Corneal Thickness- and Age-Related Biomechanical Properties of the Cornea Measured with the Ocular Response Analyzer

Aachal Kotecha,1,2 Ahmed Elsheikh,3 Cynthia R. Roberts,4,5 Haogang Zhu,1,2 and David F. Garway-Heath1,2

PURPOSE. The Ocular Response Analyzer (ORA) is a new instrument that measures the corneal biomechanical response (corneal hysteresis, CH) to rapid indentation by an air jet. CH is the difference in applanation pressures (P1, P2) between the rising and falling phases of the air jet. The investigation had two parts: a characterization study and a validation study. In the characterization study, the purposes were to investigate the intraocular pressure (IOP)–dependence of CH and to characterize the performance of the ORA. In the validation study, the purposes were to investigate the association between CH and both age and central corneal thickness (CCT) and the agreement between ORA and Goldmann applanation tonometer (GAT) IOP measurements.

METHODS. For the characterization study, data were collected from 105 untreated subjects (45 ocular hypertensive patients and 60 normal subjects; mean age, 60 years; range, 26–82). GAT and ORA measurements were performed before and after IOP lowering of one randomly selected eye with apraclonidine drops. The change in P1 and P2 (arbitrary units) in relation to change in GAT IOP was analyzed to calibrate the instrument. The relationship between P1, P2, and CCT was explored and ORA IOP was derived from the analyses. For the validation study, ORA and GAT IOP and CCT were measured in 144 eyes of 144 untreated subjects (mean age, 58 years; range, 19–83). The characterization calculations were applied to the dataset and values of CH and ORA IOP were calculated. The relationship between CH and both subject age and CCT was determined. The associations between CH and CCT and between ORA and GAT IOPs, were investigated by linear regression analysis. The agreement between measuring devices was calculated.

RESULTS. In the characterization study, P1 changed by 6.41 arbitrary units for every 1-mm Hg change in GAT IOP. CH (P1 – P2) changed by −1.60 arbitrary units for every 1-mm Hg change in GAT IOP. For each unit change in P2, P1 changed by 1.27 units. From this association a new IOP-independent corneal factor was derived [P1 − (P2/2.27)] and is termed the corneal constant factor (CCF; mm Hg). ORA IOP normalized for CCF was defined as P2 - CCF (mm Hg). The CCF (mm Hg) was associated with CCT (micrometers) and with age: CCF = [0.036 · CCT] – (0.028 · age]) + 1.06 (adjusted r² = 0.34; P < 0.0001 for CCT, P = 0.007 for age). Normalized ORA IOP measurements were not associated with CCT. GAT IOP was associated with CCT and CCF—more strongly with the latter: GAT IOP = (0.03 · CCT) + 1.52 (r² = 0.06, P = 0.002); GAT IOP = (0.65 · CCF) + 4.5 (r² = 0.13, P < 0.0001). The mean difference (95% limits of agreement) between GAT and normalized ORA IOP was 0.1 (−6.6 to +6.8) mm Hg.

CONCLUSIONS. The CCF describes an IOP-independent biomechanical property of the cornea that increases with thicker CCT and decreases with greater age. It is moderately strongly associated with CCT and yet explains more of the interindividual variation in GAT IOP than does CCT. Normalized ORA IOP measurements are not associated with CCT. (Invest Ophthalmol Vis Sci. 2006;47:5337–5347) DOI:10.1167/iovs.06-0557

G laucoma can be defined as a progressive optic neuropathy, with characteristic morphologic changes of the optic nerve head and retinal nerve fiber layer in the absence of other ocular disease and congenital abnormalities.1 Raised intraocular pressure (IOP) is the major modifiable risk factor for the development2 and progression3 of the disease. The Goldmann applanation tonometer (GAT) is currently the gold standard for measuring IOP.

When Goldmann and Schmidt introduced the GAT in 1957, they acknowledged that a variation in central corneal thickness (CCT) would influence IOP readings, but stated that in the absence of corneal disease, the CCT did not vary much around 500 µm. They also assumed that the effects of corneal rigidity would be counterbalanced by the surface tension of the tear meniscus’s drawing the tonometer tip onto the cornea when applanating a circular area of diameter 3.06 mm.1 However, Van Bahr4,5 was one of the first to report a large variation in CCT within a normal population, and studies by Ehlers et al.7 in the 1970s revealed that this variation in CCT had an effect on applanation measured IOP. Many studies since have been focused on the influence of CCT on IOP measurement, and although some have found no association,8,9 most have agreed with the findings of Ehlers et al.7 that there is an increase in measured IOP with increasing CCT and that all commonly used types of tonometer are affected.13 However, CCT alone accounts for only a small proportion of the variation in measured IOP among individuals, and it is likely that other corneal biomechanical properties have an important influence on IOP measurement.

