The Relationship between Diurnal Variations in Intraocular Pressure Measurements and Central Corneal Thickness and Corneal Hysteresis

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PURPOSE. To examine the relationship between office-hour changes in IOP, measured with the Goldmann applanation tonometer (GAT) and dynamic contour tonometer (DCT), and the corneal characteristics central corneal thickness (CCT) and corneal hysteresis (CH).

METHODS. Sixty-two eyes of 62 untreated normal subjects and patients with untreated glaucoma had IOP measurements performed with the GAT (mm Hg) and DCT (mm Hg) over an 8-hour period at 2-hour intervals beginning at 9 AM. CCT (micrometers) was measured using a noncontact optical low-coherence reflectometry (OLCR) pachymeter, and CH (mm Hg) was measured with an ocular response analyzer (ORA). The associations between IOP measurements and corneal characteristics for each patient over the measurement period were assessed by using multilevel modeling.

RESULTS. GAT and DCT IOP and CCT changed significantly during office hours (ANOVA; GAT: F = 19.9, P < 0.001; DCT: F = 4.6, P = 0.001; CCT: F = 16.4; P < 0.001). No significant changes were observed in CH (ANOVA; F = 1.8, P = 0.13). Multilevel modeling analysis of the interrelationships between CCT, CH, and age on IOP measurements revealed that both CCT and CH changes were significantly associated with GAT IOP changes (GAT IOP/CCT slope, 0.04 mm Hg/mm Hg; 95% confidence intervals [CIs], 0.02–0.06; GAT IOP/CH slope, 0.20 mm Hg/mm Hg; 95% CI, 0.01–0.39). CCT, but not CH, changes were significantly associated with DCT IOP changes (DCT IOP/CCT slope, 0.05 mm Hg/mm Hg; 95% CI, 0.00–0.05). However, although the association between CCT and GAT IOP was relatively uniform between subjects, association between CCT and DCT IOP showed greater intersubject variability. Age had no effect on the diurnal variation of IOP measured with either device.

CONCLUSIONS. Measured IOP and corneal characteristics covary during office hours. Changes in CCT and CH are associated with changes in GAT IOP and, less consistently, with DCT IOP. The data suggest that variations in corneal characteristics explain a small proportion of the change in IOP measurements made with the GAT during office hours. (Invest Ophthalmol Vis Sci. 2009;50:4229 – 4236) DOI:10.1167/iovs.08-2955

In common with most physiological systems, intraocular pressure (IOP) varies cyclically.1 Studies have shown that IOP is typically greatest in the early morning, with troughs occurring during the day, although other circadian patterns have been reported.1–4

It has also been found that central corneal thickness (CCT) follows a diurnal pattern similar to that of IOP, being thickest on awakening and reducing through the day.5–7 The influence of CCT on applanation-measured IOP is well documented.7–10 The covariation of CCT and measured IOP raises questions as to what proportion of the diurnal IOP change can be attributed to changes in true IOP, what proportion is caused by changes in corneal characteristics that affect the accuracy of measured IOP, and whether variation in CCT is induced by variation in IOP.

In studies in which the temporal relationship between diurnal changes in CCT and applanation measured IOP was assessed, no association was found between the two measures.11–14 In contrast, a recent study of diurnal changes in applanation-measured IOP and CCT in young, healthy-eyed subjects found a significant association between the two measures that persisted for the first 2 hours after awakening, but became nonsignificant thereafter.15 However, CCT accounts for only a small proportion of the variation in measured IOP between individuals, and other corneal biomechanical properties are recognized as having an important influence on IOP measurement.16,17

There have been two recent advances in IOP measuring technology that have been designed to either measure or compensate for the corneal biomechanical effects on IOP measurement. The Reichert Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Buffalo, NY) makes two measurements of the ocular response at the corneal to an air pressure pulse, the force necessary to flatten the cornea as the air pressure rises and the force at which the cornea flattens again as the air pressure falls. It has been found that the second force-out applanation occurs at a lower pressure than the first force-in applanation, and the change has been attributed to the dampening effects of the cornea. The difference between the two pressures has been termed corneal hysteresis (CH) which is thought to be a direct measure of corneal biomechanical properties. CH more completely describes the contribution of corneal resistance to IOP measurements than does CCT.18

