

Circadian Variation of Aqueous Humor Dynamics in Older Healthy Adults

Cherie B. Nau,¹ Mehrdad Malihi,¹ Jay W. McLaren,¹ David O. Hodge,² and Arthur J. Sit¹

¹Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota

²Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota

Correspondence: Arthur J. Sit, Department of Ophthalmology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905; sit.arthur@mayo.edu.

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PURPOSE. Intraocular pressure in the sitting position changes minimally during sleep, although aqueous humor flow decreases by 50% or more at night. The explanation for this apparent discrepancy has been unclear. This study investigated the roles of outflow facility, episcleral venous pressure (EVP), and uveoscleral outflow in maintaining IOP at night.

METHODS. Forty-two eyes of 21 healthy subjects (age 47–76 years, mean 59 years) were studied. Aqueous humor flow rate, IOP in the sitting position, outflow facility, and EVP were measured in each eye during the mid-diurnal period (2:00–4:00 PM). Uveoscleral flow was calculated from the other variables by using the modified Goldmann equation. These variables were remeasured during the mid-nocturnal period (2:00–4:00 AM) and compared with those measured during the diurnal period by using generalized estimating equation models.

RESULTS. Intraocular pressure did not change from the mid-diurnal period (13.9 ± 3.0 mm Hg) to the mid-nocturnal period (13.0 ± 1.8 mm Hg, mean \pm SD, $P = 0.07$), although aqueous humor flow rate decreased from 2.48 ± 0.96 μ L/min to 1.27 ± 0.63 μ L/min ($P < 0.001$). Outflow facility decreased from 0.23 ± 0.06 μ L/min/mm Hg to 0.20 ± 0.06 μ L/min/mm Hg ($P = 0.004$), and EVP was unchanged from the mid-diurnal period (7.4 ± 1.8 mm Hg) to the mid-nocturnal period (7.4 ± 2.2 mm Hg, $P = 0.95$). Uveoscleral outflow decreased 93%, from 0.94 ± 1.26 μ L/min during the mid-diurnal period to 0.07 ± 0.78 μ L/min ($P = 0.008$) during the mid-nocturnal period.

CONCLUSIONS. The nocturnal decrease in aqueous humor flow rate in older, healthy subjects is compensated by a small decrease in outflow facility and a large decrease in uveoscleral outflow to maintain a stable IOP.

Keywords: aqueous humor dynamics, uveoscleral pathway, aqueous flow, outflow facility, episcleral venous pressure

Intraocular pressure measured in the habitual positions (sitting while awake and supine during sleep) is highest during sleep in most subjects.^{1–5} When IOP is measured at night in the sitting position, it remains close to or slightly less than IOP during the day.^{1–5} This behavior is the opposite of that of aqueous humor flow, which decreases by as much as 50% during sleep.^{6–8} Because IOP is directly related to aqueous humor flow, the dramatic decrease in flow rate must be compensated by a decrease in outflow facility, an increase in episcleral venous pressure, or a diminished nonconventional outflow during sleep.

In an earlier study, outflow facility decreased somewhat at night, but the decrease was not enough to compensate entirely for the typical decrease in aqueous humor flow rate⁹ and we were not able to detect this pattern in young, healthy subjects.¹⁰ An increase in episcleral venous pressure (EVP) at night, if such an increase exists, might compensate for the decreased flow rate.^{9,10} Two studies found an increased EVP at night when measured with the subject supine at night and seated during the day.^{11,12} However, one of these studies found that EVP remained consistent if the subject did not change positions.¹¹ As well, subjective endpoints used for previous measurements of EVP have left the nocturnal pattern of EVP unclear.¹³

Differences in uveoscleral flow might also explain the minimal IOP differences between day and night. Uveoscleral flow cannot be measured directly but must be calculated from IOP, aqueous humor flow, outflow facility, and EVP measured at approximately the same time. One study estimated a large decrease in nighttime uveoscleral flow in healthy subjects, when using seated IOP, tonographic outflow facility, and an assumed nighttime EVP based on EVP measured during the day.⁹ However, the lack of objective measurement of EVP left the calculation of uveoscleral flow rate uncertain. An understanding of the relatively unchanged IOP at night, a time when aqueous humor production is naturally low, will require measurement of all variables of aqueous humor dynamics during the night as well as during the day. In this study, we investigated the circadian variation of aqueous humor dynamics in older, healthy subjects.

