M, Chung J, Azen SP, Varma R, Los Angeles Latino Eye Study Group. Blood pressure, perfusion pressure, and open-angle glaucoma: the Los Angeles Latino Eye Study. *Invest Ophthalmol Vis Sci*. 2010;51:2872–2877.
23. Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC. Hypertension, perfusion pressure, and primary open-angle glaucoma. A population-based assessment. *Arch Ophthalmol*. 1995;113:216–221.
24. Choi J, Lee JR, Lee Y, et al. Relationship between 24-hour mean ocular perfusion pressure fluctuation and rate of paracentral visual field progression in normal-tension glaucoma. *Invest Ophthalmol Vis Sci*. 2013;54:6150–6157.
25. Choi J, Kim KH, Jeong J, Cho HS, Lee CH, Kook MS. Circadian fluctuation of mean ocular perfusion pressure is a consistent risk factor for normal-tension glaucoma. *Invest Ophthalmol Vis Sci*. 2007;48:104–111.
26. Kwon J, Jo YH, Jeong D, Shon K, Kook MS. Baseline systolic versus diastolic blood pressure dip and subsequent visual field progression in normal-tension glaucoma. *Ophthalmology*. 2019;126:967–979.
27. Kwon J, Lee J, Choi J, Jeong D, Kook MS. Association between nocturnal blood pressure dips and optic disc hemorrhage in patients with normal-tension glaucoma. *Am J Ophthalmol*. 2017;176:87–101.
28. Detry M, Boschi A, Ellinghaus G, De Plaen JF. Simultaneous 24-hour monitoring of intraocular pressure and arterial blood pressure in patients with progressive and non-progressive primary open-angle glaucoma. *Eur J Ophthalmol*. 1996;6:273–278.
29. Graham SL, Drance SM, Wijsman K, Douglas GR, Mikelberg FS. Ambulatory blood pressure monitoring in glaucoma. The nocturnal dip. *Ophthalmology*. 1995;102:61–69.
30. Yoshikawa T, Obayashi K, Miyata K, Saeki K, Ogata N. Increased nighttime blood pressure in patients with glaucoma: cross-sectional analysis of the LIGHT study. *Ophthalmology*. 2019;126:1366–1371.
31. Baek SU, Kim YK, Ha A, et al. Diurnal change of retinal vessel density and mean ocular perfusion pressure in patients with open-angle glaucoma. *PLoS One*. 2019;14:e0215684.
32. Lee NY, Jung Y, Han K, Park CK. Fluctuation in systolic blood pressure is a major systemic risk factor for development of primary open-angle glaucoma. *Sci Rep*. 2017;7:43734.
33. Sung KR, Cho JW, Lee S, et al. Characteristics of visual field progression in medically treated normal-tension glaucoma patients with unstable ocular perfusion pressure. *Invest Ophthalmol Vis Sci*. 2011;52:737–743.
34. Renard E, Palombi K, Gronfier C, et al. Twenty-four hour (nyctohemeral) rhythm of intraocular pressure and ocular perfusion pressure in normal-tension glaucoma. *Invest Ophthalmol Vis Sci*. 2010;51:882–889.
35. Sung KR, Lee S, Park SB, et al. Twenty-four hour ocular perfusion pressure fluctuation and risk of normal-tension glaucoma progression. *Invest Ophthalmol Vis Sci*. 2009;50:5266–5274.
36. Choi J, Jeong J, Cho HS, Kook MS. Effect of nocturnal blood pressure reduction on circadian fluctuation of mean ocular perfusion pressure: a risk factor for normal tension glaucoma. *Invest Ophthalmol Vis Sci*. 2006;47:831–836.
37. Xu L, Wang YX, Jonas JB. Ocular perfusion pressure and glaucoma: the Beijing Eye Study. *Eye (Lond)*. 2009;23:734–736.
38. Ramdas WD, Wolfs RC, Hofman A, de Jong PT, Vingerling JR, Jansonius NM. Ocular perfusion pressure and the incidence of glaucoma: real effect or artifact? The Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2011;52:6875–6881.
39. Downs JC, Burgoyne CF, Seigfreid WP, Reynaud JF, Strouthidis NG, Sallee V. 24-Hour IOP telemetry in the nonhuman primate: implant system performance and initial characterization of IOP at multiple timescales. *Invest Ophthalmol Vis Sci*. 2011;52:7365–7375.
40. Quigley HA. Use of animal models and techniques in glaucoma research: introduction. *Methods Mol Biol*. 2018;1695:1–10.
41. Burgoyne CF. The non-human primate experimental glaucoma model. *Exp Eye Res*. 2015;141:57–73.
42. Markert JE, Jasien JV, Turner DC, Huisingh C, Girkin CA, Downs JC. IOP, IOP transient impulse, ocular perfusion pressure, and mean arterial pressure relationships in nonhuman primates instrumented with telemetry. *Invest Ophthalmol Vis Sci*. 2018;59:4496–4505.
43. Turner DC, Edmiston AM, Zohner YE, et al. Transient intraocular pressure fluctuations: source, magnitude, frequency, and associated mechanical energy. *Invest Ophthalmol Vis Sci*. 2019;60:2572–2582.
44. Turner DC, Girkin CA, Downs JC. The magnitude of intraocular pressure elevation associated with eye rubbing. *Ophthalmology*. 2019;126:171–172.
45. Turner DC, Miranda M, Morris JS, Girkin CA, Downs JC. Acute stress increases intraocular pressure in nonhuman primates. *Ophthalmol Glaucoma*. 2019;2:210–214.
46. Jasien JV, Girkin CA, Downs JC. Effect of anesthesia on intraocular pressure measured with continuous wireless telemetry in nonhuman primates. *Invest Ophthalmol Vis Sci*. 2019;60:3830–3834.
47. Jasien JV, Samuels BC, Johnston JM, Downs JC. Diurnal cycle of translaminar pressure in nonhuman primates quantified with continuous wireless telemetry. *Invest Ophthalmol Vis Sci*. 2020;61:37.
48. Jasien JV, Huisingh C, Girkin CA, Downs JC. The magnitude of hypotony and time course of intraocular pressure recovery following anterior chamber cannulation in nonhuman primates. *Invest Ophthalmol Vis Sci*. 2017;58:3225–3230.
49. Turner DC, Samuels BC, Huisingh C, Girkin CA, Downs JC. The magnitude and time course of IOP change in response to body position change in nonhuman primates measured using continuous IOP telemetry. *Invest Ophthalmol Vis Sci*. 2017;58:6232–6240.
50. He Z, Vingrys AJ, Armitage JA, Bui BV. The role of blood pressure in glaucoma. *Clin Exp Optom*. 2011;94:133–149.
51. Quaranta L, Katsanos A, Riva I, et al. Twenty-four-hour intraocular pressure and ocular perfusion pressure characteristics in newly diagnosed patients with normal tension glaucoma. *Eye (Lond)*. 2016;30:1481–1489.
52. Jasien JV, Turner DC, Girkin CA, Downs JC. Cyclic pattern of intraocular pressure (IOP) and transient IOP fluctuations in nonhuman primates measured with continuous wireless telemetry. *Curr Eye Res*. 2019;44:1244–1252.

Investigative Ophthalmology & Visual Science