

Effects of Aging on 24-Hour Intraocular Pressure Measurements in Sitting and Supine Body Positions

Kaweh Mansouri, Robert N. Weinreb, and John H. K. Liu

PURPOSE. To evaluate how aging alters 24-hour measurements of intraocular pressure (IOP) in the sitting and supine body positions.

METHODS. Fifteen older volunteers with healthy eyes (ages, 53–71 years) were each housed for 1 day in a sleep laboratory. An 8-hour accustomed sleep period was assigned to each subject. Every 2 hours, measurements of IOP were taken in the sitting and supine positions. Sitting and supine patterns of 24-hour IOP were compared. Simulated 24-hour IOP rhythms in the same body position were determined using cosine fitting of individual 24-hour data. The average postural IOP effects during the diurnal/wake period and the nocturnal/sleep period were compared. Data from this group of older subjects were compared with previously collected data from 16 healthy younger subjects (ages, 18–25 years) under the same experimental conditions.

RESULTS. Within each age group, sitting and supine patterns of 24-hour IOP were similar and parallel. Compared to the younger subjects, the phase timing (simulated peak) of 24-hour IOP was significantly delayed for the older subjects in both body positions. The postural IOP effect for the older subjects was 4.7 ± 0.8 and 4.8 ± 0.8 mm Hg during the diurnal and nocturnal periods, respectively. These postural IOP effects were not significantly different from the postural effects in the younger subjects.

CONCLUSIONS. Although aging can significantly delay the phase timing of the 24-hour IOP pattern toward the diurnal/awake period, it may not affect the postural IOP effect during the diurnal and the nocturnal periods. (*Invest Ophthalmol Vis Sci.* 2012;53:112–116) DOI:10.1167/iovs.11-8763

Intraocular pressure (IOP) in the human eye is regulated by the rate of aqueous humor formation, the resistance of aqueous humor outflow, and the episcleral venous pressure. IOP varies throughout a 24-hour period due to changes in these physiologic parameters. In healthy younger and nonmyopic subjects, there is 24-hour variation of IOP with a peak value in most individuals occurring in the late nocturnal/sleep period in either sitting or supine body position, independent of posture.^{1,2} Postural IOP effects, the difference in IOP between the sitting and supine body positions, are not significantly different

between the diurnal and nocturnal periods.¹ However, an earlier study indicated that the 24-hour IOP variation in the aging population may be different from that in the younger subjects.³

Changes in 24-hour IOP measurements may occur because of age-related structural or functional changes, including alterations in hormonal and neural activities for the regulation of IOP. Circulating levels and timing of glucocorticoids and melatonin as well as the neuronal activities such as the baroreflex may affect the IOP pattern and its variation within a 24-hour period.^{4–8} A reported age-related change is the postural IOP effect,^{9,10} which is associated with the hydrostatic response in the episcleral venous pressure and the redistribution of body fluid including the choroidal vascular volume.^{11–13} Supporting evidence, however, was limited to observations during the diurnal/wake period.^{9,11,12} The impact of aging on the postural IOP effect during the nocturnal/sleep period is unknown.

If aging alters the 24-hour IOP rhythm and has a similar influence on the postural IOP effect within 24 hours, the 24-hour IOP patterns in the sitting and supine body position should be shifted in the older subjects, but the 24-hour change patterns should remain parallel. To test this hypothesis, we prospectively collected 24-hour IOP data from a group of relatively older volunteers in both the sitting and supine positions. The phase timings (simulated peak) and the magnitudes of variation for posture-independent rhythms and the diurnal and nocturnal postural IOP effects were compared in this age group. Comparisons were also taken with data from a group of healthy younger subjects in a previous report in 2003.¹

METHODS

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of California, San Diego. Study subjects were 15 healthy, nonsmoking volunteers, recruited from university employees (age range, 53–71 years). Written informed consent was obtained. Exclusion criteria included myopia over 3 D, use of routine medication that might affect IOP, and the presence of ocular disease. All subjects underwent a complete ophthalmic examination consisting of a medical history, best corrected visual acuity, slit lamp biomicroscopy, Goldmann applanation tonometry, and dilated funduscopy during office hours. Subjects also had a normal visual field test (using Statpac II, full-threshold 24-2; Zeiss Humphrey Field Analyzer, Carl Zeiss Meditec, Inc., Dublin, CA). We sought to recruit a representative sample of older adults. Therefore, subjects taking routine medications for chronic age-related conditions, such as systemic anticholesterol, antihypertensive, antiinflammatory, antidepressant, and estrogen replacement drugs were not restricted.