METHODS

Study Subjects

Twenty-one participants in good general health, between the ages of 47 and 76 years (mean 59.0 ± 9.3 years, \pm SD), with regular sleep schedules were recruited from employees and

patients of Mayo Clinic, or local area residents. Patients with chronic disease (e.g., hypertension) were included if their condition was stable, and they did not require treatment other than medications. None of the participants were using systemic steroids or beta-adrenergic blockers, or had any history of ocular pathology including intraocular surgery or trauma, narrow angles, glaucoma, diabetic eye disease, uveitis, high myopia (>6 diopters [D]), or high hyperopia (>4 D). Each subject gave informed consent to participate after discussion of the nature and possible consequences of the study. This study followed the tenets of the Declaration of Helsinki and was approved by the institutional review board at Mayo Clinic.

Measurements

Intraocular Pressure. Intraocular pressure was measured in the sitting and supine positions by using a pneumatonometer (Model 30 Classic; Medtronic Ophthalmics, Jacksonville, FL) after instilling a topical anesthetic (proparacaine, 0.5%). Subjects remained seated or supine for at least 5 minutes prior to measurements. The mean of three consecutive measurements was recorded as the IOP.

Aqueous Humor Flow Rate. The aqueous humor flow rate was calculated from the clearance of fluorescein from the combined cornea and anterior chamber, as described previously¹⁴:

$$\text{Flow} = \frac{\Delta M_f}{C_a \Delta t} - 0.25 \mu\text{L}/\text{min} \quad (1)$$

where ΔM_f is the mass of fluorescein lost from the combined cornea and anterior chamber during interval Δt , C_a is the average concentration of fluorescein in the anterior chamber during the same interval (estimated from the initial and final concentrations, assuming an exponential decay), and $0.25 \mu\text{L}/\text{min}$ is the flow rate equivalent to the assumed diffusional loss of fluorescein from the eye.¹⁵⁻¹⁸ Fluorescein concentrations in the anterior chamber and cornea were measured by using a scanning ocular fluorophotometer,¹⁹⁻²¹ and the mass in each compartment was the product of its volume and the mean fluorescein concentration. Anterior chamber volume was measured photogrammetrically,²² and cornea volume was assumed to be $70 \mu\text{L}$ in all subjects.^{14,23}

Outflow Facility. Outflow facility was measured by using a custom digital tonometer based on an existing electronic Schiötz tonometer (Berkeley Bio-Engineering, Inc., San Leandro, CA).²⁴ A 4-minute tracing with a 5.5-g weight was recorded. Outflow facility was calculated from the beginning and end points of a second order polynomial, fitted to the pressure decay curves by a least-squares method, and standard tables.²⁵ Facility was measured at the end of the mid-diurnal and mid-nocturnal periods. Prior to tonography, topical proparacaine 0.5% was instilled in the eye as an anesthetic.

Episcleral Venous Pressure (EVP). Episcleral venous pressure was measured by using a novel device that has been described in detail elsewhere.^{15,26} In brief, the technique determined the pressure that was required to initiate collapse of an episcleral vein. A high-definition video camera captured images of the vein as it was compressed by an air chamber with a flexible and clear wall. The pressure in the chamber was increased linearly and the pressure associated with each video frame was recorded by a computer as the vein collapsed.

