Concordance of Aqueous Humor Flow in the Morning and at Night in Normal Humans

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Purpose. To test the hypothesis that an individual shows concordance of aqueous humor flow in the morning and at night in a prospective inpatient fluorophotometry study in healthy subjects.

Methods. Flow was measured in each eye every hour between 8 AM and noon and every 2 hours between midnight and 6 AM. Morning and nighttime flows were analyzed for differences between eyes and for differences between these two time points. Concordance of individual morning and nighttime flows were studied by categorization into low, medium, or high tertiles, dot plot, and ordinary least-squares regression (OLS) scatter plot.

Results. In 28 subjects, the flow was similar between eyes within a subject with healthy eyes. In the one eye examined in each subject, the average flow was 3.12 ± 1.09 μL/min in the morning, which decreased significantly to 1.59 ± 0.58 μL/min at night. During each time period, the individual flow data were normally distributed. Concordance of an individual's morning and nighttime flows was 68%. A scatter plot of morning versus nighttime flows also supported concordance with an OLS regression fit of $r^2 = 0.45$.

Conclusions. The results provide evidence that aqueous humor flow is similar between eyes, that flow variation shows a normal distribution, and that individuals show a concordance of flow in the morning and at night. These observations support the posit that aqueous humor flow, which is a factor that contributes to the important clinical risk factor of IOP variation, is amenable to study as a quantitative trait. (*Invest Ophthalmol Vis Sci.* 2006;47: 4860–4864) DOI:10.1167/iovs.06-0154

Five clinical trials have provided evidence that lowering intraocular pressure (IOP) slows glaucoma disease progression.[1–5] In addition to lowering IOP, there is evidence that IOP fluctuation contributes to disease progression. Both the Advanced Glaucoma Intervention Study (AGIS) and the Collaborative Initial Glaucoma Treatment Study (CIGTS) have shown that patients with larger IOP fluctuation have more visual field progression than do patients with smaller IOP fluctuation.[1,6,7]

Yet, there is a growing awareness of the limitations of using IOP data obtained only during clinical office hours. For example, Asrani et al.[8] showed that patients with large diurnal IOP variation and day-to-day variation detected by home tonometry showed a nearly six times increased risk for glaucomatous visual field progression compared with those with smaller IOP variation. Recently, Mosaed et al.[9] determined that the magnitude of peak nocturnal IOP in patients with untreated open-angle glaucoma (OAG) can be estimated by a supine IOP measurement in a routine office visit. Thus, given the cumulative evidence from both clinical trials and studies on the importance of lowering IOP and minimizing IOP fluctuation during glaucoma treatment, a better understanding of the basis for IOP variation is needed.

It has been well established that steady state IOP is determined by aqueous humor flow, uveoscleral outflow, outflow resistance, and episcleral venous pressure. Among these factors, aqueous humor flow has been extensively studied.[10] In a population of normal subjects, aqueous humor flow has been shown to have a Gaussian-like distribution in the morning and at night.[11] Given this distribution of flow, we designed a study to test the hypothesis that an individual will show concordance of aqueous humor flow in the morning and at night.

Methods

Design

Our study was designed to measure prospectively the variation in the circadian rhythm of aqueous humor flow in normal human subjects. It has been established that a circadian rhythm of flow can be detected using fluorophotometry between morning and night.[11] Thus, we conducted a fluorophotometry study with five morning measurements every hour from 8 AM to noon and four nighttime measurements every 2 hours from midnight to 6 AM.

Subjects

Healthy subjects were recruited as part of a protocol approved by the Institutional Review Board of the University of Michigan Medical Center, and in accord with HIPAA (Health Insurance Portability and Accountability Act) regulations and the Declaration of Helsinki. After an initial telephone screening survey, subjects underwent an ocular screening examination. The following ocular examinations were performed: Snellen visual acuity, slit lamp biomicroscopy, intraocular pressure (IOP) measured by Goldmann applanation, gonioscopy, undilated funduscopic examination of the optic disc and posterior pole of the retina, pachymetry (Ultrasonic Pachymeter 800; DGH Technology, Exton, PA), and A-scan axial length measurements (Ultrasonic Biometer 820; Allergan Humphrey-Zeiss, San Leandro, CA). Inclusion criteria included age between 18 and 50 years, any race, either sex, and healthy, normal eyes. Exclusion criteria included pregnancy, chronic eye conditions, recent eye trauma or surgery, glaucoma medical ther-