Reichert has produced an instrument, the Ocular Response Analyzer (ORA; Reichert, Corp., Buffalo, NY), that measures
the corneal response to indentation by a rapid air pulse. A feature of the ORA is that the air-jet pressure is steadily increased until the first force-in applanation event is reached. This applanation event is the trigger to switch off the air jet after some further increase in air pressure. After reaching the pressure peak, the air pressure slowly reduces until it is completely removed. The instrument makes two measurements of the corneal response to the 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.\(^1\)\(^,\)\(^2\) It has been found that the second, force-out applanation occurs at a lower pressure than the first, force-in applanation, and this effect has been attributed to the dampening effects of the cornea. The difference between the two pressures has been termed corneal hysteresis (CH; Fig. 1). Hysteresis is a measure of the energy absorption during the "loading–unloading, stress–strain cycle of viscoelastic materials (see Fig. A1, Appendix). CH is a direct measure of the corneal biomechanical properties and therefore may more completely describe the contribution of corneal resistance to IOP measurements than does CCT alone. The contribution of the anterior ocular structures to the observed ocular hysteresis under ORA pressure is likely to be small due to the direct pressure application on the cornea. For this reason, we refer to hysteresis as a corneal response in this article.

The primary purpose of this study was to investigate the effect of IOP on CH, with a view to using CH measurements to normalize the ORA IOP measurements for the effect of corneal biomechanical properties. The secondary aims were to investigate the association between CH values and both CCT and IOP-related effects on P1, P2, and CH were investigated in a group of 105 eyes in which IOP was pharmacologically lowered (Table 1).

Data were collected from 105 subjects who were not using topical hypotensive medication (45 patients with ocular hypertension and 60 volunteers with normal IOP) and attended the Glaucoma Research Unit Ocular Hypertension clinic between October 2002 and July 2003. Subjects were included if they had \(\pm 2\) D of corneal astigmatism and no previous history of intraocular or corneal surgery. GAT and ORA measurements were performed before and after the IOP was pharmacologically lowered in one randomly selected eye. Two GAT and three ORA measurements were performed on both eyes by one observer (AK). The ORA readings were obtained consecutively, and only good-quality readings were stored. The ORA displays a graphic representation of the corneal response after each measurement. The manufacturer defines good-quality readings as both force-in and force-out applanation signal peaks on the ORA waveform being fairly symmetrical in height (Fig. 1). ORA measurements were performed before GAT measurements to eliminate the possible effect that applanation tonometry may have on the hysteresis value. GAT IOP measurements were

\[ \text{P1 - P2. (1)} \]

where \(k\) is the corneal constant factor (CCF), which is defined in the next section.

**Characterization of the ORA**

IOP-related effects on P1, P2, and CH were investigated in a group of 105 eyes in which IOP was pharmacologically lowered (Table 1).

**Definitions**

P1 is the force-in applanation pressure; P2 is the force-out applanation pressure; \(P_{\text{max}}\) is maximum applied air-jet force; CH, as mentioned, is corneal hysteresis, which is calculated as

\[ (P1 - k)P2. \quad (2) \]

\(n = 105.\)

\[ \text{P1 - P2. (1)} \]

The corneal factors are calculated as

\[ (P1 - k)P2. \quad (2) \]

where \(k\) is the corneal constant factor (CCF), which is defined in the next section.

**TABLE 1. Demographic Data of Characterization Group**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye (n, left)</td>
<td>50/105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (n, females)</td>
<td>47/105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>59.9</td>
<td>13.8</td>
<td>25.5–81.8</td>
</tr>
<tr>
<td>CCT apraclonidine ((\mu m))</td>
<td>564.8</td>
<td>36.2</td>
<td>482–653</td>
</tr>
<tr>
<td>CCT control ((\mu m))</td>
<td>564.6</td>
<td>36.8</td>
<td>478–655</td>
</tr>
<tr>
<td>Mean keratometry apraclonidine (mm)</td>
<td>7.7</td>
<td>0.23</td>
<td>7.2–8.3</td>
</tr>
<tr>
<td>Mean keratometry control (mm)</td>
<td>7.7</td>
<td>0.24</td>
<td>7.1–8.3</td>
</tr>
<tr>
<td>Baseline GAT IOP apraclonidine (mm Hg)</td>
<td>17.8</td>
<td>4.5</td>
<td>9–31</td>
</tr>
<tr>
<td>Baseline GAT IOP control (mm Hg)</td>
<td>17.8</td>
<td>4.5</td>
<td>9–29</td>
</tr>
<tr>
<td>Change/reduction GAT IOP apraclonidine (mm Hg)</td>
<td>5.8</td>
<td>3.0</td>
<td>−0.5–12.5</td>
</tr>
<tr>
<td>Change/reduction GAT IOP control (mm Hg)</td>
<td>1.2</td>
<td>1.9</td>
<td>−2.5–7</td>
</tr>
</tbody>
</table>