Episcleral veins were identified in the video stream as being straighter and deeper than the conjunctival vessels and were often bifurcated. The vein was assumed to be uncollapsed prior to increasing the pressure in the balloon above ambient pressure. As the chamber inflated, the vein began to collapse, and this was seen in the video sequence as a reduction of

vessel intensity and sometimes as vessel narrowing. Image processing techniques were used to identify the frame where the vessel just began to collapse, and we assumed that the pressure associated with that frame corresponded to EVP.

Uveoscleral Flow Rate. Uveoscleral flow rate cannot be measured directly in humans, but must be estimated from the modified Goldmann equation:

$$\text{IOP} = \text{EVP} + \frac{1}{c}(Q - U) \quad (2)$$

where Q is the aqueous humor flow rate, c is the conventional (trabecular) outflow facility, and U is the pressure-insensitive uveoscleral outflow rate. Uveoscleral outflow can be estimated by rearranging equation 2:

$$U = Q - c(\text{IOP} - \text{EVP}) \quad (3)$$

This expression represents the difference between the production rate of aqueous humor measured by fluorophotometry and the estimate of trabecular flow from outflow facility and the pressure difference that moves aqueous humor out of the eye.

Blood Pressure. Blood pressure was measured with an automated blood pressure cuff (Model HEM-780; Omron Healthcare, Inc., Vernon Hills, IL). The cuff was placed on the right arm, with the subject in the seated position, immediately after the seated IOP measurements.

Experimental Protocol

All subjects received a dilated eye examination and an updated medical history was recorded. One-week prior to the study session, subjects were asked to maintain a regular sleep cycle of 8-hours sleep and 16-hours wake. Subjects were equipped with a wrist actigraph to monitor physical activity (Actiwatch AW-L; Mini Mitter, Bend, OR). They were also asked to maintain a log with sleep and wake times. Subjects' sleep patterns were used to adjust the actual time of the measurements during the study, so that the nocturnal period for each subject was equivalent to 11 PM to 7 AM. Contact lens wearers (3 subjects) were asked to stop wearing their contact lenses one-week prior to the study in order to minimize any potential effect on permeability of the corneal epithelium to fluorescein and provide a consistent initial depot of fluorescein in the stroma.

On the day of the study the subjects were asked to maintain a regular schedule with normal activities. The subjects instilled 2 to 3 drops of topical 2% fluorescein in each eye between 1 AM and 2 AM on the study day, and carefully cleaned excess fluorescein from their lashes and lid margins prior to returning to sleep.

Subjects reported to the Clinical Research Unit (CRU) at Mayo Clinic at 1 PM on the day of the study. Meals were provided and subjects continued normal activities while at the CRU, except that they were asked to refrain from exercise. They were permitted to leave the CRU for brief periods but were asked to remain in the hospital.

At the beginning of the mid-diurnal period (2:00 PM), fluorescein concentration was measured in the anterior segment of each eye by using fluorophotometry. This measurement was repeated at end of the mid-diurnal period (4:00 PM). Episcleral venous pressure was measured immediately after fluorophotometry, and this was followed by measurement of IOP and blood pressure. Outflow facility was measured at the end of the diurnal measurements. Immediately after tonography, subjects instilled another 2 to 3 drops of 2% fluorescein in each eye, depending on the measured anterior chamber fluorescein concentration. After cleaning excess fluorescein from the lashes and lid margins,

TABLE 1. Study Population Baseline Characteristics

Parameter	Value
Number of patients	21
Number of eyes	42
Age, y \pm SD	58.5 \pm 9.1
Range	47–76
Sex	
Female, number	12
Male, number	9
IOP, mm Hg \pm SD, at screening	14.7 \pm 2.9
Refractive error, diopters \pm SD	−0.30 \pm 1.0
Range	1.8 to −3.4 (spherical equivalent)
Central corneal thickness, μ m \pm SD	541.9 \pm 26.2
Range	488–629

subjects lay supine for 20 minutes with eyes closed to ensure a uniform distribution of the fluorescein in the cornea. Subjects were asked to go to sleep at their regular times based on their sleep patterns from the previous week.