Individuals were selected who had a regular daily sleep cycle of approximately 8 hours. Before the laboratory session, they were instructed to maintain an accustomed 8-hour sleep period with lights off and lying down for 7 days. They were required to wear a wrist-mounted device (Actiwatch; Mini Mitter, Sunriver, OR) to monitor physical activity and light exposure and to keep a wake-sleep log. They were instructed to abstain from alcohol for 3 days and coffee intake for 1 day before the laboratory session.

From the Hamilton Glaucoma Center and Department of Ophthalmology, University of California, San Diego, La Jolla, California.

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Corresponding author: John H. K. Liu, University of California, San Diego, Department of Ophthalmology, 9500 Gilman Drive, La Jolla, CA 92093-0946; joliu@ucsd.edu.

Subjects reported to the sleep laboratory at approximately 2 PM and stayed indoors for 24 hours. Laboratory conditions were strictly controlled as in the previous study that enrolled healthy younger subjects whose data were available for comparisons.¹ The 8-hour period of darkness in each subjects' room was adjusted to correspond to the individual's sleep cycle, and the clock times for the IOP measurements were individualized correspondingly. For data presentation, clock times were normalized as if each subject slept from 11 PM to 7 AM.¹

Measurements of IOP in both eyes were taken by experienced examiners every 2 hours in the supine and then in the sitting position with a pneumatonometer (Model 30 Classic; Reichert, Depew, NY). The device has a resolution of 0.5 mm Hg. Measurements were always taken first in the right eye. Proparacaine 0.5% was applied to the eye for local anesthesia. A hard-copy printout was produced for each measurement and inspected visually. Before the nocturnal/sleep period, IOP was measured at 3:30, 5:30, 7:30, and 9:30 PM. The subjects were instructed to lie on the bed for 5 minutes before the supine IOP measurements, and they then assumed a vertical position for 5 minutes before the sitting IOP measurements. Blood pressure and heart rate were measured immediately before the IOP measurements. Lights were turned off at 11:00 PM and nocturnal measurements were taken at 11:30 PM and 1:30, 3:30, and 5:30 AM. The subjects were awakened, if necessary, and the supine measurements were taken immediately under dim light (<10 lux). The subjects resumed the sitting position, and IOP was measured 5 minutes later. Their light exposure was kept to a minimum during the nocturnal period. When the assigned nocturnal period ended at 7 AM, room lights were turned on and subjects were awakened, if necessary. IOP measurements were taken again at 7:30, 9:30, and 11:30 AM and 1:30 PM, as described previously. Food and water were available at all times, and meal times were not regulated. Room activities were continuously videotaped for 24 hours by infrared cameras.

The IOPs from both eyes were averaged and used for data analysis. The mean sitting and supine IOPs were calculated at each time point. Postural IOP changes were determined as the difference between the IOP measured in the sitting and supine positions. The postural IOP changes were averaged for the diurnal period (eight data points) and the nocturnal period (four data points). Using the best-fitting cosine curve,¹⁴ we estimated the 24-hour IOP rhythms for each subject in both body positions. With the 12 IOP time points, a cosine-fit curve was generated for each subject in sitting and supine. The cosinor method was used to characterize the 24-hour rhythm of variables and measure acrophase and amplitude. The acrophase (cosinor analysis-derived peak time) represented the phase timing. The amplitudes (half distance between the cosine-fit maximum and minimum) provided the simulated variation for the 24-hour rhythm. The null hypothesis that phase timings were distributed randomly in 24 hours was tested with the Rayleigh test.¹⁵ A significant difference would indicate a synchronized 24-hour rhythm for the group. The acrophases and amplitudes of supine IOP and sitting IOP were compared using the Wilcoxon signed

rank test for paired data within the group of older subjects. $P < 0.05$ indicated statistically significant. Results from this group of older subjects were compared with results from a group of healthy younger subjects whose data were collected under the same conditions.¹