The Pascal Dynamic Contour Tonometer (DCT; Swiss Microtechnology AG, Port, Switzerland) uses a transcorneal...
method to measure IOP. The device is slit-lamp-mounted in a fashion similar to the Goldmann applanation tonometer (GAT). The contact surface of the DCT is concave such that the corneal surface assumes the contour of the tip, thus reducing the corneal mechanical effects on IOP measurement. The DCT gathers 100 IOP readings per second over a 5- to 8-second period to record dynamic IOP. Recent studies have shown that DCT IOP measurements are less affected by CCT than those by GAT. 19-22

The purpose of this study was to examine the relationship of diurnal IOP changes with CCT and CH, measured with GAT and DCT during normal office hours.

METHODS

The study population comprised two groups of subjects: normal subjects and patients with untreated glaucoma (having pathologic, glaucomatous optic neuropathy [GON] with or without visual field loss) attending the Glaucoma Research Unit at Moorfields Eye Hospital for all-day IOP phasing. Subjects were excluded if they were using IOP-lowering medication, had a history of previous ocular surgery or injury, or had astigmatism of greater than 2 D. Contact lens wearers were instructed not to use their lenses for a minimum of 24 hours before participation. Sixty-two eyes of 62 subjects were included. Demographic data are presented in Table 1. The study had local ethics committee approval, and informed consent, according to the tenets of the Declaration of Helsinki, was obtained from each subject before examination.

The patients underwent measurements of CCT and CH, and IOP was measured with both the DCT and GAT every 2 hours from 9 AM to 5 PM.

CCT measurements were performed with the noncontact optical low coherence reflectometry (OLCR) pachymeter (Haag-Streit, Bern, Switzerland). The OLCR pachymeter was programmed to take the average of 8 out of 10 consecutive readings and at each time interval measurements were repeated until the standard deviation of the eight readings was <1 µm. CH measures were made with a prototype Reichert ORA, and three good-quality measurements were recorded at each time interval. The manufacturer defines good-quality readings as both force-in and force-out applanation signal peaks, which are fairly symmetrical in height on the ORA waveform. The average of three measurements was used for the analysis. The order of CCT and corneal hysteresis measurements was randomized, and both were performed before tonometry to avoid possible changes in corneal behavior with anesthesia.

After topical corneal anesthesia (proxymetacaine hydrochloride 0.5% with fluorescein sodium 0.25%) two GAT IOP and three DCT IOP measurements were made in a randomized order. Only DCT measurements with a quality score of 1 or 2 were accepted, and the first DCT reading was discarded, in accordance with the manufacturer’s guidance. The average of two IOP readings was calculated for each subject at each time point and used in the analyses. All measurements were performed by a single observer (AK) who was masked to the previous corneal measurements, but not to the previous IOP measurements.

Participants were advised that they could leave the hospital clinic between measurements, but were advised to avoid consumption of alcohol and limit their caffeine intake.

Data Analysis

A two-way ANOVA was used to consider the changes in group average GAT IOP and DCT IOP, using the subjects as data blocks in the study, with the five time points (9 AM, 11 AM, 1 PM, 3 PM, and 5 PM) being the main factor. The same analysis was used to assess changes in corneal characteristic across the five time points.

Multilevel modeling (MLM), a modern statistical technique commonly used in the medical, social, and educational sciences, was chosen to analyze the relationships between the variables and the variability of that relationship between subjects. 23 This method is equivalent to ordinary multiple linear regression in that a model describing the relationship between several predictor variables and a single outcome variable may be developed and estimated. Ordinary linear regression analysis makes the assumption that all outcome observations are independent of each other. However, in the data presented, each subject contributes five measurements, and therefore measurements are nested within subjects and thus are not independent. An analysis ignoring this grouping, or nonindependence, of the measurements will result in the underestimation of the standard errors of regression coefficients, giving overly low probabilities, whereas a patient-level analysis (e.g., using a simple mean of the five measurements) loses potentially valuable information. MLM adjusts for the hierarchical structure of the data, explicitly modeling the way in which measurements are grouped within subjects. In MLM, subjects are regarded as a random sample from the population of all patients, and inference is made about the variation between patients in general. Both the intercepts and slopes of the fitted regression lines can vary randomly between subjects.

