Aqueous Humor Dynamics During the Day and Night in Volunteers With Ocular Hypertension

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Objective: To evaluate the differences in aqueous humor dynamics between nighttime and daytime in participants with ocular hypertension.

Methods: Thirty participants (mean [SD] age, 59.2 [11.1] years) with ocular hypertension were enrolled in the study, which included 1 daytime and 1 nighttime visit. During each visit, measurements included central cornea thickness by ultrasound pachymetry, intraocular pressure (IOP) by pneumatonometry, aqueous flow by fluorophotometry, outflow facility by tonography, and blood pressure by sphygmomanometry. Uveoscleral outflow was calculated using the Goldmann equation. Daytime measurements were made only of episcleral venous pressure by venomanometry, anterior chamber depth by A-scan, and outflow facility by fluorophotometry. Repeated-measures analysis of variance and 2-tailed t tests were used for statistical comparisons.

Results: Compared with daytime seated IOP (21.3 [3.5] mm Hg), nighttime seated IOP (17.2 [3.7] mm Hg) was reduced (P < .001) and nighttime supine IOP (22.7 [4.6] mm Hg) was increased (P = .03). Central cornea thickness was increased at night from 570 (39) µm to 585 (46) µm (P < .001). There was a 48% nocturnal reduction in aqueous flow from 2.13 (0.71) µL/min during the day to 1.11 (0.38) µL/min at night (P < .001). Uveoscleral outflow was significantly reduced (P = .03) by 0.61 µL/min at night when using supine IOP, tonographic outflow facility, and episcleral venous pressure adjusted for postural changes in the Goldmann equation. All other measurements had no significant changes.

Conclusions: Significant ocular changes occur at night in individuals with ocular hypertension, including a reduction in seated IOP but an increase in habitual IOP, thickening of the cornea, and decreases in aqueous flow and uveoscleral outflow. Outflow facility does not change significantly at nighttime.

Arch Ophthalmol. 2011;129(9):1162-1166

Elevated intraocular pressure (IOP) is a risk factor for glaucoma,1 the second leading cause of blindness worldwide.2 Elevated IOP can be traced predominantly to abnormalities in aqueous humor outflow pathways.3 Studies4-5 of patients with pigment dispersion syndrome, exfoliation syndrome, ocular hypertension (OHT), glaucomatocyclitic crisis, and glaucoma have shown that the cause of the IOP increase is predominantly a reduction in outflow facility or uveoscleral outflow. A less common cause for elevated IOP is increased episcleral venous pressure associated with Sturge-Weber syndrome.6 It has been well established for decades that IOP has a distinctive 24-hour pattern established in part by a nocturnal reduction in aqueous flow.8 Recent studies9,10 have shown that drainage of aqueous humor in healthy individuals also exhibits a 24-hour pattern. Although it has been reported9,8,11 that patients with OHT have reduced trabecular outflow facility and uveoscleral outflow during the daytime compared with age-matched controls, there is little information about nocturnal changes in outflow facility or uveoscleral outflow that accompany the nocturnal changes in IOP.

Elevated nocturnal IOP might play a significant role in the development of glaucomatous damage.12,13 A study of physiologic patterns of 24-hour aqueous humor dynamics in OHT can provide insights into the pathophysiologic characteristics of nocturnal IOP elevation. It also can be used to help select the optimal time to administer IOP-lowering medications to ensure adequate continuous IOP control and attenuate progression of glaucoma-related optic nerve damage. The present study investigated daytime vs nighttime differences in IOP, aqueous humor dynamics, central corneal thickness, and blood pressure in participants with OHT.

Methods: Individuals having a diagnosis of OHT for at least 6 months were invited to participate in the study. For the purpose of this study, OHT was defined as at least 2 prior recordings (sepa-
rated by ≥1 month) of IOP above 20 mm Hg, without use of any IOP-lowering medications. In addition, the elevated IOP had to be unrelated to trauma or corticosteroid use. For some participants, elevated IOP may have been recorded before antiglaucoma treatment was initiated. Ocular medications were not discontinued to determine whether an individual’s IOP was above the necessary level for eligibility. All participants had normal appearance of the optic disc and no glaucomatous defects on visual field testing. This study was approved by the University of Nebraska Institutional Review Board before enrollment of any participants. After providing informed consent, potential participants were given a screening examination, which included gonioscopy, tonometry, and a dilated fundus examination. They were excluded for history of glaucoma, chronic or recurrent severe ocular inflammation, previous ocular surgical procedures, a gonioscopic angle less than Schaffer grade 2,14 IOP higher than 35 mm Hg or lower than 20 mm Hg without medication, or a cup-disc ratio larger than 0.8.