RESULTS

Fifteen older volunteers with healthy eyes were recruited, and all completed the study. Their demographic data are presented in Table 1. Twelve older subjects were routinely using anticholesterol, antihypertensive, antiinflammatory, antidepressant, and estrogen replacement drugs. Figure 1 shows the 24-hour sitting and supine IOP profiles for both age groups. In both groups, there was a gradual decrease in IOP in the diurnal period, and it reached the trough before sleep time at 11 PM. In the older group, a gradual increase in IOP occurred during the nocturnal period, reaching a peak in the morning hours after awakening. In the younger group, the peak was reached at the end of the nocturnal period before awakening. For each age group, change patterns of 24-hour sitting and supine IOP curve were parallel by visual inspection. Compared with the younger subjects, change patterns of 24-hour IOP in the older group were shifted to the right.

Cosine fits of sitting and supine IOP data were computed for each subject and the acrophases and amplitudes were determined. Synchronized 24-hour IOP rhythms appeared for the elder group as well as for the younger group (Rayleigh test). Figure 2 summarizes all the individual phase timings from various calculations. Phase timings were 10:20 AM \pm 205 minutes (mean \pm SD) in the sitting position and 10:23 AM \pm 268 minutes in the supine position for the older groups. Phase timings were 6:35 AM \pm 202 minutes and 5:31 AM \pm 211 minutes for the younger group in the sitting and supine positions, respectively. Compared to younger subjects, the phase timings for 24-hour IOP rhythms were significantly delayed for the older subjects both in the sitting ($P = 0.004$) and in the supine position ($P = 0.002$). Simulated 24-hour IOP fluctuations showed no difference between the older and younger subjects in either body position (data not shown).

The diurnal and nocturnal IOP levels for the older and younger subjects are summarized in Table 2. For the older group, the postural IOP effect during the diurnal period was 4.7 ± 0.8 mm Hg, which was not significantly different from the postural IOP effect of 4.8 ± 0.8 mm Hg during the nocturnal period ($P = 0.961$). These postural IOPs in the older group were not significantly different from those in the younger group, which were 4.3 ± 1.5 mm Hg during the diurnal period ($P = 0.300$) and 3.9 ± 2.3 mm Hg during the nocturnal periods ($P = 0.180$).

The mean nocturnal arterial blood pressure was lower than the diurnal levels in older subjects (91.4 ± 17.0 vs. 97.3 ± 12.7

TABLE 1. Demographic Characteristics

	Older Group ($n = 15$)	Younger Group ($n = 16$)	P^*
Sex, female	9 (60%)	8 (50%)	0.722
Ancestry	13 White, 1 Asian, 1 Black	8 White, 5 Asian, 2 Black, 1 Hispanic	0.100
Age, y	60.5 ± 5.3	21.5 ± 2.1	<0.001
Height, in	67.1 ± 4.2	68.0 ± 4.9	0.616
Weight, lb	172.7 ± 39.3	150.9 ± 25.5	0.076
Body mass index, lb/in ²	26.8 ± 4.6	22.9 ± 1.9	0.004
CCT, μ m	567.7 ± 32.1	542.9 ± 39.2	0.065
Axial length, mm	23.4 ± 1.1	23.3 ± 0.7	0.761
Refractive state, D (range)	0.6 ± 1.2 (-2.3 to +2.6)	-0.4 ± 0.8 (-1.8 to +0.9)	0.010
IOP, mm Hg (range)	15.7 ± 2.4 (11-20)	15.4 ± 3.9 (10-21)	0.824

Values are the mean \pm standard deviation. CCT central corneal thickness.

* Fisher's exact test for sex and ancestry and Student's *t*-test for other parameters.