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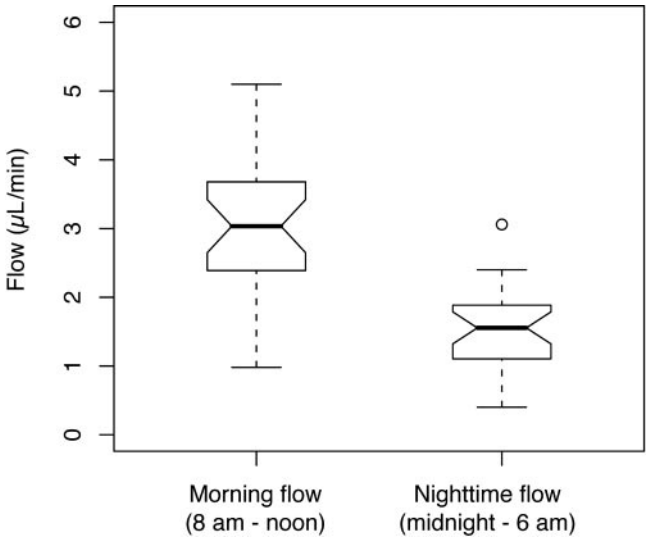

FIGURE 1. Aqueous humor flow in normal human subjects ($N = 28$) in the morning and at night. A Tukey box-and-whiskers plot of morning and nighttime flows with notches showing the approximate 95% CI around the individual medians. (○) Outlier.

apy, corneal scarring, inability to cooperate for fluorophotometry measurements, less than 30 days stable dosage of systemic medications before participation in the study, anatomically narrow angle determined by gonioscopy, or recent use of glucocorticoids.

Fluorophotometry

Scanning for fluorophotometry was conducted in an inpatient setting at the University of Michigan General Clinical Research Center. Excess fluid intake[12] and exercise[13] affect IOP and may affect flow; hence, these environmental factors were controlled in this inpatient setting during the scanning period. Subjects were sedentary, with explicit orders for no exercise. Activities were limited to television, phone conversation, reading, computer use, and sleeping. Fluid intake was controlled by provisions from nursing and dietary services. Caffeine intake does not affect aqueous humor flow,[14] so this dietary factor was not restricted. The scanning was performed in a room without windows and under darkroom conditions with a dim red light.

In brief, fluorescein 2% drops were administered topically to each subject's eye 4 to 6 hours before the scan, to allow for adequate steady state distribution. Using a previously established protocol,[15] the dose of the fluorescein varied by age (i.e., 1 drop every 5 minutes for a total of 5 drops for subjects <25 years of age; 4 drops total for subjects between 26 and 35 years of age; and 3 drops total for subjects between 36 and 50 years of age). Subjects were instructed to close their eyes between the fluorescein drops, and excess fluorescein was removed from their eyelids. Subjects wearing contact lenses were instructed to remove the lenses before instillation of the topical fluorescein and to refrain from contact lens use until the last scan for the morning and nighttime fluorophotometry study.

After proper fluorescein application for the morning flow, subjects were scanned with a fluorophotometer (Fluorotron; OcuMetrics, Mountain View, CA) hourly between 8 AM and noon. Afterward, subjects were on leave from the General Clinical Research Center and were instructed on the proper fluorescein dose for the nighttime flow. For the nighttime flow, subjects slept at the General Clinical Research Center, where they were awakened for scans every 2 hours between midnight and 6 AM.

Analysis and Statistics

Aqueous humor flow rates were calculated for each eye, with previously established protocols.[16] The data acquired from each eye were evaluated for quality of scan, accidental fluorescein reapplication due to incomplete removal from the subject's eyelids, and inability to refrain from blinking during the scanning procedure despite multiple repeat scans. Because the flow in one eye mimics that in the fellow eye in normal healthy eyes,[11] the flow data from one eye, selected in each subject based on the quality of the data as just described, was used for analysis.

The difference between the morning and nighttime flow rates were compared by using the paired Student's *t*-test.[17] The distribution of the individual flow rates was examined with box-and-whiskers plots,[18] ordinary least-squares regression (OLS),[18] and a kernel density plot.[19] In principle, the kernel density plot provides a graphic summary of a data set and its shape as local relative frequency. Each observation is replaced with a function centered on the observation and scaled by a chosen factor. The kernel density estimate is then the sum of these individual kernel functions.