Participants were instructed to cease all IOP-lowering medications for 6 weeks before the study’s start. During this washout period, they returned to the clinic every 2 weeks to ensure that their IOP remained within safe limits, as determined by the treating physician. The 30 participants (10 men and 20 women) had a mean (SD) age of 59.2 (11.1) years (range, 38-81 years). Twenty-two were white, 6 were African American, 1 was Native American, and 1 was Hispanic. Seventeen individuals who were using topical ocular medications discontinued the treatment before the screening visit. Twenty-nine participants completed all study visits; 1 individual withdrew from the study after the daytime visit because of a previously unknown allergy to acetazolamide.

After a screening visit, participants were scheduled for 2 study visits: 1 daytime visit (day 1) and 1 nighttime visit (day 3). The night before the daytime measurements, start- ing between 10 PM and midnight, participants self-administered 1 drop of fluorescein sodium, 2%, in each eye every 5 minutes, for a total of 6 to 8 drops. At 9 AM, central corneal thickness was measured by ultrasound pachymetry, anterior chamber depth by A-scan (Pacscan Series 300; Sonomed, Lake Success, New York), and seated IOP by pneumatonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, New York). Aqueous flow was determined during the next 3 hours by fluorophotometry, using an ocular fluorophotometer (Fluorotron Master; OcuMetrics, Mountainview, California) every 45 minutes to measure the fluorescence of the cornea and anterior chamber. At noon, IOP was measured and oral acetazolamide, 500 mg, was administered. One hour later, fluorophotometry was performed, and repeated for 2 more measurements, from approximately 1 PM to 2:30 PM; IOP was measured after each set of afternoon scans. Outflow facility was calculated at each of the 3 measurements as the ratio of the change in aqueous flow to the change in IOP. The mean of the 3 values was the reported outflow facility.16 At approximately 2:30 PM, outflow facility was measured by 2-minute tonography, using the tonography setting on the pneumatonometer. Seated blood pressure was measured by sphygmomanometry at noon. Episcleral venous pressure was measured by an episcleral venometer (Eyetech, Morton Grove, Illinois) at 10 AM. The episcleral veins were identified as those that did not move when the probe was applied and were deeper than conjunctival vessels. Some vessels were seen to contain aqueous humor. Vessels were observed near the temporal limbus. The pressure behind the probe was increased until the vessel half-collapsed, then the pressure was reduced slightly to let the vessel refill and then increased again until half-collapse again. That was the point at which the pressure was recorded.17 If the difference between the 2 measurements was more than 2 mm Hg, a third measurement was taken. With the exception of tonography, all measurements during the daytime visit were made with the participant in the seated position.

Two days later, the participants returned for the nighttime measurements. This part of the study was performed in a 2-room suite of a hospital-based hotel on campus. At 5 PM, 1 drop of proparacaine hydrochloride, 0.5%, and 3 to 4 drops of fluorescein sodium, 2%, were instilled into each eye by an investigator. Participants continued their normal activities until 10 PM, when they were asked to lie in bed. In the dimly lit hotel room, IOP was measured with the participants in the supine and then seated position at 10 PM, midnight, 2 AM, and 5 AM. Fluorophotometric scans of the cornea and anterior chamber were performed immediately after each IOP measurement. Participants were instructed to return to sleep between readings. Corneal thickness and seated blood pressure were measured at 2 AM. Two-minute tonography was the last measurement of the night (3 AM). Outflow facility was not assessed by the fluorophotometric method at night because aqueous flow suppressants did not reduce IOP at night in 50% of participants in a previous study.10

During tonography, the IOP was recorded at a rate of 40 measurements per second. All IOPs during the 2 minutes that the standard weight was applied to the eye were imported into a computerized spreadsheet (Excel; Microsoft Corporation, Redmond, Washington), and a regression line of all points was generated. Outflow facility was calculated using the same formulas as the pneumatonometer software except that the initial and final IOPs were taken from the regression line at 0 and 2 minutes.

