Comparative Human Aqueous Dynamics Study between Black and White Subjects with Glaucoma

Laura Beltran-Agullo, Pouya Alaghband, Safina Rashid, Josee Gosselin, Adanna Obi, Rabat Husain, and Kin Sheng Lim

PURPOSE. To compare the baseline aqueous humor dynamics in white Caucasians and patients of African origin with previously untreated primary open-angle glaucoma (POAG) or ocular hypertension (OHT).

METHODS. Ninety-one participants were enrolled in this prospective, observational controlled study: 34 black subjects with POAG or OHT, 32 white Caucasian participants with POAG or OHT, and 12 black and 13 white healthy volunteers as the controls. All aqueous humor parameters were taken between 9 AM and 12 noon on the same day. Intraocular pressure (IOP) was measured by pneumotonometer; morning aqueous humor flow rate was measured by fluorophotometry and trabecular outflow facility by electronic Schiotz tonography. Uveoscleral outflow was calculated by using Goldmann’s equation with assumed episcleral venous pressure of 8, 9, 10, and 11 mm Hg. Differences among groups were analyzed with parametric and nonparametric tests and the relationship between aqueous dynamics parameters were evaluated with linear regression analyses.

RESULTS. The POAG/OHT groups had similar IOP (white, 24.6 ± 3.0 mm Hg; black, 24.3 ± 4.0 mm Hg; comparison by Holm’s sequential Bonferroni method (HBonf); \( P_{\text{HBonf}} = 0.51 \)), outflow facility (white, 0.13 ± 0.09 \( \mu \text{l/min/mm Hg} \); black, 0.13 ± 0.07 \( \mu \text{l/min/mm Hg} \); \( P_{\text{HBonf}} = 0.87 \)), aqueous flow (white, 2.56 ± 0.63 \( \mu \text{l/min} \); black, 2.55 ± 0.53 \( \mu \text{l/min} \); \( P_{\text{HBonf}} = 0.95 \)), and uveoscleral outflow (white, 0.42 ± 1.59 \( \mu \text{l/min} \); black, 0.58 ± 1.17 \( \mu \text{l/min} \); \( P_{\text{HBonf}} = 1.78 \)). POAG/OHT groups had significantly higher IOP and lower outflow facility than their healthy counterparts \( (P < 0.01) \). Black participants had significantly thinner corneas \((540 ± 37 \, \mu\text{m} \text{ vs. } 564 ± 36 \, \mu\text{m})\) than those of white participants \( (P = 0.002) \).

CONCLUSIONS. The aqueous humor dynamics of black African and white Caucasian patients with POAG or OHT have no significant differences. However, the significantly thinner corneas of the black patients may be masking potential differences in outflow facility and IOP measurements between the racial groups. (Invest Ophthalmol Vis Sci. 2011;52:9425–9430) DOI: 10.1167/iovs.10-7130

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The night before (10 PM) the fluorophotometric scans, participants self-administered from 3 to 6 drops of fluorescein sodium 2% (Minims; Bausch & Lomb, Kingston-upon-Thames, UK) topically into both eyes at 5-minute intervals depending on their ages (age, ≤25 years, 5 to 6 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops).15 Fluorophotometry was performed in both eyes with a scanning oculair fluorophotometer from 9 AM to 12 noon (FM-2, Fluorotron Master oculair fluorophotometer; OcuMetrics, Mountain View, CA). The aqueous flow rate was determined using dedicated software provided with the fluorophotometer (Appendix). Duplicate or triplicate scans were collected and repeated at 1-hour intervals for four measurements to determine the aqueous flow rate ($F_a$). Following each set of scans, IOP was measured using pneumotonometer (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); IOP was recorded as the arithmetic mean of a total of 12 measurements per eye: 3 measurements every hour alternating between eyes. Patients with IOP >21 mm Hg on the screening day may have had IOP of 21 mm Hg or less thereafter.