Statistical analyses were performed with commercial software (SPSS ver.10; SPSS Inc., Chicago, IL). MLM was performed with MLwiN software, version 2.02 (Multilevel Models Project, Institute of Education, London, UK). 24

RESULTS

The mean IOP and corneal characteristics of the 62 eyes at baseline are given in Table 1. There was a significant difference between average GAT IOP and DCT IOP measurements in GON and normal eyes. However, the mean and range of corneal characteristics between the groups was similar.
Variations in IOP Measurements and Corneal Characteristics at Each Time Point

Average GAT IOPs varied with the time point at which they were measured, with a statistically significant difference between morning and afternoon measurements: average (range) GAT IOP measurement at each time point: 9 AM, 16.5 mm Hg (10.0–28.0); 11 AM, 15.9 mm Hg (9.0–28.0); 1 PM, 14.9 mm Hg (7.5–26.0); 3 PM, 14.7 mm Hg (7.5–26.0); 5 PM, 14.9 mm Hg (7.5–24.0); ANOVA $F = 19.9; P < 0.001$. A similar effect, but less pronounced, was observed with average DCT IOP measurements across the time points: DCT IOP measurements: 9 AM, 19.0 mm Hg (11.8–27.6); 11 AM, 18.9 mm Hg (12.2–32.3); 1 PM, 18.2 mm Hg (10.1–29.3); 3 PM, 18.3 mm Hg (11.7–35.3); 5 PM, 18.2 mm Hg (11.5–33.1); ANOVA; $F = 4.59 P = 0.001$ (Fig. 1). Average CCTs also varied with the time point at which they were measured, with small but significant mean differences between early morning and late afternoon: CCT: 9 AM, 548.7 μm (471.8–628.2); 11 AM, 546.9 μm (476.3–627.1); 1 PM, 546.1 μm (472.5–624.3); 3 PM, 545.2 μm (472.7–625.2); 5 PM, 544.7 μm (469.6–625.3); ANOVA $F = 16.4; P < 0.001$. These data revealed no evidence of CH values varying with measurement time: CH: 9 AM, 12.2 mm Hg (7.3–17.6); 11 AM, 11.9 mm Hg (6.8–16.5); 1 PM, 11.8 mm Hg (6.5–16.2); 3 PM, 11.8 mm Hg (6.5–17.6); 5 PM, 11.9 mm Hg (6.5–17.6); ANOVA $F = 1.79; P = 0.13$; Fig. 2.

GAT IOP changes were, on average, greater than DCT IOP changes (Table 2). Differences in IOP measurement between the two devices became significant after the 11 AM time point. Changes in IOP measurements and corneal characteristics are illustrated in Figure 3. Both measured IOP and CCT decreased during the day, and CH appeared to decrease only slightly. Figure 3 suggests that, on average, DCT IOP changes were smaller than those recorded with the GAT.

Further analyses showed that greater IOP changes occurred over the 8-hour period in subjects with higher baseline IOP for both control subjects and patients with glaucoma. The relationship between baseline IOP level and degree of office-hour IOP change was similar in both control subjects and patients with glaucoma. Therefore, data from both these groups were pooled for the MLM analysis.

Multilevel Modeling

The results for the MLM using GAT IOP as the outcome variable with CCT, CH and age as the explanatory variables is given in Table 3. This model has a much better fit to the data compared with an ordinary linear regression model of all the data (i.e., 62 × 5 measurements) which takes no account of the hierarchical structure of the data and assumes that all measurements are independent, which is clearly not the case. This is supported by the reduction in the model deviance ($-2 \times$ log-likelihood measure) which improves from 1572 to 1291, indicating a much better fit of the more elaborate model to the data. Age was shown to have no significant effect on the change in GAT IOP during the day, whereas there was a small but statistically significant association of CCT with GAT IOP: an average decrease in CCT of 1 μm was associated with a decrease of 0.04 mm Hg. A change in CH also had a statistically significant association with GAT IOP: a decrease in 1 mm Hg CH was associated with a decrease in 0.20 mm Hg IOP. An important value from the model is the square root of the
variance of the residuals, which is 1.50 units, a value that is several orders of magnitude greater than the estimate of the main effect: the variability around the average line is therefore very large.