The subjects were awakened at the beginning of the mid-nocturnal period (2:00 AM) for a third set of fluorophotometry measurements, after which subjects returned to sleep. At the end of the midnocturnal period (4:00 AM), anterior segment fluorescence was measured a fourth and final time. This was followed by measurement of EVP, IOP, blood pressure, and outflow facility. During the nocturnal period, all measurements were performed under reduced light conditions (approximately 10 lux white light). Previous reports indicated that a moderate light exposure at night does not affect the nocturnal IOP pattern.²⁷ Total time for all measurements in the midnocturnal period was 20 to 30 minutes.

Statistical Analysis

Mean IOP, aqueous humor flow rates, EVP, outflow facility, and uveoscleral flow rates from the midnocturnal period were compared with the same variables from the middiurnal period. Both eyes were included in statistical analysis, and diurnal and nocturnal data were compared by using generalized estimating equation models to account for possible correlation between the two eyes of each subject.

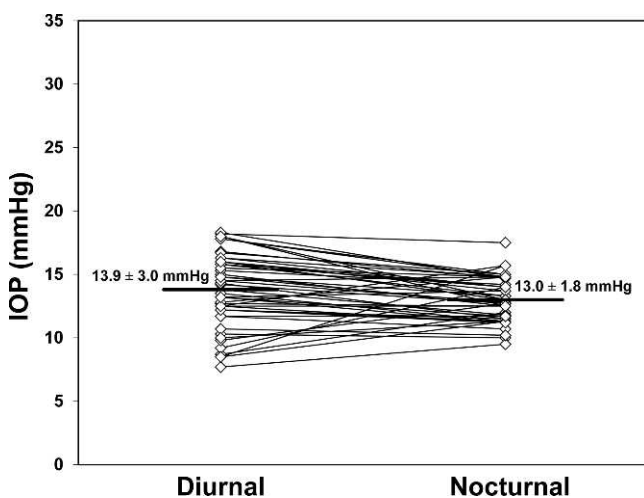


FIGURE 1. Intraocular pressure measured in the sitting position did not change from the middiurnal period (13.9 \pm 3.0 mm Hg) to the midnocturnal period (13.0 \pm 1.8 mm Hg, mean \pm SD, $P = 0.07$).

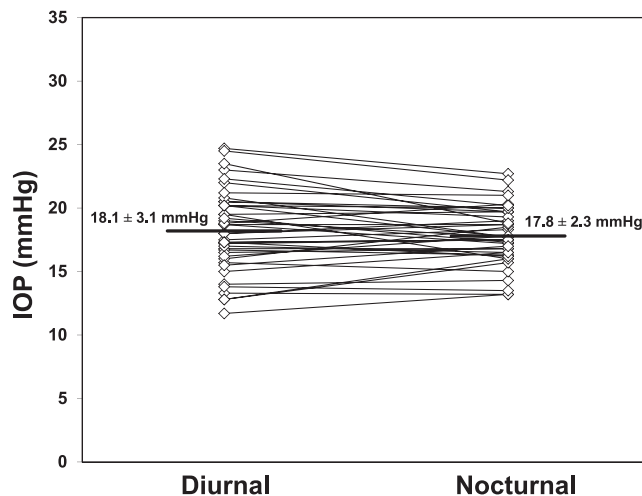


FIGURE 2. Intraocular pressure measured in the supine position did not change from the middiurnal period (18.1 \pm 3.1 mm Hg) to the midnocturnal period (17.8 \pm 2.3 mm Hg, mean \pm SD, $P = 0.42$).

period was compared with blood pressure in the midnocturnal period using paired t -tests. Differences were considered significant if P was less than 0.05.