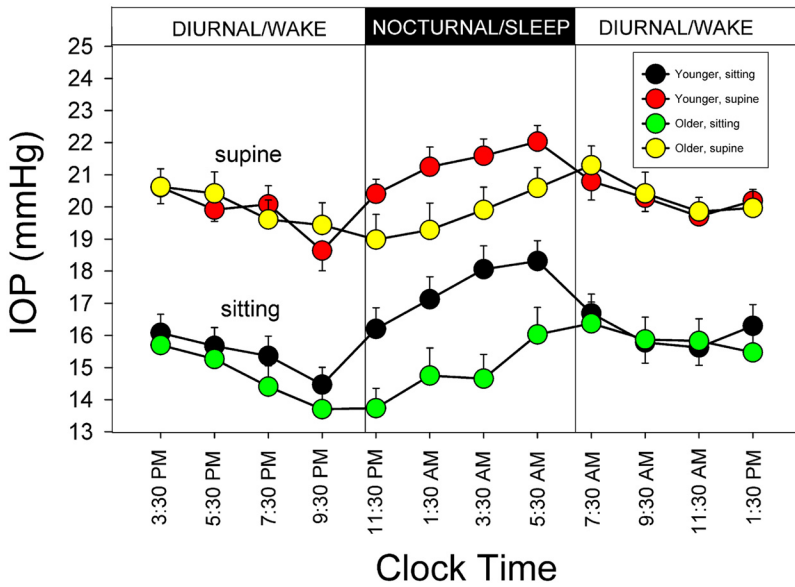


FIGURE 1. Twenty-four-hour patterns of mean IOP in healthy older ($n = 15$) and healthy younger ($n = 16$) adults in the sitting and supine positions. Error bars, SEM.

mm Hg; $P < 0.05$) and in younger subjects (87.7 ± 8.1 vs. 92.9 ± 8.7 mm Hg; $P < 0.01$) when measured in the habitual body positions. The mean heart rate, measured in habitual body positions, was 71.6 ± 9.5 (beats/min) during waking and 70.0 ± 9.3 during sleeping hours in older subjects ($P = 0.63$) vs. 72.6 ± 9.3 and 65.3 ± 8.0 in younger subjects ($P = 0.02$).

DISCUSSION

The present study shows that aging can significantly delay the phase timing of the 24-hour IOP pattern toward the diurnal/awake period and may not affect the magnitude of the postural IOP effect during the diurnal and the nocturnal periods. A concern of IOP investigations during sleep is the necessity of

waking patients up for the IOP measurements. It is possible that the effect of arousal introduced certain artificial changes in the nocturnal IOP. However, data from continuous IOP recordings using a contact lens IOP sensor in uninterrupted sleeping patients show that the potential artifact is not significant and does not seem to affect nocturnal IOP patterns.¹⁶ With both age groups undergoing testing under the same controlled environmental conditions, we assumed that other environmental effects on IOP patterns would be similar between the two study groups.

Several studies have reported that IOP increases with a postural change from sitting to supine with magnitudes ranging from as little as 0.3 ± 1.8 to 5.6 ± 1.7 mm Hg in healthy adults.^{17,18} Jain and Marmion⁹ observed a direct relationship between age and postural IOP change in normal and glaucomatous eyes. However, similar to the above-mentioned studies,^{17,18} they assessed only IOP at limited time points during the day. Mosaed et al.,¹⁹ reported a relatively small difference in the postural IOP effect between healthy younger subjects and older subjects with healthy eyes when IOP readings were taken at several time points during office hours (average sitting and supine IOP differences were 4.2 and 4.6 mm Hg, respectively). The present study shows again that there was no difference between the postural IOP effect for 24 hours in a group of older subjects, similar to the observation in healthy younger subjects.¹

The nocturnal IOP elevation in the habitual body position appears to be associated with the postural change from vertical to recumbent at night.^{16,20} Other physiological determinants for the change of IOP at night remain unclear. Excluding the postural effect on episcleral venous pressure¹¹ and the chori-