\(n = 105.\)

**TABLE 2. Demographic Data of Validation Group**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.7</td>
<td>15.1</td>
<td>19.4–83.4</td>
</tr>
<tr>
<td>CCT ((\mu m))</td>
<td>553.0</td>
<td>36.6</td>
<td>464.1–634.3</td>
</tr>
<tr>
<td>GAT IOP (mm Hg)</td>
<td>17.3</td>
<td>4.1</td>
<td>9.5–28.0</td>
</tr>
<tr>
<td>Diagnosis (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular hypertension</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigment dispersion syndrome</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect normal tension glaucoma</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect primary open angle glaucoma</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(n = 144.\)
taken within 5 minutes of ORA measurements. After this, 1 drop of apraclonidine hydrochloride (Iopidine 0.5%; Alcon Laboratories, Fort Worth, TX) was administered to one randomly selected eye. To account for the possibility that topical apraclonidine may have a direct effect on the CH measurement, we administered a control drop of the same composition, but without apraclonidine, to the other eye. GAT and ORA readings were repeated after 3 hours.

CCT was measured by ultrasound pachymetry (Altair 2000; Optikon Corp., Kitchener, ON, Canada) after baseline IOP measurements. The mean of three readings was recorded.

The raw applanation pressure (arbitrary units) output by the ORA software was analyzed. The instrument gives readings for force-in and force-out applanation pressures, \( P_1 \) and \( P_2 \), respectively (see Fig. 1). The differences between GAT, \( P_1 \), and \( P_2 \) at baseline and after 3 hours are expressed as \( \Delta \text{GAT} \), \( \Delta P_1 \), and \( \Delta P_2 \), respectively.

The relationship between \( \Delta P_1 \) and \( \Delta P_2 \) (arbitrary units) versus \( \Delta \text{GAT} \) (mm Hg) was investigated in both eyes. The results of this relationship were used to convert the applanation pressures \( P_1 \) and \( P_2 \) into pressure units (mm Hg). The relationship between \( \Delta P_1 \) and \( \Delta P_2 \) was determined, to establish the value for \( k \) (see equation 2) that resulted in an IOP-independent factor. This was termed the corneal constant factor (CCF). The associations among \( P_1 \), \( P_2 \), and CCT were explored and the value of \( k \) that resulted in the strongest association with CCT was identified. This corneal response parameter was termed the corneal resistance factor (CRF). A normalized ORA IOP measurement was defined using the new values for \( P_1 \) and \( P_2 \) (mm Hg). The repeatability (coefficient of variation) of GAT and ORA measurements was calculated.

**Validation Studies**

**Study I: The Associations of CH.** For the second part of the study, GAT IOP, ORA IOP, CH, and CCT were measured in 144 eyes of 144 untreated subjects, with inclusion criteria as for the subjects in the characterization study (Table 2). ORA measurements were performed before the application of topical corneal anesthesia. CCT was measured after tonometry. All measurements were performed by the same observer (AK). ORA IOP, CCF, and CRF were calculated by using the relationships determined in the characterization study. The relationship between CCF and both age and CCT was determined. Associations of CCT and CCF with GAT measurements were investigated.

**Study II: Agreement between Measuring Devices.** The agreements among GAT, normalized ORA IOP measurements (ORA IOP(CCF)), and the manufacturer’s ORA IOP (IOP(CC)) were assessed. The relation between GAT/ORA(CCF) and GAT/ORA(CC) differences and CCF and CCT were explored.

**Data Analysis**

A least-squares linear regression analysis was used to determine the relationship between variables, when the measurement variability of the independent variable was much less than that of the dependent variable. When the measurement variability of the two variables was more equal, an orthogonal regression was used (Principal Components Analysis method available in MatLab, ver. 7.0 R14, The MathWorks, Inc., Natick, MA). The coefficient of variation was calculated as the average SD/mean of all measurements \( \times 100 \). Multiple linear regression analyses were used to investigate the relationship between a dependent variable and multiple independent variables. Bland-Altman plots were used to assess the agreement between IOP measurement techniques.\(^{16}\) The mean difference and 95% limits of agreement were

**TABLE 3. Repeatability of CCT and IOP Measurements Made with GAT and ORA P1 and P2**

<table>
<thead>
<tr>
<th></th>
<th>CCT CoV (%)</th>
<th>GAT CoV (%)</th>
<th>P1 CoV (%)</th>
<th>P2 CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.6</td>
<td>2.1</td>
<td>3.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

P1, ORA first applanation event; P2, ORA second applanation event (see Fig. 1); CoV, coefficient of variation.