Our sample size was estimated to detect a 20% difference between diurnal and nocturnal outflow facilities. If diurnal outflow facility in our sample were 0.25 \pm 0.10 μ L/min/mm Hg (mean \pm SD), similar to that of other healthy subjects,^{8,10,28–30} 34 eyes would be required to have a 90% chance of detecting a 20% difference between diurnal and nocturnal outflow facility if such a difference actually exists (paired t -test, $\alpha = 0.05$, $\beta = 0.10$).

RESULTS

Experimental Results

Forty-two eyes of 21 subjects were enrolled in the study and analyzed. The ages of the subjects ranged from 47 to 76 years (58.5 \pm 9.1 years, mean \pm SD). The baseline characteristics of subjects are shown in Table 1. One subject was being treated for hypertension with hydrochlorothiazide, eight subjects were taking daily aspirin, and six were being treated with simvastatin.

Intraocular pressure in the sitting position (Fig. 1) did not change from the middiurnal period (13.9 \pm 3.0 mm Hg, mean \pm SD) to the midnocturnal period (13.0 \pm 1.8 mm Hg, $P = 0.07$). Intraocular pressure in the supine position (Fig. 2) also did not change from the middiurnal period (18.1 \pm 3.1 mm Hg) to the midnocturnal period (17.8 \pm 2.3 mm Hg, $P = 0.42$). During both the middiurnal and midnocturnal periods, the supine IOP was higher than the sitting IOP ($P < 0.001$).

Seated systolic blood pressure decreased from 130.5 \pm 14.0 mm Hg during the middiurnal period to 121.0 \pm 10.4 mm Hg during the midnocturnal period ($P = 0.006$). Seated diastolic blood pressure did not change, from the middiurnal period (83.5 \pm 9.1 mm Hg) to the midnocturnal period (80.7 \pm 7.8 mm Hg, $P = 0.562$).

Aqueous humor flow rate (Fig. 3) decreased from 2.48 \pm 0.96 μ L/min during the middiurnal period to 1.27 \pm 0.63 μ L/min ($P < 0.001$) during the midnocturnal period. The mean outflow facility (Fig. 4) was 0.23 \pm 0.06 μ L/min/mm Hg during the middiurnal period, and decreased to 0.20 \pm 0.06 μ L/min/mm Hg ($P = 0.004$) during the midnocturnal period. Episcleral venous pressure (Fig. 5) did not change from the middiurnal

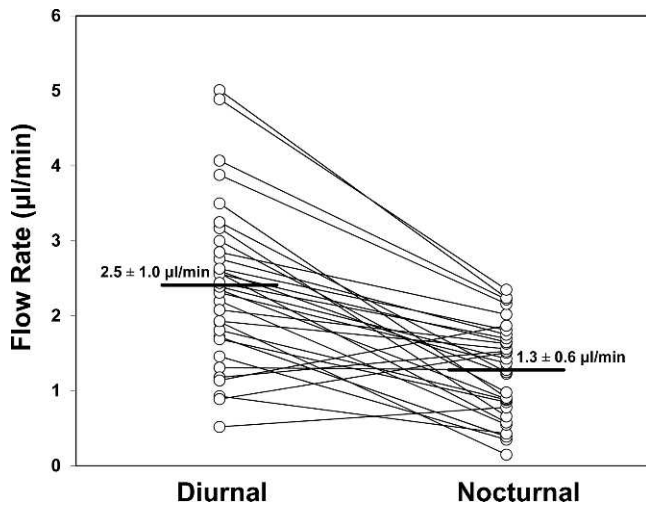


FIGURE 3. Aqueous humor flow rate decreased from $2.5 \pm 1.0 \mu\text{L}/\text{min}$ (mean \pm SD) during the mid-diurnal period to $1.3 \pm 0.6 \mu\text{L}/\text{min}$ during the mid-nocturnal period ($P < 0.001$).