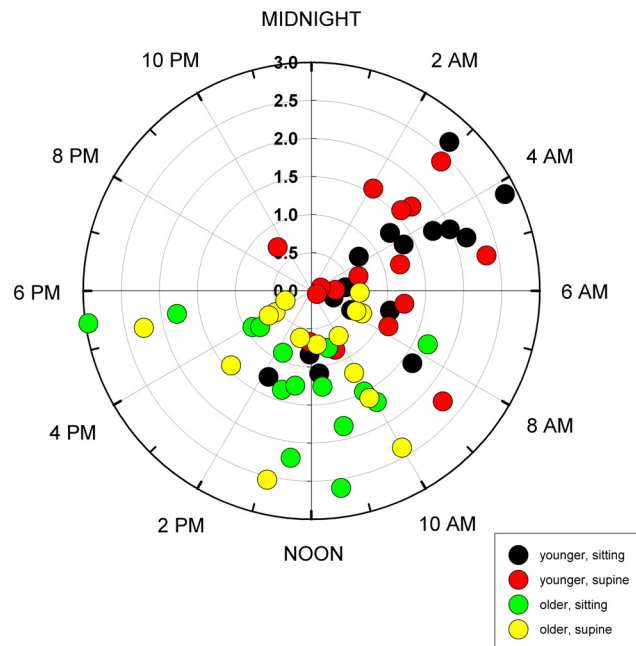


FIGURE 2. Estimated 24-hour rhythm of IOP in the sitting and supine positions in healthy older ($n = 15$) and healthy younger ($n = 16$) adults. The clock time of the acrophase (phase timing) is shown with the amplitude in the radial scale (mm Hg).

TABLE 2. Diurnal and Nocturnal IOP in Both Body Positions

	Older Group ($n = 15$)	Younger Group ($n = 16$)	P (t -test)
Diurnal sitting	15.3 ± 2.4	15.8 ± 1.9	0.594
Diurnal supine	20.1 ± 2.2	20.0 ± 1.2	0.950
Nocturnal sitting	14.8 ± 2.9	17.4 ± 2.3	0.008
Nocturnal supine	19.6 ± 2.8	21.3 ± 1.6	0.036

IOPs (mm Hg) are mean \pm standard deviation and represent the average of both eyes. The diurnal period was 7 AM to 11 PM, and the nocturnal period was 11 PM to 7 AM.

dal blood volume,¹³ there is a potential influence of the sympathetic nervous system and baroreflex on IOP in the present study. The baroreflexes are the primary mechanism by which blood pressure can be rapidly modulated when body position is changed. Although the exact interplay between blood pressure and IOP is still unclear, the altered response in blood pressure due to the baroreflex may influence or accompany changes in aqueous humor dynamics and IOP. Singleton et al.,²¹ have reported that in patients who had orthostatic hypotension with autonomic imbalance, postural change produced significant IOP changes by sudden fluctuation of blood pressure. Aging is known to be associated with a decreased sensitivity of the parasympathetic but not the sympathetic arm of the baroreflex.²² This age-related change may have physiological consequences, such as the reduced ability to deal with an acute challenge to the maintenance of blood pressure. Although the magnitude of blood pressure change from the supine to the sitting position is associated with lower levels of baroreceptor sensitivity in older adults,²² this specific consideration of the baroreflex function seems to have limited influence on the postural IOP effect throughout the 24 hours.

Cortisol and melatonin are important regulators of human circadian rhythm, and both can have a direct effect on IOP.^{4-6,8} The endogenous release of each hormone is characterized by a circadian rhythm that is controlled by the output of the suprachiasmatic nucleus.²³ As the body ages, there is a rearrangement of the internal circadian phase timings among rhythms of cortisol, melatonin, and sleep.²⁴ In this context, the circadian rhythmicity and posture-independent IOP rhythm could be influenced by factors associated with the timing of cortisol and melatonin release, relative to the timing of sleep and provide certain explanations for the delayed phase timing of 24-hour IOP rhythm observed in the elderly.

Endogenous glucocorticoids, such as cortisol, are associated with a rise in IOP in normal subjects.^{5,25,26} It has also been suggested that chronically higher levels of cortisol and its metabolic byproducts can cause slowly progressive changes in the trabecular meshwork and lead to elevated IOP and glaucomatous optic neuropathy.^{27,28} In healthy younger subjects, plasma cortisol peaks during morning hours before or after awakening and troughs around midnight.²³ The onset and midpoint but not the offset of nocturnal cortisol secretion is advanced in the elderly compared to younger controls.^{24,29} A relative advance in the phase timing of cortisol with age can potentially influence the circadian IOP rhythm. However, the fact that our older subjects had a phase-delay in their peak IOP seems to contrast with the expected aging effect of cortisol on IOP.