**FIGURE 2.** Orthogonal regression analysis showing the relationship between \( \Delta P_1 \) and \( \Delta P_2 \) in both treated and untreated eyes.
calculated. All statistical analyses were performed in commercial software (Medcalc, ver. 8.2.1.0; Medcalc Software, Mariakerke, Belgium).

RESULTS

Characterization of ORA

A change in GAT IOP and P1 and P2 was observed in eyes treated with apraclonidine, as well as in control eyes, and in subsequent analyses, both treated and control eyes were assessed together. There was a change in CH (arbitrary units) for every 1-mm Hg change in GAT IOP of \(1.7\) (eyes treated with apraclonidine), \(1.8\) (eyes treated with placebo), and \(1.6\) (both eyes together). This illustrates that CH is dependent on IOP, although the relationship is relatively weak.

The coefficients of variation of CCT (\(\mu m\)), GAT IOP (mm Hg), and ORA P1 and P2 (arbitrary units) measurements are tabulated (Table 3). An orthogonal regression was performed to evaluate the relationship between \(\Delta P1\) and \(\Delta P2\) (Fig. 2). There was no association between the residuals from the regression and GAT IOP or CCT.

The orthogonal regression analysis showed that P1 and P2 were linearly related, and the equations generated from this analysis were

\[
\Delta P2 = -6.70 + (1.3 \cdot \Delta P1),
\]

for apraclonidine treated eyes,

\[
\Delta P2 = -4.2 + (1.8 \cdot \Delta P1),
\]

for placebo treated eyes.

\[
\Delta P2 = -3.7 + (1.5 \cdot \Delta P1),
\]

for both eyes together.

FIGURE 3. Change in CH with change in GAT- eyes treated with apraclonidine and placebo. Regression line: \(y = -1.60x + 1.39; r^2 = 0.19\).

FIGURE 4. Coefficient of determination of the relationship between \((P1 - kP2)\) and CCT for different values of \(k\). Solid line: value of \(k\), determined in the characterization study; dashed line: value of \(k\), resulting in the greatest association with CCT.
for control eyes, and

$$\Delta P_2 = -3.98 + (1.27 \cdot \Delta P_1),$$

for both eyes together.

Because of the small value of the constant in the equation 3, the relationship between $\Delta P_1$ and $\Delta P_2$ may be approximated by

$$\Delta P_2 = 1.27 \cdot \Delta P_1 \quad (4)$$

or

$$\Delta P_1 = 0.79 \cdot \Delta P_2.$$

The calculations demonstrate that for every unit change in $P_1$ resulting from a change in IOP, there is a corresponding, proportional change in $P_2$, accounting for the finding that $CH (P_1 - P_2)$ changes as IOP changes (Fig. 3). To obtain a corneal factor that does not change with IOP (CCF), we used a constant $k$ to adjust $P_2$ with the coefficient derived from equation 4 (i.e., 0.79), so that:

$$CCF = P_1 - kP_2, \quad (5)$$

where $k = 0.79$.

To evaluate the association of $(P_1 - kP_2)$ with CCT, various values of $k$ were explored, including 1 (i.e., raw $P_1$ and $P_2$ data output from the ORA), 0.79 (determined from the character-
The relationship between \( \Delta P1 \) and \( \Delta GAT \) was found to be linear, and the equations generated by the analysis were

\[
\Delta P1 = (5.85 \cdot \Delta GAT) + 11.85,
\]

for apraclonidine treated eyes,

\[
\Delta P1 = (6.18 \cdot \Delta GAT) + 5.93,
\]

for control eyes, and

\[
\Delta P1 = (6.41 \cdot \Delta GAT) + 7.85.
\]

for both eyes together.

Due to the small value of the constant in the equations, the relationship between \( \Delta P1 \) and \( \Delta GAT \) may be approximated as

\[
\Delta P1 = 6.41 \cdot \Delta GAT.
\]

From equations 4 and 6, \( P1 \) and \( P2 \) (arbitrary units) were converted into GAT equivalents (mm Hg), and the corneal constant factor (CCF; mm Hg) was defined by

\[
P1_{mm Hg} = P1_{arbitrary units}/6.41
\]

(8)

\[
P2_{mm Hg} = P2_{arbitrary units}/6.41 \cdot 0.79
\]

(9)

and

\[
CCF_{mm Hg} = P1_{mm Hg} - P2_{mm Hg}.
\]

(10)

To assess the independence of CCF (mm Hg) from IOP, these calculations were applied to the characterization dataset, and the difference between CCF at baseline and after 3 hours (\( \Delta CCF \)) was determined. The relationship between \( \Delta CCF \) and \( \Delta GAT \) confirmed the IOP independence of CCF (slope 0.013, 95% confidence interval [CI] for slope -0.04 to 0.04; \( r^2 = 0.001, P = 0.64 \)).