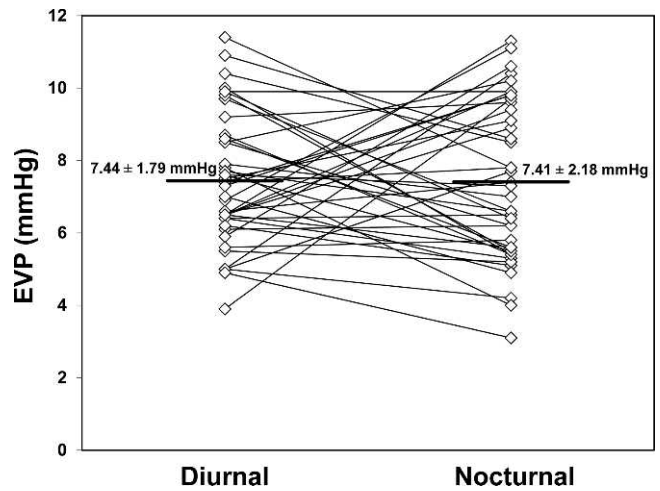


FIGURE 5. Episcleral venous pressure measured by using objective venomanometry did not change from the mid-diurnal period ($7.44 \pm 1.79 \text{ mmHg}$) to the mid-nocturnal period ($7.41 \pm 2.18 \text{ mmHg}$, mean \pm SD, $P = 0.95$).

period ($7.44 \pm 1.79 \text{ mmHg}$) to the mid-nocturnal period ($7.41 \pm 2.18 \text{ mmHg}$, $P = 0.95$)

The uveoscleral outflow rate (Fig. 6) calculated by using Equation 3 was $0.94 \pm 1.26 \mu\text{L}/\text{min}$ during the mid-diurnal period and decreased to $0.07 \pm 0.78 \mu\text{L}/\text{min}$ during the mid-nocturnal period ($P = 0.008$, Table 2), a reduction of 93%. Uveoscleral outflow rate comprised 38.0% of total aqueous humor flow during the mid-diurnal period, and 5.6% of total flow during the mid-nocturnal period.

DISCUSSION

Diurnal rhythms in IOP have been well documented, starting with the work of Drance in the 1960s.³¹ Subsequent investigators examined the 24-hour pattern of IOP variation and found that glaucoma patients exhibited a drop in IOP during the night.^{32,33} However, these studies measured IOP in the sitting position by Goldmann applanation tonometry. Liu et al.^{1,2,5} demonstrated that IOP measured in the habitual

positions (sitting while awake and supine while asleep) was significantly higher during the nocturnal period than the diurnal period. When IOP was measured in the supine position during the day and night, glaucoma patients showed a slight decrease in IOP at night,⁵ while healthy subjects showed a slight increase.^{1,2} In the present study, we did not find any change in the nocturnal IOP compared with diurnal IOP when both were measured in the same body position. However, when measured in habitual positions (sitting while awake, supine during sleep), nocturnal IOP was significantly higher than diurnal IOP, consistent with the work of Liu et al.^{1,2,5}

In this study, outflow facility decreased somewhat from day to night, and this is similar to the decrease in nocturnal outflow facility in healthy subjects reported by Lui et al.⁹ and ocular hypertensive subjects reported by Fan et al.¹² It is not clear why outflow facility decreases at night. Acute changes in outflow facility can be induced with parasympathomimetics, such as pilocarpine.³⁴ However, nocturnal parasympathetic activity is typically higher than diurnal activity,³⁵ as is ciliary muscle tone.³⁶ This would be expected to increase outflow