Melatonin has been shown to decrease IOP by reducing aqueous humor secretion.^{6,8,30} Although the age-related decline of endogenous melatonin levels is uncertain,^{31,32} there is generally agreement on the aging effect on the circadian rhythm of melatonin secretion. Duffy et al.,³³ investigated the relationship between the timing of plasma melatonin and the sleep-wake cycle in healthy young and old volunteers. They found a longer interval between usual bedtime and plasma melatonin midpoint in the older subjects. The awake times of the elderly began significantly earlier, which indicates that the plasma melatonin levels may be phase-delayed within the sleep of older subjects. Zhou et al.,³⁴ showed that the acrophase of melatonin levels was similar in all age groups but they also found significantly higher offset levels in older subjects, indicating a delayed melatonin offset phase. They further show that these changes start to occur around the age of 40 years. It is not known how the delayed and higher offset levels of plasma melatonin reflect the rhythm of ocular melatonin. A delayed phase of ocular melatonin offset can be associated with the phase delay of the 24-hour IOP rhythm. Further

biochemical studies are needed to investigate the potential interaction of melatonin and 24-hour IOP pattern.

A limitation of the present study is that six individuals in the older adult group (versus none in the younger adult group) used routine medication to control blood pressure and blood cholesterol. These medications may have some effects on the timing and magnitude of the 24-hour IOP rhythm and the related postural IOP response. We also assume that uncontrolled factors inside and outside the sleep laboratory have no significant impact on the comparison between the older subjects and the younger subjects whose data were collected approximately 9 years apart.

In conclusion, these experimental data verified our hypothesis that the 24-hour IOP rhythms in the sitting and supine body position are shifted in the older subjects, whereas the 24-hour IOP change patterns remain parallel. This information should be taken into consideration in clinical studies evaluating circadian IOP at different time points between age groups.

References

- Liu JH, Boulogny RP, Kripke DF, Weinreb RN. Nocturnal elevation of intraocular pressure is detectable in the sitting position. *Invest Ophthalmol Vis Sci.* 2003;44:4439-4442.
- Loewen NA, Liu JH, Weinreb RN. Increased 24-hour variation of human intraocular pressure with short axial length. *Invest Ophthalmol Vis Sci.* 2010;51:933-937.
- Liu JH, Kripke DF, Twa MD, et al. Twenty-four-hour pattern of intraocular pressure in the aging population. *Invest Ophthalmol Vis Sci.* 1999;40:2912-2917.
- Rosenberg S, Levene R. Suppression of plasma cortisol in normal and glaucomatous patients. *Arch Ophthalmol.* 1974;92:6-9.
- Schwartz B, Seddon JM. Increased plasma cortisol levels in ocular hypertension. *Arch Ophthalmol.* 1981;99:1791-1794.
- Serle JB, Wang RF, Peterson WM, et al. Effect of 5-MCA-NAT, a putative melatonin MT3 receptor agonist, on intraocular pressure in glaucomatous monkey eyes. *J Glaucoma.* 2004;13:385-388.
- Monahan KD. Effect of aging on baroreflex function in humans. *Am J Physiol Regul Integr Comp Physiol.* 2007;293:R3-R12.
- Samples JR, Krause G, Lewy AJ. Effect of melatonin on intraocular pressure. *Curr Eye Res.* 1988;7:649-653.
- Jain MR, Marmion VJ. Rapid pneumatic and Mackey-Marg applanation tonometry to evaluate the postural effect on intraocular pressure. *Br J Ophthalmol.* 1976;60:687-693.
- Wuthrich UW. Postural change and intraocular pressure in glaucomatous eyes. *Br J Ophthalmol.* 1976;60:111-114.
- Friberg TR, Sanborn G, Weinreb RN. Intraocular and episcleral venous pressure increase during inverted posture. *Am J Ophthalmol.* 1987;103:523-526.
- Chiquet C, Custaud MA, Le Traon AP, et al. Changes in intraocular pressure during prolonged (7-day) head-down tilt bedrest. *J Glaucoma.* 2003;12:204-208.
- Prata TS, De Moraes CG, Kanadani FN, et al. Posture-induced intraocular pressure changes: considerations regarding body position in glaucoma patients. *Surv Ophthalmol.* 2010;55:445-453.
- Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinorhythmometry. *Chronobiologia.* 1979;6:305-323.
- Zar JH. *Biostatistical Analysis.* 5th ed. Upper Saddle River, NJ: Pearson Prentice Hall; 2010:624-626.
- Mansouri K, Shaarawy T. Continuous intraocular pressure monitoring with a wireless ocular telemetry sensor: initial clinical experience in patients with open angle glaucoma. *Br J Ophthalmol.* 2011;95:627-629.
- Anderson DR, Grant WM. The influence of position on intraocular pressure. *Invest Ophthalmol.* 1973;12:204-212.
- Tsukahara S, Sasaki T. Postural change of IOP in normal persons and in patients with primary wide open-angle glaucoma and low-tension glaucoma. *Br J Ophthalmol.* 1984;68:389-392.
- Mosaed S, Liu JH, Weinreb RN. Correlation between office and peak nocturnal intraocular pressures in healthy subjects and glaucoma patients. *Am J Ophthalmol.* 2005;139:320-324.