A second corneal factor was defined using \( k = 0.68 \), which resulted in the strongest association between CCT and \( (P1 - kP2) \), and was termed the corneal resistance factor (CRF):

\[
P1_{mm Hg} = P1_{arbitrary units}/6.41,
\]

(11)

\[
P2_{mm Hg} = P2_{arbitrary units}/6.41 \cdot 0.68,
\]

(12)

\[
CCF_{mm Hg} = P1_{mm Hg} - P2_{mm Hg}.
\]

(13)

This factor, derived using \( k = 0.68 \), is similar to that defined by the manufacturer (Luce D, personal communication, 2005):

\[
CRF_{mm Hg (reichert)} = 0.1324[(P1 - 0.7)P2] - 7.46
\]

(14)

\[
CRF_{mm Hg (present study)} = 0.1560[(P1 - 0.68)P2].
\]

(15)

where \( P1 \) and \( P2 \) are in arbitrary units.

Having determined the corneal constant factor, we wanted to explore the relationship between \( P1, P2, \) and CCF with CCT, to determine a method of removing the corneal effect from the IOP measurement (Fig. 6).

The relationship between CCT and \( P1, P2, \) and CCF were found to be linear, and the equations generated from the analysis were as follows:

\[
P1 = (0.091 \cdot CCT) - 5.0; r^2 = 0.23,
\]

(16)

\[
P2 = (0.056 \cdot CCT) - 6.0; r^2 = 0.12,
\]

(17)

and

\[
CCF = (0.033 \cdot CCT) + 1.0; r^2 = 0.34.
\]

(18)

### Table 5. Multiple Regression Analysis of Corneal Factors and Age against GAT IOP Measurements

<table>
<thead>
<tr>
<th>Corneal Factor</th>
<th>Coefficient</th>
<th>P for Corneal Factor</th>
<th>Age Coefficient</th>
<th>P for Age</th>
<th>Adjusted ( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH (( k = 1.0 )) (arbitrary units)</td>
<td>-0.037</td>
<td>0.122</td>
<td>0.032</td>
<td>0.16</td>
<td>0.02</td>
</tr>
<tr>
<td>CCF (( k = 0.79 )) (mm Hg)</td>
<td>0.679</td>
<td>&lt;0.0001</td>
<td>0.047</td>
<td>0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>CRF (( k = 0.68 )) (mm Hg)</td>
<td>0.911</td>
<td>&lt;0.0001</td>
<td>0.041</td>
<td>0.03</td>
<td>0.34</td>
</tr>
</tbody>
</table>
CCF was moderately strongly associated with CCT. As P2 appeared to be less affected by CCT than P1 and to a similar extent as the CCF, we hypothesized that subtracting this corneal factor from P2 would reduce the CCT dependence of P2 measurements.

Therefore, “normalized” ORA IOP was defined as

\[
\text{ORA IOP(CCF)} = \frac{P_2}{C CF} \text{ mm Hg} / \text{constant. (19)}
\]

The mean difference between GAT IOP measurements and ORA IOP measurements was used to determine \(k\), to adjust the ORA IOP measurement so that the mean difference between the two techniques was 0. The constant was 11 mm Hg. These characterizations were then applied to the validation group.

Validation Studies

Study I: Associations of Corneal Factors. The characterization study determined that the corneal factor could be defined as \(P_1 - kP_2\). For the validation study, the relationships of the corneal factor calculated using \(k = 1\) (i.e., original/raw hysteresis value), \(k = 0.79\) (CCF), and \(k = 0.68\) (CRF) were determined. Multiple linear regression analysis showed that the corneal factors were dependent on CCT and age (Table 4).

Previous work has suggested that increasing age induces a measurement error additional to that of CCT on GAT IOP measurements, because of the likely effect of age on the material properties of the cornea.\(^{15,19}\) A multiple linear regression analysis indicated that GAT IOP measurements were associated with both the corneal factor and age, although the association with the latter was weaker (Table 5, Fig. 7).

Study II: Agreement between Measuring Devices. For the following analyses, agreement between ORA IOP (CCF) and GAT IOP measurements were determined. In addition, the manufacturer’s value for corneal compensated ORA IOP was determined from the following formulas provided by Reichert (D. Luce, personal communication, October 2005), and was termed ORA IOP (CCF):

\[
\text{ORA IOP(CC)} = \frac{P_2}{0.202} - \frac{(0.430 \cdot P_1)}{0.633} + 11.0. (20)
\]

For comparison, equation 19 which defines ORA IOP (CCF) can be rewritten as

\[
\text{ORA IOP(CCF)} = \frac{P_2}{0.79} - \frac{(0.79 \cdot P_1)}{6.41} + 11.0. (21)
\]

where \(P_1\) and \(P_2\) are in arbitrary units, as in equation 20.