<table>
<thead>
<tr>
<th>Table 1. Study Schedule and Proceduresa</th>
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<tbody>
<tr>
<td><strong>Procedure</strong></td>
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<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Instill fluorescein sodium, 2%</td>
</tr>
<tr>
<td>Pachymetry</td>
</tr>
<tr>
<td>Episceral venomometry</td>
</tr>
<tr>
<td>Tonometry</td>
</tr>
<tr>
<td>Seated</td>
</tr>
<tr>
<td>Fluorophotometry</td>
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<tr>
<td>Aqueous flow</td>
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<tr>
<td>Outflow facility</td>
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<tr>
<td>Administer acetazolamide</td>
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<tr>
<td>BP measurement</td>
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<tr>
<td>Tonography</td>
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</table>

Abbreviations: BP, blood pressure; ND, not determined.

a Times are approximate.
For both the daytime and nighttime studies, uveoscleral outflow was calculated from the modified Goldmann equation, \( F_u = C \cdot (P_{ev} - P_{in}) \), in which \( F_u \) is uveoscleral outflow, \( F_s \) is aqueous outflow, \( C \) is outflow facility, and \( P_{ev} \) is episcleral venous pressure. Because daytime outflow facility was determined by both fluorophotometry and tonography, \( F_u \) was calculated twice, once with tonographic \( C \) and once with fluorophotometric \( C \) in the equation. Nighttime \( P_{ev} \) was not measured but was calculated as 2 mm Hg less than the individual daytime value for the seated position and 1.5 mm Hg higher than the daytime value for the supine position. These values are based on 2 previous studies \(^{18,19} \) reporting the diurnal and postural variations of episcleral venous pressure. Thus, at night, uveoscleral outflow was calculated in 2 ways: seated \( P_{ev} \) and IOP values as well as supine \( P_{ev} \) and IOPs in the Goldmann equation.

During the day and night, mean ocular perfusion pressure (MOPP) was calculated by the equation \( \text{MOPP} = \frac{2}{3}(DBP + \frac{\text{SBP} - \text{DBP}}{3}) - \text{IOP} \), in which DBP and SBP are diastolic and systolic blood pressure, respectively, and IOP is that measured at the time of the blood pressure measurement in the seated position (noon and 2 AM). For each participant, the values of each variable for both eyes were averaged. If measurements were available from just 1 eye, data from only that eye were included in the analysis. Daytime seated IOPs vs nighttime seated or supine IOPs were compared with repeated-measures analysis of variance. Two-tailed paired t tests were used to compare day vs night values for all other measures. \( P \) values <.05 were considered statistically significant.

**RESULTS**

Compared with mean (SD) daytime seated IOP (21.3 [3.5] mm Hg), mean nighttime seated IOP (17.2 [3.7] mm Hg) was reduced (\( P < .001 \)) and mean nighttime supine IOP (22.7 [4.6] mm Hg, habitual position) was increased (\( P = .03 \), **Figure**). There was a 48% nocturnal reduction in aqueous flow from 2.13 (0.71) µL/min during the day to 1.11 (0.38) µL/min at night (\( P < .001 \), **Table 2**). Uveoscleral outflow was significantly (\( P = .03 \)) reduced by 0.61 µL/min at night when using tonographic \( C \), supine IOP, and \( P_{ev} \) adjusted for postural change in the Goldmann equation (**Table 3**).
The circadian rhythm of aqueous flow remains elusive. Day and night aqueous flow rates in participants with OHT in the current study were not significantly different from those in healthy volunteers of similar age in a previous study,\textsuperscript{10} providing evidence that aqueous flow and its diurnal pattern remain normal in OHT. In the current study, there was a significantly reduced nocturnal uveoscleral outflow when calculated using daytime seated IOP, nighttime supine IOP, and adjusted nighttime supine episcleral pressure (habitual position) in the Goldmann equation. Episcleral venous pressure is markedly influenced by posture and is higher when a person is lying down than when seated.\textsuperscript{19,30} Interestingly, the higher the assumed \( P_{ev} \) value in the calculation, the greater the reduction in uveoscleral outflow at night (Table 3). A similar pattern has been reported elsewhere.\textsuperscript{37} Measurement of \( P_{ev} \), using currently available noninvasive techniques, is imprecise and highly variable. As a result, it is not unprecedented when calculating uveoscleral outflow by the Goldmann equation to obtain numerically negative \( F_u \) values. This error appears to affect all cases equally, as noted by the similar day and night differences in \( F_u \) reported in Table 3. The important finding is not the absolute value of uveoscleral outflow but that it decreased at night no matter which \( P_{ev} \) was used in the calculation. The nighttime reduction in uveoscleral outflow in participants with OHT appears to be similar to the changes reported in healthy individuals of similar age.\textsuperscript{10}