The results for the MLM using DCT IOP as the outcome variable, with CCT, CH, and age as the explanatory variables, is given in Table 4. Age was shown to have no significant effect on the change in DCT IOP measurements during the day, although there was a small but statistically significant association of CCT with DCT IOP: an average decrease in CCT of 1/9262 m9262 was associated with a decrease of 0.03 mm Hg. CH did not have a statistically significant association with DCT IOP. The square root of the variance of the residuals is 1.46 units, a value that is much greater than the estimate of the main effect: the variability around the average line is therefore relatively very large.

Figure 4 shows the predicted values of GAT-measured IOP for the MLM of the effect of CCT on GAT IOP. This plot differs from an ordinary regression plot, in that each line represents the variation in both GAT IOP and CCT measurements for a single subject, with the slopes of all subjects varying around the average relationship between GAT IOP and CCT. The slopes of the lines indicate the magnitude of the relationship between CCT and GAT IOP. The length of the line indicates the degree of measurement change. Parallel lines indicate that the relationship between CCT and GAT IOP over the five time points is the same in all patients (close to the average relationship). On closer inspection, this result seems generally to be the case. In MLM this relationship is known as a complex level 2 variation, and it tells us about the consistency of the relationship. Figure 5 shows the predicted DCT IOPs for the MLM of the effect of CCT on DCT IOP. On inspection, fewer lines seem parallel compared with those in Figure 4; there is greater level 2 complex variation. In other words, the association of CCT with DCT IOP is less consistent across subjects compared with the association of CCT with GAT IOP. The association of individual subject IOP/CCT regression slopes with individual subject mean diurnal IOP was not significant for either GAT or DCT across the range of IOPs in this study.

These analyses suggest that the DCT IOP/CCT relationship is not uniform among subjects, unlike the GAT IOP/CCT relationship. This subtle but important difference in the relationship between GAT IOP and CCT compared with that between DCT IOP and CCT is supported by a value from the covariance matrix of the MLM results, which can be interpreted as the variation in the slopes across the patient’s summary lines. For GAT and CCT, this value is 0.001 (SE, 0.001) and for DCT and CCT it is four times greater (0.004; SE, 0.002).

**DISCUSSION**

In this study there were small but significant changes in both GAT and DCT IOP during office hours. There were small yet

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**Table 2. Change in GAT and DCT Measured IOP at Each Time Interval**

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>ΔGAT (mmHg)</th>
<th>ΔDCT (mmHg)</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 AM–9 AM</td>
<td>-0.66 (1.99)</td>
<td>-0.13 (2.24)</td>
<td>0.55</td>
<td>0.102</td>
</tr>
<tr>
<td>1 PM–9 AM</td>
<td>-1.58 (2.02)</td>
<td>-0.83 (2.36)</td>
<td>0.75</td>
<td>0.012</td>
</tr>
<tr>
<td>3 PM–9 AM</td>
<td>-1.87 (2.44)</td>
<td>-0.70 (2.94)</td>
<td>1.18</td>
<td>0.001</td>
</tr>
<tr>
<td>5 PM–9 AM</td>
<td>-1.62 (2.53)</td>
<td>-0.84 (2.60)</td>
<td>0.78</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Data are the mean mm Hg (SD) [range].

* Paired t-test.

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**Figure 3.** Change in IOP measurements and corneal characteristics from baseline at each time-point. Error bars, 95% CI.
significant changes in CCT, but not in CH, in the group studied. MLM analysis showed that both CCT and CH were significantly associated with the changes seen in GAT IOP measurements, and the slope of the association was relatively consistent between subjects. In contrast, DCT IOP measurements were only modestly associated with CCT, and not with CH. Furthermore, the association of CCT with DCT-measured IOP showed greater intersubject variability when compared with CCT with GAT-measured IOP.