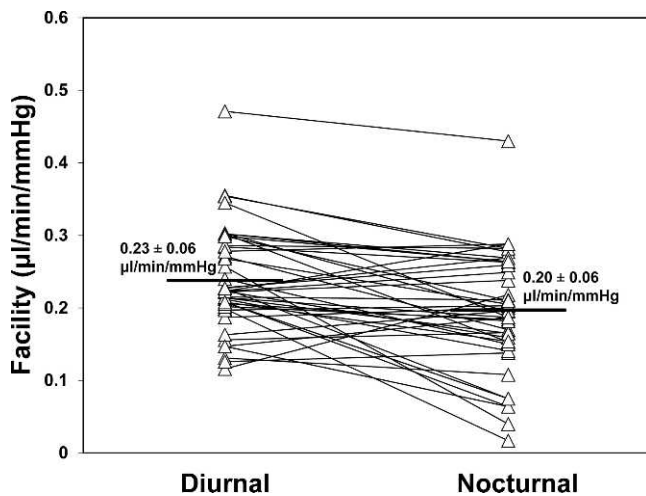


FIGURE 4. Outflow facility measured by using tonography decreased significantly from $0.23 \pm 0.06 \mu\text{L}/\text{min}/\text{mmHg}$ (mean \pm SD) during the mid-diurnal period to $0.20 \pm 0.06 \mu\text{L}/\text{min}/\text{mmHg}$ during the mid-nocturnal period ($P = 0.004$).

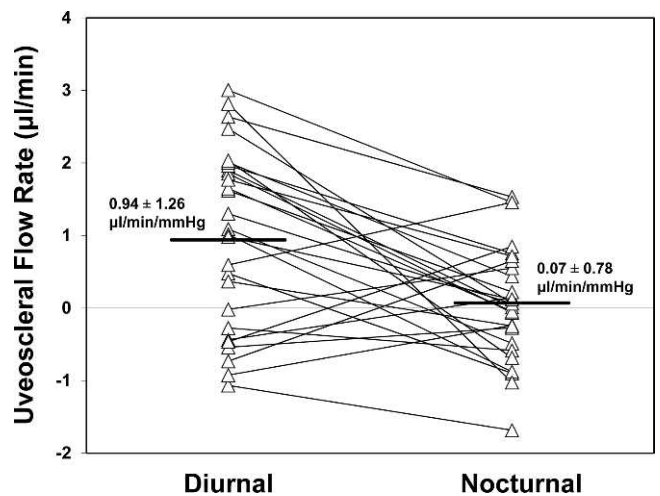


FIGURE 6. Uveoscleral outflow rate calculated by using the modified Goldmann equation decreased from $0.94 \pm 1.26 \mu\text{L}/\text{min}$ during the mid-diurnal period to $0.07 \pm 0.78 \mu\text{L}/\text{min}$ during the mid-nocturnal period ($P = 0.008$), a reduction of 93%.

TABLE 2. Aqueous Humor Dynamics Parameters During the Middiurnal and Midnocturnal Periods (\pm SD)

	Sitting IOP, mm Hg	Aqueous Humor Flow Rate, μ L/min	Outflow Facility, μ L/min/mm Hg	EVP, mm Hg	Uveoscleral Outflow, μ L/min
Diurnal	13.9 \pm 3.0	2.5 \pm 1.0	0.23 \pm 0.06	7.4 \pm 1.8	0.94 \pm 1.26
Nocturnal	13.0 \pm 1.8	1.3 \pm 0.6	0.20 \pm 0.06	7.4 \pm 2.2	0.07 \pm 0.78
<i>P</i>	0.07	<0.001	0.004	0.95	0.008

facility rather than decrease it as we observed. As well, our previous study of younger, healthy subjects did not find a change in outflow facility from day to night. It is possible that as subjects get older, the day–night differences in outflow facility may increase, as suggested by Liu et al.⁹ The mean age of subjects in the previous study was 29 years, which was younger than the mean age of 59 years in this study. However, the reasons for decreases in outflow facility at night are unknown at this time.