Tonographic outflow facility ($C$) was performed with an electronic Schiotz tonograph (model 720; Berkeley Bioengineering, Inc., San Leandro, CA) at 10 AM. The facility of outflow was measured from the rate of decay of IOP in the supine position during application of a recording Schiotz tonometer over a period of 4 minutes with a standard 5.5-g weight.16 The $R$ values of the curve at every 30-second time point were manually entered into the McLaren tonography computer program.19 The program fits a second-degree polynomial by least-squares to the nine data points and determines by extrapolation the best-fit values for time 0 and time 4 minutes.

Uveoscleral outflow was calculated using Goldmann’s equation20 with an assumed episcleral venous pressure of 8, 9, 10, or 11 mm Hg.18,19 $F_v$ is the rate of aqueous humor formation, $C$ is the tonographic facility of outflow, IOP is the intraocular pressure, $P_e$ is the episcleral venous pressure, and $F_a$ is the uveoscleral outflow.

$$F_v = C (IOP - P_e) + F_a$$

$$F_u = F_v - C (IOP - P_e)$$

Only one randomly (Excel random number generator; Microsoft, Redmond, WA) chosen eye per participant was included in the data analysis, when both eyes fulfilled the inclusion criteria.

### Data Collection and Outcome Measures

Data including age, sex, race, IOP, tonographic outflow facility, aqueous flow rate, CCT, axial length, anterior chamber depth, cup-to-disc ratio, mean deviation in visual field testing and amount of fluorescein drops and time of instillation were recorded. Outcomes measures were outflow facility, IOP, aqueous flow rate, and uveoscleral outflow.

### Data Analysis

Histograms and a Shapiro-Wilk test were performed to test for normality of distribution of data. A Shapiro-Wilk $W > 0.05$ was evidence of normal distribution. Student’s $t$ test and one-way analysis of variance (ANOVA) were used to compare continuous variables among groups. When data did not follow normality, nonparametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Post hoc comparisons among groups were made when appropriate, by Holm’s sequential Bonferroni method (HBonf). The $95%$ confidence intervals (CI) for the mean and median difference between pairs for each outcome measure were calculated. The median difference $95%$ CI were estimated using Hodges-Lehman methodology. Linear regression analyses were used to determine the correlation of one parameter versus another parameter of aqueous humor dynamics and the correlation of age versus each parameter. Fisher’s exact test was used to compare the number of patients with POAG and OHT in each racial group. $P < 0.05$ was considered statistically significant (all analyses, SPSS 16.0; SPSS, Chicago, IL).

### RESULTS

One hundred and one patients with OHT/POAG and 32 healthy volunteers were recruited into the study. Only 66 affected subjects and 25 controls were included in the data analysis. Poor-quality Schiotz tonography tracings ($n = 14$), with excess noise or extreme excursions, or poor fluorophotometric scans ($n = 27$), with extremely high or low baseline corneal fluorescence concentration, were identified in 41 participants and were therefore excluded from the analysis. Another participant was excluded for not having slept the night before the measurements were taken.22

In the black POAG/OHT group, 13 (38.2%) subjects had OHT and 21 (61.8%) had POAG, compared with 18 (56.2%) subjects with OHT and 14 (43.8%) subjects with POAG in the white affected group ($P = 0.21$).

The baseline characteristics from each group are shown in Table 1. The mean age of the study population was 58.7 ± 13.0 years. Age did not correlate with the outcome measures in any of the affected groups or in all participants combined ($IOP = 0.58; C = 0.08; F_v = 0.56$; and $F_u = 0.06$).

Subjects with POAG/OHT exhibited significantly higher IOP than their control counterparts ($P < 0.001$), but the black and white affected groups had comparable IOP ($P = 0.51$; 95% CI, −1.0 to 2.0). Black POAG/OHT subjects had significant thinner corneas than did the white POAG/OHT group ($P_{HBonf} = 0.009$; 95% CI, 8.4–43) but no other difference regarding CCT was observed between the rest of the groups. The mean CCT of all black participants was $540 ± 37 \mu m$ compared with $564 ± 36 \mu m$ in all white participants together (Table 2; $P = 0.002$; 95% CI, 8.4–40). CCT did not correlate significantly with any of the aqueous dynamics parameters.