Unlike healthy people of similar age,\textsuperscript{10} outflow facility in participants with OHT did not change significantly from day to night. The 9% decrease seen in our study may not have reached statistical significance because of the small sample size and large standard deviations. An alternative explanation is that daytime outflow facility already is low in patients with OHT\textsuperscript{5,31} and is not further reduced at night.

In agreement with other studies,\textsuperscript{38-41} the current study found that the central cornea was thicker at night compared with during the day. Changes in corneal metabolism may occur under the closed eyelids during the night, such as hypoxia, increased lactate levels, decreased osmolarity, and corneal swelling.\textsuperscript{42-44} These changes appear to be unrelated to OHT because they have been found in healthy volunteers as well as participants with OHT.

Blood pressure is reported to be lower at night than during the day because of a nocturnal reduction in sympathetic nerve activity.\textsuperscript{45,46} It is higher in the seated position than in the supine position, mainly as a result of the
carotid sinus reflex due to hydrostatic effects.\textsuperscript{47} Ocular perfusion pressure is higher in the nocturnal period in healthy persons, as well as in patients with glaucoma and OHT.\textsuperscript{48-50}

The present study did not find a nocturnal dip in seated blood pressure and showed no significant change in the mean ocular perfusion pressure between day and night. This may be the result of several factors. First, only 1 blood pressure measurement was taken during the day and night in the current study, which could have missed the peak or trough values. Another factor is that the participants were awakened and seated for approximately 10 minutes before their blood pressure was measured. Pneumotonometry and fluorophotometry were performed first and sphygmomanometry was done last while the patient was seated. Thus, they may have lost some of the effects of sleep when the blood pressure was measured.

This study was based on the assumption of conservation of IOP for at least short periods for the results to be applicable in a meaningful way to the general population. However, we are aware that such conservation of circadian rhythm with IOP has been questioned recently.\textsuperscript{51,52} We made multiple measurements during the daytime visit. Considering the safety of the patients and the requirements of the methods, day and night measurements were separated by 55 hours.

In summary, the current study identified several significant nocturnal ocular changes in people with OHT, including thickening of the central cornea, decrease in aqueous flow and uveoscleral outflow, and reduction in seated IOP but increase in habitual IOP. Unlike volunteers of similar age with normal IOP, participants with OHT did not have a significant decrease in outflow facility at night.

Submitted for Publication: October 7, 2010; final revision received March 17, 2011; accepted March 22, 2011.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by Research to Prevent Blindness and an unrestricted grant from Pfizer, Inc.

Table 3. Uveoscleral Outflow Calculated With Different Adjustments Applied to the Measured \( P_{ev} \) in the Goldmann Equation\textsuperscript{a}

<table>
<thead>
<tr>
<th>( P_{ev} ), mm Hg</th>
<th>Day (Seated IOP) ((n=29))</th>
<th>Night (Supine IOP) ((n=29))</th>
<th>( \Delta F_u )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured</td>
<td>-0.59 (1.67)</td>
<td>-1.20 (1.34)</td>
<td>-0.61</td>
<td>.03</td>
</tr>
<tr>
<td>Measured + 2</td>
<td>-0.14 (1.51)</td>
<td>-0.80 (1.18)</td>
<td>-0.66</td>
<td>.02</td>
</tr>
<tr>
<td>Measured + 4</td>
<td>0.32 (1.36)</td>
<td>-0.39 (1.04)</td>
<td>-0.71</td>
<td>.01</td>
</tr>
<tr>
<td>Measured + 6</td>
<td>0.77 (1.23)</td>
<td>0.01 (0.92)</td>
<td>-0.76</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: \( F_u \), uveoscleral outflow; IOP, intraocular pressure; \( P_{ev} \), episcleral venous pressure.

\( a \)Goldmann equation: \( F_u=F_a-C(IOP-P_m) \), in which \( F_a \) indicates aqueous flow, and \( C \), tonographic outflow facility.

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