The coefficients of variation for IOP measurements are shown in Table 6.

Linear regression analysis indicated that ORA IOP (CCF) measurements were not associated with CCT (slope 0.002, 95% CI for slope -0.02 to +0.01; \(r^2 = 0.002, P = 0.85\)). There was a

![Figure 7. Relationship between age and corneal factors. The graph illustrates the relationship between age and the residuals of the linear regression analysis of corneal factors (mm Hg) against central corneal thickness. Regression lines: CCF versus CCT residuals = (-0.020 \times age) + 1.1; \(r^2 = 0.02\).](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933233/)
nonsignificant trend of association between ORA IOP(CCF) and CCT (slope 0.02, 95% CI for slope −0.003 to +0.003; $r^2 = 0.02$, $P = 0.09$). GAT IOP measurements were significantly associated with CCT (slope 0.03, 95% CI for slope 0.01 to 0.05; $r^2 = 0.06$, $P = 0.002$), and more strongly associated with corneal factors (Tables 5, 7; Fig. 8).

Agreement between GAT and ORA IOP was poor and is tabulated in Table 8. There was a significant relationship between GAT IOP/ORA IOP(CCF), GAT IOP/ORA IOP(CC), and ORA IOP(CCF)/ORA IOP(CC) differences and mean IOP. GAT overestimates both ORA IOP(CCF) and ORA IOP(CC) measurements at low IOP and underestimates them at higher IOP. In addition, ORA IOP(CCF) underestimated ORA IOP(CC) with increasing IOP (Fig. 9).

Linear regression analysis indicated that GAT IOP/ORA IOP(CCF) differences were not associated with CCT, but were positively associated with CCF, indicating that GAT overestimates ORA IOP(CCF), with increasing CCF. The lack of association between GAT IOP/ORA IOP(CCF) differences and CCT may be explained by the positive, though nonsignificant, association between ORA IOP(CCF) and CCT, which was similar to the association between GAT IOP and CCT.

GAT IOP/ORA IOP(CCF) differences, however, were associated with both corneal parameters, showing that with increasing CCT and CCF, GAT IOP overestimates ORA IOP(CCF). ORA IOP(CCF)/ORA IOP(CC) differences were also associated with both CCT and CRF, so that ORA IOP(CCF) underestimates ORA IOP(CC) with increasing CCT and CCF. These two relationships suggest that the ORA IOP(CCF) measurement is less affected by corneal material properties.

### Table 7. Linear Regression Analysis of CCT and Corneal Factors against GAT IOP Measurements

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT (µm)</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>CH ($k = 1.0$) (arbitrary units)</td>
<td>-0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>CCF ($k = 0.79$) (mm Hg)</td>
<td>0.65</td>
<td>0.13</td>
</tr>
<tr>
<td>CRF ($k = 0.68$) (mm Hg)</td>
<td>0.91</td>
<td>0.32</td>
</tr>
</tbody>
</table>

### Discussion

The Reichert ORA measures the corneal response to indentation by a rapid jet of air, giving a measure of the corneal biomechanical properties, termed CH. In this study, we determined that CH ($P_1 - kP_2$, where $k = 1$) varies with IOP (Fig. 2). This IOP dependence may be physiological or reflect an artifact of the instrument measurement, or both. The finding that ($P_1 - kP_2$) becomes independent of IOP when $k = 0.79$ and that the strongest association between ($P_1 - kP_2$) and CCT was achieved with $k = 0.68$, suggests that the IOP dependence of CH may be in part related to the instrument measurement technique.

A feature of the instrumentation is that the maximum air pressure ($P_{max}$) applied to the cornea varies between subjects, and is dependent on P1, which is in turn determined by both the true IOP and the structural resistance of the individual eye, which may contribute to the observed CH change with IOP change. As the cornea is a viscoelastic material, another instrument-related factor that may influence the corneal material property at different IOP levels is the rate of application of air jet pressure to the cornea. The rate of application of force increases with P1, and this may affect the hysteresis. We attempted to isolate the instrument-related corneal response from the structurally related response by modulating the IOP within eyes and measuring the change in P1 and P2 recorded. With this method, we took an empiric approach to recalculating the IOP measurement made by the ORA, and by doing so have significantly reduced the corneal effect on measured IOP: our normalized ORA IOP(CCF) is independent of CCT (Fig. 8), whereas P1 is strongly related to CCT (Fig. 6).