Both the black and white groups with POAG/OHT had significantly lower outflow facility than their matched control group (black: $P_{HBonf} < 0.001$; 95% CI, −1.1 to −0.2; white, $P_{HBonf} < 0.001$; 95% CI, −0.17 to −0.03), but no differences were found between the affected groups ($P_{HBonf} = 0.51$; 95% CI, −0.03 to 0.04). A weakly significant inverse relationship was observed between outflow facility and IOP, with a reduction of 0.008 ± 0.002 µL/min/mm Hg ($R^2 = 0.16; P < 0.001$; Fig. 1). This relationship remained after adjustment for age ($R^2 = 0.21; P < 0.001$). Facility of outflow appeared not to be linearly correlated to the stage of glaucoma damage measured by mean deviation (MD) in visual field testing ($R^2 = 0.002; P = 0.72$) and cup-to-disc ratio ($R^2 = 0.007; P = 0.51$).

The mean aqueous flow rates for each group ranged from 1.81 to 2.36 µL/min. The black control subjects had significantly lower aqueous production than did the black affected subjects ($P_{HBonf} = 0.01$; 95% CI, 0.17–0.90). The aqueous production did not differ significantly between the black and white affected groups ($P_{HBonf} = 0.95$; 95% CI, −0.29 to 0.30). Aqueous flow was independent of IOP ($R^2 = 0.03; P = 0.07$).

The uveoscleral outflow was not different among groups, regardless of the assumed episcleral venous pressure. The $95%$ CI for the difference between the affected group medians for $F_u = 10$ mm Hg) was $-0.74$ to $0.58 \mu L/min$. Averted patients, independent of their racial origin ($n = 66$), had a mean $F_u$ of $0.75 ± 1.36 \mu L/min (median, 0.50; range, −4.04 to 2.68) compared with 0.47 ± 0.95 µL/min (median, 0.52; range, −1.51 to 2.19; $P = 0.51$) in all control subjects ($n = 25$). Contrary to outflow facility, the relationship between uveoscleral outflow ($F_u$) and IOP ($R^2 = 0.007; P = 0.44$) did not reach significance. Uveoscleral outflow and stage of glaucomatous damage were not related. Similar results were obtained with assumed episcleral venous pressures of 8, 9, and 11 mm Hg (Table 1).
**TABLE 1. Comparison of Baseline Aqueous Dynamics Parameters in Patients with POAG or OHT and Healthy Volunteers**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>POAG/OHT (Black: n = 34)</th>
<th>POAG/OHT (White: n = 32)</th>
<th>Controls (Black: n = 12)</th>
<th>Controls (White: n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP, mm Hg</td>
<td>Median (Mean)</td>
<td>IQR (SD)</td>
<td>Range (Min, Max)</td>
<td>Median (Mean)</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>23.51</td>
<td>14.01</td>
<td>20.10</td>
<td>19.10</td>
</tr>
<tr>
<td>Fp, μL/min</td>
<td>2.36</td>
<td>0.94</td>
<td>2.37</td>
<td>1.65</td>
</tr>
<tr>
<td>C, μL/min/mm Hg</td>
<td>0.12</td>
<td>0.08</td>
<td>0.36</td>
<td>0.18</td>
</tr>
<tr>
<td>Fu8, μL/min</td>
<td>0.46</td>
<td>1.66</td>
<td>6.17</td>
<td>0.13</td>
</tr>
<tr>
<td>Fu9, μL/min</td>
<td>0.61</td>
<td>1.55</td>
<td>5.92</td>
<td>0.15</td>
</tr>
<tr>
<td>Fu10, μL/min</td>
<td>0.76</td>
<td>1.46</td>
<td>5.69</td>
<td>0.45</td>
</tr>
<tr>
<td>Fu11, μL/min</td>
<td>0.89</td>
<td>1.41</td>
<td>5.46</td>
<td>0.57</td>
</tr>
</tbody>
</table>

IQR, interquartile range; FuN, uveoscleral outflow with an assumed episcleral venous pressure of 8, 9, 10, and 11 mm Hg.