Concordance of individual flow in the morning and at night was evaluated by using two approaches. The first was based on categorization into low, medium, or high tertiles and a dot plot of morning and nighttime flows. Specifically, tertiles were established by rank ordering the morning and nighttime flow data and then dividing the flow data into three tertiles corresponding to low, medium, and high flow. The ranges for the flow tertiles are as follows: low, 0.00 to 2.58 μL/min for morning and 0.00 to 1.20 μL/min for night; medium, 2.59 to 3.37 μL/min for morning and 1.21 to 1.69 μL/min for night; and high, 2.60 to 5.10 μL/min for morning and 1.70 to 3.06 μL/min for night. The second approach was based on a dot plot with different symbols representing morning and nighttime flows of each paired set of measurements (ordered by pair means).[20,21]

RESULTS

Twenty-eight subjects (15 men, 13 women) were studied, with an average age of 27.9 ± 9.4 years (range, 18–45). Among the 28 subjects studied, 18 were white, 3 were black, 5 were Asian, and 2 were Hispanic. For the biometric data used to calculate flow, the average anterior chamber depth was 3.4 ± 0.3 mm (range, 2.8–4.0). The calculated anterior chamber volume average was 253.6 ± 43.3 μL (range, 183.0–228.0). The average central corneal thickness was 542.2 ± 33.9 μm

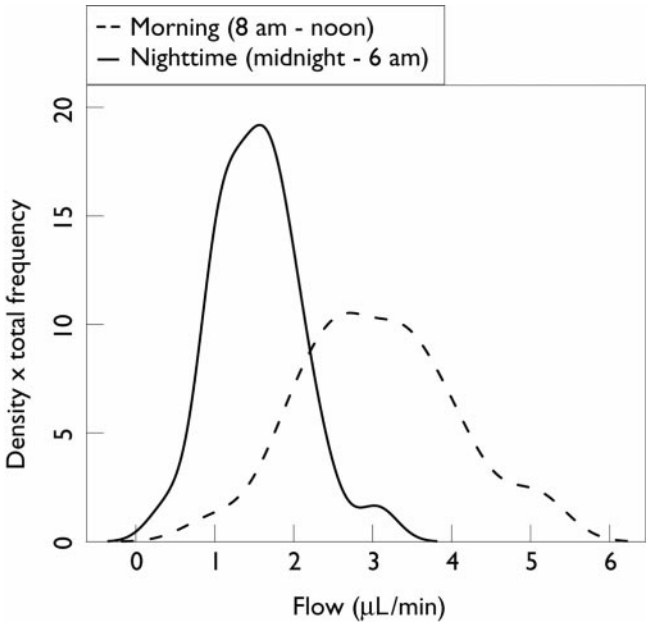

FIGURE 2. Density plot of morning and nighttime flows in normal human subjects ($N = 28$).

TABLE 1. Data Categorized into Tertiles as Low, Medium, or High Flows

Nighttime Flow (Midnight-6 AM)	Morning Flow (8 AM-Noon)			
	Low	Med	High	Total
Low	**6**	3	0	9
Med	2	**6**	1	9
High	1	2	**7**	10
Total	9	11	8	28

A 3 × 3 Contingency Table. The bold statistics represent the number of individuals whose morning and nighttime flows were concordant (19/28; 68%).

(range, 456.0–608.0), and the calculated cornea volume average was 73.3 ± 4.5 μL (range, 62.0–82.0). These biometric values are larger than the assumed volumes of the anterior chamber (174 μL), and cornea (70 μL), which was determined from a Japanese population.[22] Because the individuals in our study were of various ethnoracial populations, the individual calculated anterior chamber volume and cornea volumes could be smaller, equivalent, or larger than the assumed volumes, which were calculated from Japanese subjects, depending on the given individual's biometric measurements. Given our goal to study individual variation in flow, we calculated aqueous humor flow rates for each eye using biometric data from the subjects' eyes.[16]

There was no statistically significant difference in flow between right and left eyes in the morning or at night, which replicates previous findings.[11] In comparing the two eyes, the means and standard deviations of right and left eyes were 3.14 ± 1.26 and 2.88 ± 1.21 μL/min, respectively, in the morning, and 1.62 ± 0.83 and 1.58 ± 0.88 μL/min, respectively, at night. Thus, in general, flow was similar between eyes in these normal subjects.

In one eye of each subject, the mean ± SD of morning flow was 3.12 ± 1.09 μL/min, which significantly decreased ($P < 0.0001$, paired Student's *t*-test) at night to 1.59 ± 0.58 μL/min. The median morning flow was 3.04 μL/min (range, 0.98–5.10), and the median nighttime flow was 1.56 μL/min (range, 0.94–3.06), with one outlier at 3.06 μL/min (Fig. 1).

To examine the variation of the flow data further, we used a kernel density plot of morning and nighttime flows that shows similarity in distribution, but a difference in the spread of variability (Fig. 2).