The results of both treated and control eyes were analyzed together, as the regression coefficients found when analyzing each group separately were very similar and using both eyes increased the range of values (because the IOP in one eye had not been artificially modulated), adding robustness to the analysis. The method used to characterize the ORA and convert arbitrary pressure units to mm Hg makes the assumption that
any IOP-related change in biomechanics will have the same effect on both GAT and ORA measurements. When exploring the relationship between ΔP1 and ΔGAT, we found that the regression line did not pass through 0, illustrating that a small change in P1 occurs when there is no change in GAT IOP (Fig. 5). The GAT measure is a static measure, whereas the P1 and P2 generated by the ORA are dynamic measures. The former is affected by the elastic properties of the cornea, and the latter is affected by both the elastic and viscous properties. In our study, there was a 3-hour interval between measurements while waiting for the apraclonidine to take effect. It is possible that in conjunction with IOP changes, corneal hydration changes may have occurred during that period which in turn may alter the elastic and viscous properties of the cornea in different proportions, as is suggested by the data in Figure 5. It is also possible that these corneal properties vary diurnally; the relationship between diurnal GAT and ORA (P1 and P2) measurements may have occurred due to that period which in turn may alter the elastic and viscous properties of the cornea in different proportions, as is suggested by the data in Figure 5.

The relationship between ΔP1 and ΔP2 determined that for each eye there was a corneal factor that did not change with a change in IOP (equations 3, 4, 5). We defined this factor as the CCF. We also defined a factor, the CRF, that is determined by finding the factor that has the strongest association with CCT. The formulas determined in our characterization study were applied to a second dataset from untreated individuals, and the relationships of both corneal factors (CCF and CRF) showed a strong association with CCT, although the latter is to be expected, as it was designed to be so. The CCF increased 0.36 mm Hg per 10 μm CCT, indicating that thicker corneas have greater dampering properties. CCF also decreased by approximately 0.28 mm Hg per decade. Experimental ex vivo studies have shown an age-related change in corneal collagen fibril properties that may contribute to an increased stiffness of the cornea with age,17,18 and in vivo endothelial specular microscopic studies have demonstrated corneal signs that indicate an increased corneal stiffness with age.19 The CCF is a measure of the corneal material properties, which include both stiffness and viscoelasticity. The observed negative association between corneal viscoelastic properties with advancing age may be further evidence of an increase in cross-linkage of collagen fibrils within the cornea, making it a stiffer and less viscoelastic structure.

However, ageing changes in this study cohort should be interpreted cautiously. Normal ageing changes may be confounded by pathologic corneal biomechanical changes associated with ocular hypertension (23% of our cohort), possible glaucoma, and pigment dispersion syndrome (44% of our cohort). Viscoelastic materials exhibited both creep and stress relaxation and, as a biological material, the cornea may remodel in response to raised IOP. This in turn may have an effect on the hysteresis. Studying a normal ageing population may yield different results.

Using the CCF, we redefined the ORA IOP measurement, and assessed the relationships between this measurement (ORA IOPCCF), the ORA IOP measurement determined from the manufacturer’s calibration (ORA IOPCC), and GAT IOP measurements with CCT. The relationship between GAT IOP measurements and CCT was in agreement with previous findings. Our study showed an increase of 0.30 mm Hg per 10-μm increase CCT, whereas a previous study has reported slopes of 0.19 mm Hg to 0.37 mm Hg per 10-μm increase in CCT in different populations.13 However, although the association is statistically significant, the r² value indicates that only 6% of the measured variation is explained by CCT. A greater proportion of between-subject variation in GAT IOP (13%) was explained by the CCF. GAT IOP measurements increased with increasing CCF by 1 mm Hg per 0.65-mm Hg increase in CCF. An important point to note, however, is that GAT measurements are primarily affected by the elastic properties of the cornea and that ORA measures the viscoelastic response of the cornea (and potentially anterior segment) to rapid deformation. It is uncertain to what extent the elastic and viscous properties are covariates. For instance, although both GAT IOP and CH increased with increasing CCT, CH declined with both higher IOP and greater age, and both these factors are likely to increase corneal stiffness.