* Kruskal-Wallis test.
† One-way ANOVA.
Table 2. Descriptive Parameters of Black and White Patients with POAG or OHT and Healthy Volunteers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>POAG/OHT Controls</th>
<th>Median (SD)</th>
<th>IQR (SD)</th>
<th>Range (Min, Max)</th>
<th>Age, y (Mean)</th>
<th>Median (SD)</th>
<th>IQR (SD)</th>
<th>Range (Min, Max)</th>
<th>Median (SD)</th>
<th>IQR (SD)</th>
<th>Range (Min, Max)</th>
<th>Median (SD)</th>
<th>IQR (SD)</th>
<th>Range (Min, Max)</th>
<th>Median (SD)</th>
<th>IQR (SD)</th>
<th>Range (Min, Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black (n = 34)</td>
<td></td>
<td></td>
<td></td>
<td>60.0 (58.2)</td>
<td>23.6 (12.9)</td>
<td>23.6 (12.9)</td>
<td>48.2 (30.8, 79.1)</td>
<td>61.6 (61.8)</td>
<td>22.2 (13.2)</td>
<td>47.8 (40.3, 88.1)</td>
<td>49.6 (51.1)</td>
<td>8.1 (10.6)</td>
<td>36.7 (38.1, 74.8)</td>
<td>61.5 (59.8)</td>
<td>16.4 (13.2)</td>
<td>45.4 (33.8, 79.2)</td>
</tr>
<tr>
<td></td>
<td>White (n = 32)</td>
<td></td>
<td></td>
<td></td>
<td>61.6 (61.8)</td>
<td>22.2 (13.2)</td>
<td>22.2 (13.2)</td>
<td>47.8 (40.3, 88.1)</td>
<td>61.6 (61.8)</td>
<td>22.2 (13.2)</td>
<td>47.8 (40.3, 88.1)</td>
<td>49.6 (51.1)</td>
<td>8.1 (10.6)</td>
<td>36.7 (38.1, 74.8)</td>
<td>61.5 (59.8)</td>
<td>16.4 (13.2)</td>
<td>45.4 (33.8, 79.2)</td>
</tr>
<tr>
<td></td>
<td>Black (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td>49.6 (51.1)</td>
<td>8.1 (10.6)</td>
<td>8.1 (10.6)</td>
<td>36.7 (38.1, 74.8)</td>
<td>61.5 (59.8)</td>
<td>16.4 (13.2)</td>
<td>45.4 (33.8, 79.2)</td>
<td>0.11*</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>White (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td>49.6 (51.1)</td>
<td>8.1 (10.6)</td>
<td>8.1 (10.6)</td>
<td>36.7 (38.1, 74.8)</td>
<td>61.5 (59.8)</td>
<td>16.4 (13.2)</td>
<td>45.4 (33.8, 79.2)</td>
<td>0.48†</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

IQR, interquartile range; ACD, anterior chamber depth; AXL, axial length.

* Significant, according to one-way ANOVA.† Kruskal-Wallis test.

Table 3 summarizes the aqueous dynamics parameters of the POAG and OHT subjects, which showed nonsignificant differences.

Despite the prestudy sample size calculation based on the results of another study,19 post hoc analysis of our data showed that our study would be able to detect a difference of 10.2% in IOP, 9.7% in aqueous flow, and only 38% in outflow facility, with a power of 80% and α (two-sided) = 0.05, if those differences existed.

**Discussion**

Previous aqueous dynamics studies have included black participants, but this is the first study that specifically compares the aqueous dynamics between Africans/African Caribbeans and white Caucasians. Despite the anatomic differences reported between black and white populations, this study found no significant differences in the aqueous dynamics among patients with POAG/OHT of both racial groups.