Given the similar pattern of distribution of aqueous humor flow as shown earlier,[11] we analyzed the concordance of an individual's aqueous humor flow in the morning and at night. In other words, if a subject shows an average flow in the morning, does the same subject show a lesser, but average flow at night? Evidence for concordance of individual flow was examined by categorizing data into tertiles as low, medium, or high flows in a 3 × 3 contingency table (Table 1).

With this approach, 68% (19/28) of the subjects' paired flow rates for the morning and night were concordant. If flow rates in the morning and at night were simply random, one would expect to find approximately three subjects in each of the nine cells of Table 1. In another analytical approach for concordance, the dot plot also supported a trend of concordance in individuals by ordering the difference in an individual's paired morning and nighttime flows (Fig. 3).

Another tool for analyzing concordance of our paired data is the scatter plot. A pattern not readily apparent from the scatter plot emerged when a local regression fitting method was used. In Figure 4, we can see a pattern emerge in which the morning versus nighttime flows show a small incremental increase in nighttime flow as morning flow increases, compared with a larger incremental nighttime flow in cases in which morning flow increases over 3 μL/min, a pattern obscured by using a simple OLS fitting. The relationship revealed by the Loess fit, which shows a correlation coefficient of 0.62, can be estimated using a two-piece OLS fit—one for those with morning flows less than 3 μL/min and another for those with flows greater than 3 μL/min. This distribution of individual paired data is curious and may be related to the sample size in our study.

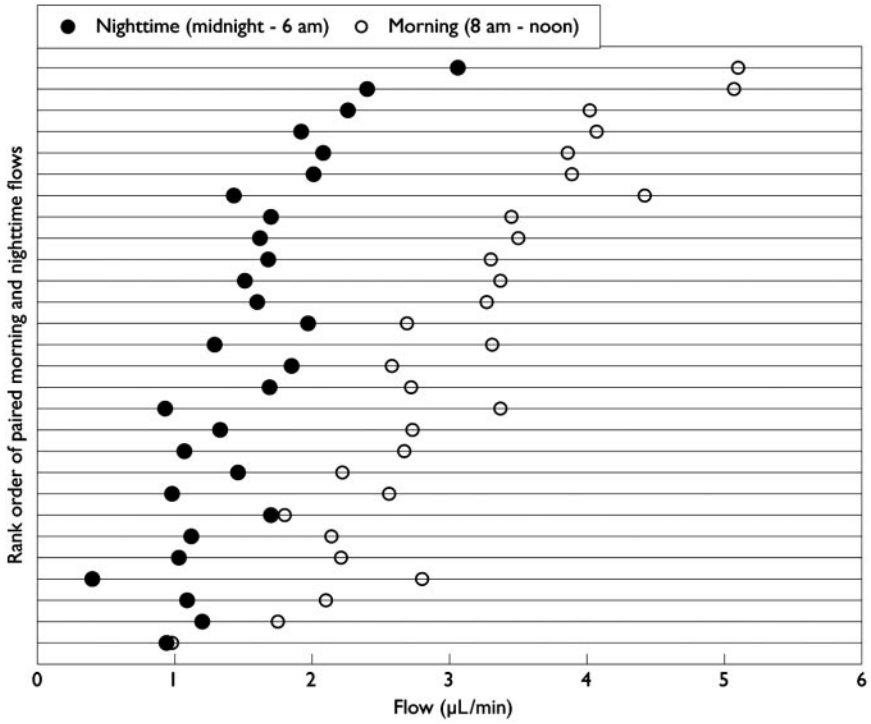

FIGURE 3. Rank order dot plot based on difference between morning and nighttime flows. Individuals are ordered by their pair-wise flow rates. Each circle represents an individual's flow: *open circles*, morning flow; *closed circles*, nighttime flow.