Agreement between GAT, ORA IOPCCF, and ORA IOPCC measurements was poor. The differences between devices were associated with CCT and CCF (Table 9). It has been established that the GAT will overestimate IOP in patients with thick corneas and underestimate IOP in those with thin corneas.10-12 GAT IOP/ORA IOPCCF differences increased with both increasing CCF and CCT, suggesting that in eyes with thicker and stiffer

### Table 8. Agreement between Measuring Devices

<table>
<thead>
<tr>
<th>IOP Differences</th>
<th>Mean Difference (mm Hg)</th>
<th>Limits of Agreement (mm Hg)</th>
<th>Slope</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT - ORA(CCF)</td>
<td>0.1</td>
<td>-6.6 to 6.8</td>
<td>-0.22</td>
<td>0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>GAT - ORA(CC)</td>
<td>-1.7</td>
<td>-7.6 to 4.3</td>
<td>-0.28</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ORA(CCF) - ORA(CC)</td>
<td>-1.8</td>
<td>-4.1 to 0.5</td>
<td>-0.07</td>
<td>0.08</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

Slope refers to the relationship between IOP differences and average IOP. Mean and 95% limits of agreement are shown.
cornes, the GAT IOP measurement overestimates the ORA IOP\(_{\text{CCF}}\) measurement. GAT IOP/ORA IOP\(_{\text{CC}}\) measurement differences were not significantly associated with CCT, suggesting that the corneal biomechanical effect is not completely eliminated from the manufacturer’s ORA IOP\(_{\text{CC}}\) measurement. This is supported by the finding that the differences between ORA IOP\(_{\text{CCF}}\) and ORA IOP\(_{\text{CC}}\) measurements are negatively associated with both CCT and CCF, indicating that ORA IOP\(_{\text{CC}}\) overestimates our calibration (ORA IOP\(_{\text{CCF}}\)) in patients with thicker and stiffer corneas.

The coefficient of variation of ORA IOP measurements, however, was four to five times greater than that of GAT IOP measurements, which is similar to a previous report of the repeatability of noncontact tonometer devices.\(^{20}\) Noncontact devices measure the IOP within 1 to 3 ms, approximately one-fifth hundredth of the cardiac cycle. As a result, the ocular pulse becomes a significant source of variability, with repeated readings producing a range of between 1 and 3 mm Hg.\(^{21}\) In addition, the present study used P2 to derive IOP, and this pressure was more variable than P1 (Table 3). ORA IOP\(_{\text{CCF}}\) measurements may be more accurate in comparison with GAT IOP measurements, but measurement variability may result in reduced precision in comparison with GAT. However, averaging repeated IOP measurements may mitigate the variability of single measurements to some extent. An additional possible reason for high variability may be that the ORA used in this study was a prototype unit. Further studies assessing the repeatability of IOP measurements on production ORA units are needed.

The approach taken in this study was to use empiric data to adjust for IOP-related changes in the measured CH and to establish the relation between CH and CCT and the subsequent calculation of a normalized IOP. However, the relation between the corneal factor CCT, measured IOP, and true IOP should be confirmed by an in vivo manometry study, and such a study is under way. These data will be used to develop a finite element model of the ORA IOP measurement process so that IOP measurement errors may be predicted in a wide range of clinical circumstances.

In summary, we have presented a technique that reduces the corneal biomechanical effect on ORA IOP measurement and defines a term, the CCF, that is moderately strongly associated with CCT and explains more of the interindividual variation in GAT IOP than does CCT. The normalized ORA IOP was independent of CCT and CCF and seems to better reduce the corneal effect from the IOP measurement than the manufacturer’s calibration. Further study is needed to establish whether this measure of corneal viscoelasticity may be influenced by globe biomechanics.

**Acknowledgments**

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**References**


**Appendix**

The air jet is switched off at the first applanation signal. The ORA software records the air-jet pressure at first (P1) and second (P2) applanation and at maximum pressure (P_max). The level of P_max is not constant and increases with increasing P1 (slope 1.20, 95% CI for slope 1.14–1.22, $r^2 = 0.90$, $P < 0.0001$). This variation in P_max has implications for the corneal loading and unloading cycle (Figure A1).

Figure A1 shows actual strip extensiometry data from porcine corneas tested with a 5-mm/min strain rate of up to 1.0, 1.5, and 2.0 N/mm². The loading and unloading cycles for each specimen show that the unloading stiffness is almost constant regardless of both loading stiffness and the load level. The area between the curves, or hysteresis, however, changes.

The rate of air-jet application is nonlinear, and it is also possible to determine the rate of air-jet pressure change from the software output. Using custom software written in ANSI standard C, we extracted the slope of the air-jet pressure curve during each applanation peak. Plotting the rate of application (arbitrary units/millisecond) for various levels of P1 revealed a linear relationship (slope 0.010, 95% CI for slope 0.009–0.011, $r^2 = 0.91$, $P < 0.0001$). As the cornea is viscoelastic, the more rapid applanation at higher pressure may result in a relative stiffening of the cornea and a change in CH.

Our calculations suggest that IOP-related changes CH recorded by the ORA may be as a result of instrument-related variables. Figure A1 shows that it is also related to material behavior.

**Figure A1.** Illustration of corneal loading and unloading curves for porcine corneal strips subjected to differing stress levels. While the unloading curves remain constant, the hysteresis alters.