Despite the number of patients with POAG being higher in the black affected group than in the white affected group, this disproportion between both racial groups would not have interfered in our analysis, since OHT and POAG patients have comparable aqueous dynamics.24

Contrary to some studies,3,13,14 we found black and white participants with POAG/OHT had similar IOP, but this is likely to be due to the bias in our sampling as patients with IOP higher than 35 mm Hg at the screening visit, according to our ethics committee recommendations, would have been excluded from the study, to avoid delaying the instauration of the medical treatment. Also, since IOP measured with the pneumotonometer increases approximately 0.40 mm Hg for every 10-μm increase in CCT, and the black participants had thinner corneas than the white participants had, the IOP could have been slightly underestimated.

To measure the trabecular outflow facility, we used the classic electronic Schiotz tonometer because compared to pneumotonomography or fluorophotometry techniques, the Schiotz tonometer gives an accurate assessment of outflow facility with less variability over time.19 Rejection rate of 14 of 101 subjects due to poor-quality tonography is only slightly higher than that found in a previous study by the same researchers.19

Since Grant and Becker27 reported a reduction in outflow facility in patients with glaucoma many years ago, other groups have come to the same conclusion.23-24 We found a similar reduction in the outflow facility in both affected racial groups without a significant increase in the aqueous production. The measurements performed with the Schiotz tonometer, are affected by ocular rigidity.28,29 Indentation tonography makes no compensation for individual variations in ocular rigidity. The stiffer the eye, the greater the force needed to indent the cornea and displace the aqueous. Corneal hysteresis factor (CH) and corneal resistance factor (CRF) are dynamic measurements of the viscoelastic properties of the cornea that have been found to be lower in subjects of black African descent than in whites.16-19 Several studies have shown an increase in CH and CRF with increasing CCT.16,31,32 The higher the CH, the longer the cornea takes to return to its original shape, implying greater stiffness. Therefore, higher outflow facility measurements could have been obtained in the black participants on the basis of decreased ocular rigidity rather than the true equality found among the groups. On the other hand, Pallikaris et al.33 measured in vivo the rigidity coefficient in 79 living human eyes and found no statistically significant correlation between ocular-rigidity coefficient and CCT. They reported that despite the low power to detect that correlation, CCT may have an effect on the corneal biomechanics, but may have less impact in ocular rigidity which involves the whole eye and not only the cornea.
Larsson et al.\textsuperscript{34} and Toris et al.\textsuperscript{23} showed that the rate of aqueous production was not different in patients with glaucoma or ocular hypertension compared with healthy age-matched controls. This study confirmed this finding, and we also found no racial difference in the aqueous production rates.

The uveoscleral outflow was not significantly different between the white and black groups or the affected subjects and healthy volunteers, although it has been found to be reduced in beagle dogs with advanced glaucoma\textsuperscript{35} and elevated in monkeys with laser-induced glaucoma.\textsuperscript{36} In humans with POAG, Toris et al.\textsuperscript{23} reported that uveoscleral outflow could be reduced in early stages of glaucoma, together with reduced trabecular outflow, but increases as the disease progresses to prevent the IOP from increasing further, but we did not find any differences between patients with OHT and POAG compared with normal subjects.

In summary, African/African-Caribbean patients with POAG or OHT have baseline aqueous humor dynamics similar to those of white Caucasians patients. Subjects with African ancestry have thinner corneas that may have masked some of the potential aqueous dynamics differences between the studied racial groups.