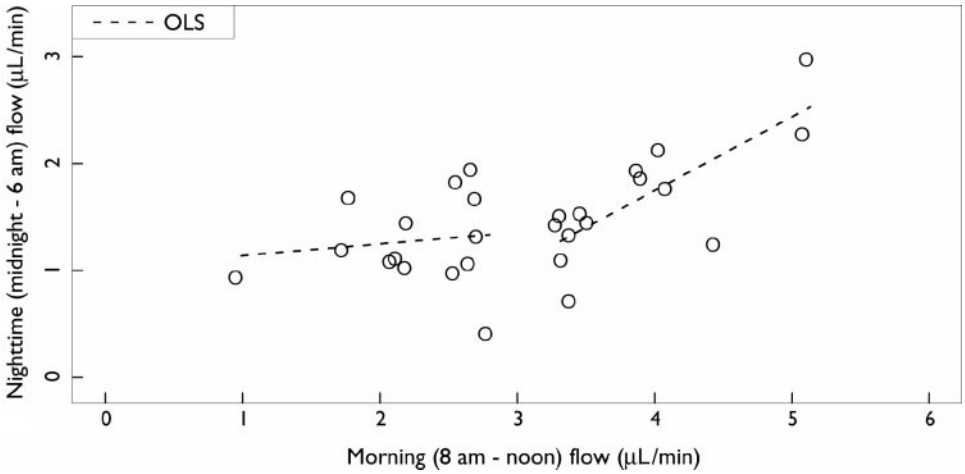

FIGURE 4. Scatter plot of individual morning versus nighttime flow data, which were best fit in a two-piece ordinary least-squares regression (OLS) analysis to estimate a Loess fit ($r^2 = 0.62$). As morning flow increased, so did nighttime flow, with a steeper pace of increase in those individuals in whom the morning flow was ≥3 μL/min.

Regardless, Figure 4 supports our hypothesis of concordance in normal individuals.

DISCUSSION

Our results show that there is concordance of aqueous humor flow in the morning and at night in an individual with normal healthy eyes (Table 1; Figs. 3, 4). In other words, individuals with a high, medium, or low flow in the morning also show a lower, but relatively high, medium, or low flow at night. Thus, aqueous humor flow is a phenotype with the following features: (1) similar flow between eyes of a normal individual; (2) a circadian pattern (Fig. 1); (3) a normal distribution (Fig. 2), as previously shown in a much larger sample size of normal subjects[11]; and (4) a pattern of concordance of morning and nighttime flow rates in an individual (Table 1; Figs. 3, 4). This latter point of concordance supplements an earlier finding that reported pooled data for flow at night and in the morning without determining whether an individual within the pooled data set showed concordance in his or her flow at these two time points.[11]

Although readily measured quantitatively with various instruments, IOP is a complex trait. This quantitative trait is determined by several factors as represented by the modified Goldmann equation, $P_o = (F - US)/C_{trab} + P_v$, where P_o is the IOP, F is the rate of aqueous humor flow, US is the rate of uveoscleral outflow, P_v is the episcleral venous pressure, and C_{trab} is the resistance to outflow.[23] C_{trab} has also been described as the inverse of trabecular outflow resistance. All these contributing factors may contribute to variation in IOP.

Among these four factors, aqueous humor flow has been studied most extensively. The variation in flow has been studied in humans with respect to circadian pattern, and the effects of age, disease state, hormones, and drugs.[10,11] Episcleral venous pressure is known to change in response to body position.[24,25] A similar mechanism may be involved in the elevated IOP and uveal engorgement observed in individuals who play high-resistance musical instruments.[26] More recently, Selbach et al.[27] showed that episcleral venous pressure differs between age-matched healthy control subjects, patients with primary OAG (POAG), and patients with normal tension glaucoma (NTG). An increase in trabecular outflow resistance accounts for the variation in IOP in patients with OAG.[28] In patients with ocular hypertension, Toris et al.[29] showed that reduction in trabecular outflow facility and uveoscleral outflow causes increased IOP. Uveoscleral outflow varies with age in primates.[30]

Although it is appreciated that all four factors are variable and contribute to steady state IOP, we still have not been able to explain their relative effects on the circadian rhythm of IOP.[31,32] Furthermore, we do not yet have a comprehensive understanding of how all these factors vary in healthy subjects with aging and in patients with glaucoma.

Both our data (Fig. 2) and a larger sample size of normal subjects[11] exhibit a normal distribution in morning and nighttime flows. Such a distribution is amenable for genetic studies.[33,34] Family studies, individual studies, and large epidemiologic studies of POAG show that there are genetic determinants of IOP.[35–37] More recently, three studies have supported this concept. The Beaver Dam Eye Study investigators reported that IOP is heritable[37] and have identified two genetic loci that contribute to IOP.[38] The Salisbury Eye Evaluation study confirmed that IOP is heritable.[39] A family study showed significant linkage for IOP to chromosome 10, region q22.[40]

In conclusion, we have demonstrated that normal individuals show concordance of flow in the morning and at night. With the recent evidence showing heritability of IOP and finding several genetic loci for IOP, we propose that the complex factors that contribute to variation in IOP are quantitative traits. Because flow is a factor that determines IOP, discovering the underlying genetic markers of this trait holds promise for understanding its contribution to IOP variation. Further aqueous humor dynamic measurements in family and twin studies will demonstrate whether aqueous flow has genetic determinants. Finding clinical and genetic markers that are predictive for wide IOP variation would be advantageous in managing patients with glaucoma, to minimize the risk of disease progression.

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