Episcleral venous pressure did not change from day to night, and this is consistent with recent work by Lui et al.⁹ Uveoscleral outflow, calculated from the other measured variables, decreased by over 90% from the middiurnal period to the midnocturnal period. However, the reason for this decrease is unclear because our understanding of uveoscleral outflow continues to evolve. One possible explanation is related to the increase in ciliary muscle tone at night.³⁶ Pilocarpine decreases uveoscleral flow in monkeys, likely by closing intramuscular spaces in the ciliary body, and as a consequence decreasing flow through this path.³⁷ It is also possible that active contractions of the ciliary muscle are required to move fluid through the uveoscleral system, similar to microlymphatic vessels, which lack muscular walls and any significant pressure gradient and requires muscle contraction to move fluid.³⁸ If this is the case, then increased ciliary muscle tone at night may reduce active contractions, reducing movement of aqueous humor through the uveoscleral path. However, the nature of uveoscleral outflow and its changes at night continue to be elucidated.

The circadian change in aqueous humor dynamics is important for glaucoma therapy if similar patterns exist in glaucoma patients. For example, aqueous humor flow decreases at night most likely because of the nocturnal lack of sympathetic tone. Beta-adrenergic antagonists cannot reduce aqueous humor flow rate further at night because there is not sufficient sympathetic stimulation to block.^{39,40} Unlike beta-antagonists, prostaglandin analogs reduce pressure at night, but not as well as they do during the day.^{41–44} Prostaglandin analogs, however, may reduce pressure through two actions, one that improves outflow facility (although this has not been a universal finding) and the other that increases uveoscleral flow.^{45–47} A study by Gulati et al.⁴⁸ suggested that latanoprost might increase uveoscleral outflow at night, although their study was not sufficiently powered to show a statistically significant difference. If prostaglandin analogs increase uveoscleral flow by a percentage, rather than by a fixed amount, then the nocturnal effect on absolute uveoscleral flow rate would be small, because uveoscleral flow is markedly reduced at night. This small change in absolute uveoscleral flow rate would be more difficult to detect, consistent with the findings of Gulati et al.⁴⁸ In contrast, outflow facility decreased by only approximately 15% at night in our subjects, which suggests that improving outflow facility may be a possible mechanism for reducing nocturnal IOP. It is possible that prostaglandin analogs, which have persistent nocturnal efficacy, may use this mechanism. Other medications that improve outflow facility, such as parasympathomimetics, may also be

good options for lowering IOP for similar reasons, but their nocturnal efficacy needs to be investigated.

Uveoscleral outflow was calculated from the difference in trabecular flow, estimated from our measurements of outflow facility, IOP, and EVP, and the total aqueous humor flow calculated from fluorescein clearance, and this difference is sensitive to all of the variables measured. At night, the estimate of aqueous humor flow rate is accurate because it is based on clearance of fluorescein during the 2-hour interval that subjects slept, and aqueous humor flow is independent of body position.⁴⁹ However, IOP, EVP, and outflow facility were measured during a short time and could be affected by transients shortly after subjects were awoken. For example, the process of waking can transiently elevate IOP with a rapid decay to baseline over several minutes. It is unclear if the nocturnal IOP that we measured was affected by this transient or was representative of true IOP during sleep. Subjects were awake for approximately 5 to 10 minutes during fluorophotometry before EVP measurements, and even longer before IOP and outflow facility measurements, sufficient time for transients to return to baseline in most subjects. However, the question of whether these measurements represent a sleeping, awake, or a transient state cannot be answered at this time. Any systematic difference between our estimate of IOP, EVP, or outflow facility, and the true value during sleep could systematically shift the estimate of flow through the trabecular meshwork and this would influence our estimate of uveoscleral flow. Ideally, we would measure uveoscleral outflow by a method independent of the other variables, although such a noninvasive method, suitable for use in human volunteers, is not available.

In summary, aqueous humor flow rate decreases by approximately 50% during the midnocturnal period as compared with the middiurnal period. In contrast, IOP remains remarkably similar from day to night after accounting for differences in body position. This stable pressure seems to be maintained by a small reduction in outflow facility accompanied by a large reduction in uveoscleral flow rate. Further research is required to determine if glaucoma patients experience similar changes.

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