\section*{APPENDIX}

The Yablonski Protocol of estimating aqueous humor flow rate was used.\textsuperscript{37} Aqueous humor flow is the volume of aqueous humor produced by the ciliary body per unit of time.

\[ F_t = K_{ov} V_a \]

where, \( F_t \) is the aqueous humor flow (\( \mu \text{L/min} \)), \( V_a \) is the volume of the anterior chamber (\( \mu \text{L} \)), and \( K_{ov} \) is the loss coefficient due to

\begin{table}[h]
\centering
\caption{Comparison of Baseline Aqueous Dynamics Parameters between Patients with POAG Versus Those with OHT}
\begin{tabular}{llllllllll}
\hline
 & \multicolumn{3}{c}{POAG (\( n = 35 \))} & \multicolumn{3}{c}{OHT (\( n = 31 \))} & \multicolumn{1}{c}{\( P^* \)} \\
 & Median (Mean) & IQR (SD) & Range (Min, Max) & Median (Mean) & IQR (SD) & Range (Min, Max) & \\
\hline
Age, y & 62.7 (62.6) & 19.7 (12.9) & 55.0 (33.1, 88.1) & 54.9 (56.9) & 21.9 (12.9) & 52.6 (30.8, 83.4) & 0.07\textsuperscript{†} \\
IOP, mm Hg & 25.0 (25.7) & 4.0 (2.8) & 12 (19.0, 31.0) & 26.0 (25.3) & 6.4 (4.1) & 20 (19.5, 39.0) & 0.07 \\
\( F_t \), \( \mu \text{L/min} \) & 2.38 (2.40) & 0.89 (0.58) & 2.67 (1.02, 3.69) & 2.18 (2.30) & 0.81 (0.58) & 2.49 (1.20, 3.69) & 0.46\textsuperscript{†} \\
\( C_{ov} \), \( \mu \text{L/min/mmHg} \) & 0.12 (0.13) & 0.07 (0.07) & 0.41 (0.03, 0.44) & 0.11 (0.13) & 0.14 (0.09) & 0.36 (0.03, 0.39) & 0.98 \\
\( F_{u8} \), \( \mu \text{L/min} \) & 0.55 (0.38) & 1.30 (1.39) & 7.48 (4.92, 2.56) & 0.32 (0.07) & 2.71 (1.63) & 6.54 (4.00, 2.54) & 0.46 \\
\( F_{u10} \), \( \mu \text{L/min} \) & 0.67 (0.51) & 1.28 (1.32) & 7.10 (4.48, 2.62) & 0.43 (0.21) & 2.45 (1.56) & 6.30 (3.72, 2.58) & 0.46 \\
\( F_{u11} \), \( \mu \text{L/min} \) & 0.79 (0.64) & 1.26 (1.25) & 6.72 (4.04, 2.68) & 0.54 (0.35) & 2.27 (1.49) & 6.06 (3.44, 2.62) & 0.44 \\
CCT, \( \mu \text{m} \) & 549 (547) & 51 (39) & 196 (438, 654) & 554 (556) & 50 (35) & 129 (492, 621) & 0.35 \\
MD, dB & −3.99 (−5.62) & 5.22 (5.70) & 30.52 (−29.97, 0.55) & −1.30 (−1.46) & 2.24 (2.56) & 15.53 (−7.58, 7.95) & <0.001 \\
\hline
\end{tabular}
\end{table}

\footnotesize

\begin{itemize}
\item IQR, interquartile range; \( F_{u8−11} \), uveoscleral outflow with an assumed episcleral venous pressure of 8–11 mmHg.
\item * Mann-Whitney U test.
\item † Student \( t \)-tests.
\end{itemize}
bulk flow and diffusion from the anterior chamber (minutes⁻¹). $K_c$ can be thought of as the fraction of anterior chamber volume cleared of fluorescein every minute, due to aqueous flow.

To calculate the aqueous flow, the program uses default variables that can be changed by the operator as necessary:

- Corneal volume default value, 50 $\mu$L
- Anterior chamber volume default value, 174 $\mu$L

CCT of each patient, in micrometers (We introduced each patient’s corneal thickness instead of using the 500-$\mu$m default value to correct for the depth of the focal diamond. However, the corneal volume does not change if a different corneal central thickness is entered).

Once the relationship between cornea and anterior chamber concentrations of fluorescein becomes steady, the program can determine $K_c$ and aqueous flow ($f$), as described in detail elsewhere. 37

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References