The diurnal variation of IOP is well established, but it is not known to what extent changes in corneal properties influence the amplitude of measured IOP change. In the present study, GAT IOP measurements were greatest first thing in the morning and reduced gradually during the day. It has been suggested that the peak in measured IOP often seen in the early morning may be explained in part by the effects of a hydration-related corneal thickness increase induced by overnight eye closure. Studies have shown an association between GAT IOP and corneal hydration changes (determined by changes in CCT) induced by contact lens wear that mimics conditions encountered during sleep. However, corneal thickness changes induced by overnight swelling are of a greater magnitude than the changes seen during the rest of the day and usually diminish within 2 hours of awakening. In the present study, CCT was greatest in the morning and followed a similar pattern of reduction over the course of the day. The magnitude of change, however, was small (~1%), in agreement with other studies assessing CCT changes during office hours. In prior studies, investigators have failed to find a temporal relationship between CCT and measured IOP during office hours. This may be due to a combination of factors, including measurement imprecision that would mask any relationships that exist. In the present study, CCT was measured with the OLCR pachymeter (Haag-Streit). The instrument offers an advantage over ultrasound pachymetry, in that readings are only obtained if the probe is centered on the optical axis. Studies have found it to provide repeatable measures that show less variability compared with standard contact ultrasound techniques. A further advantage of the OLCR pachymeter is that, due to its noncontact design, no corneal anesthesia is necessary for measurements. Studies have indicated that the use of corneal anesthesia and repetitive contact of the cornea during contact pachymetry may induce changes in CCT. However, even with highly precise measures of CCT, the small magnitude of change in parameters and large interindividual measurement variability may mask any temporal relationship between changes in measured IOP and corneal characteristics when data are grouped. These difficulties may be overcome by the use of MLM techniques. MLM allows data to be grouped for analysis, but keeps individual subject data nested together so that patterns in the repeated measurement may be observed. MLM techniques analyze the extent to which IOP measurement variability in the group is attributable to differences in characteristics both between and within subjects. Using this analysis, corneal characteristics, but not age, were shown to be significantly associated with GAT IOP measurements made during office hours (1 mm Hg decrease in CCT was associated with a 0.04 mm Hg decrease in GAT IOP). The MLM analyses show that the relationship between DCT IOP and CCT was less consistent between subjects. In some subjects, changes in CCT had little association with DCT IOP, whereas in others the association was quite prominent (Fig. 5). The reasons for this are as yet unclear, and we may only speculate on our findings at this stage. The DCT has been shown to be less strongly associated with...
CCT, and so perhaps our data show that the “true” IOP changes significantly during the day in some individuals but not in others. It is possible that such significant changes in true IOP may alter corneal hydration, causing alterations in both CCT and corneal biomechanics. These speculations should be explored further and will be the subject of further study.

It has only recently become possible to measure the diurnal changes in corneal biomechanics since the introduction of the Reichert ORA. In agreement with other studies, the changes in CH found in the present study were small and not statistically significant. However, incorporating CH within the MLM analysis showed that these subtle changes in CH were significantly associated with GAT IOP measurements (an increase in 1 mmHg CH was associated with an increase in 0.20 mm Hg IOP), but were not associated with DCT IOP measurements during the day, and provides further evidence that the DCT may be more robust to corneal biomechanical effects.

DCT IOP measurements were on average 2.6 mm Hg higher than those made with the GAT. Other studies have reported a higher DCT IOP reading compared with the GAT, and this has been attributed to the calibration technique; the DCT is calibrated with a direct manometric measurement against a hydrostatic pressure standard, without any corneal surface. A recent study has shown DCT IOP measurements to be concordant with intracameral IOP.

After the incorporation of age, the MLM analysis found that age did not explain any of the diurnal variation in IOP measurements between subjects. Previous work has shown that there is an age-related increase in corneal stiffness, and this may induce a further measurement error with GAT, although our results suggest that any age-related error does not
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vary during the day. However, in contrast with ex vivo studies, clinical studies require a significant sample size to detect age effects due to clinical measurement variability. As ageing effects were not a primary outcome measure, the present study may be insufficiently powered to detect an effect, and this should be explored in further studies.

In conclusion, our study of untreated eyes shows that there is a covariation of measured IOP and corneal characteristics during normal office hours. IOP measurement changes made with the GAT are significantly associated with changes in both CCT and CH, whereas the DCT IOP measurement appears less strongly associated with diurnal changes in these corneal characteristics. The study suggests that diurnal variations in corneal characteristics may be responsible for only a small proportion of the observed diurnal variation in IOP.

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References