20. Liu JH, Weinreb RN. Monitoring intraocular pressure for 24 h. *Br J Ophthalmol*. 2011;95:599–600.
21. Singleton CD, Robertson D, Byrne DW, Joos KM. Effect of posture on blood and intraocular pressures in multiple system atrophy, pure autonomic failure, and baroreflex failure. *Circulation*. 2003;108:2349–2354.
22. James MA, Potter JF. Orthostatic blood pressure changes and arterial baroreflex sensitivity in elderly subjects. *Age Ageing*. 1999;28:522–530.
23. Selmaoui B, Touitou Y. Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: a study of three different 24-h cycles over six weeks. *Life Sci*. 2003;73:3339–3349.
24. Kripke DF, Youngstedt SD, Elliott JA, et al. Circadian phase in adults of contrasting ages. *Chronobiol Int*. 2005;22:695–709.
25. Weitzman ED, Henkind P, Leitman M, Hellman L. Correlative 24-hour relationships between intraocular pressure and plasma cortisol in normal subjects and patients with glaucoma. *Br J Ophthalmol*. 1975;59:566–572.
26. Boyd TA, McLeod LE. Circadian rhythms of plasma corticoid levels, intraocular pressure and aqueous outflow facility in normal and glaucomatous eyes. *Ann N Y Acad Sci*. 1964;117:597–613.
27. Clark AF, Wordinger RJ. The role of steroids in outflow resistance. *Exp Eye Res*. 2009;88:752–759.
28. Weinstein BI, Iyer RB, Binstock JM, et al. Decreased 3 alpha-hydroxysteroid dehydrogenase activity in peripheral blood lymphocytes from patients with primary open angle glaucoma. *Exp Eye Res*. 1996;62:39–45.
29. Kripke DF, Elliott JA, Youngstedt SD, Rex KM. Circadian phase response curves to light in older and young women and men. *J Circadian Rhythms*. 2007;5:4.
30. Alarma-Estrany P, Guzman-Aranguiz A, Huete F, et al. Design of novel melatonin analogs for the reduction of intraocular pressure in normotensive rabbits. *J Pharmacol Exp Ther*. 2011;337:703–709.
31. Zeitzer JM, Daniels JE, Duffy JF, et al. Do plasma melatonin concentrations decline with age? *Am J Med*. 1999;107:432–436.
32. Haimov I, Laudon M, Zisapel N, et al. Sleep disorders and melatonin rhythms in elderly people. *BMJ*. 1994;309:167.
33. Duffy JF, Zeitzer JM, Rimmer DW, et al. Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. *Am J Physiol Endocrinol Metab*. 2002;282:E297–E303.
34. Zhou JN, Liu RY, van Heerikhuizen J, et al. Alterations in the circadian rhythm of salivary melatonin begin during middle-age. *J Pineal Res*. 2003;34:11–16.