Choroidal Blood Flow in the Foveal Region of the Human Ocular Fundus

Charles E. Riva, Stephen D. Cranstoun, Juan E. Grunwald, and Benno L. Petrig

Purpose. To develop a noninvasive method for the investigation of choroidal blood flow (ChBF) and its regulation in the foveal region of the human ocular fundus.

Methods. Measurement of ChBF was based on the technique of laser Doppler flowmetry (LDF). Sixteen normal subjects (age range, 20 to 64 years), with normal eye examination results, were asked to fixate on a diode laser beam (wavelength = 811 nm, 60 μW at the cornea) delivered to the undilated eye through a fundus camera. Light scattered by red blood cells in the tissue volume sampled by the incident laser beam was detected at the fundus image plane of the camera by an optical fiber. The diameter of the beam at the fundus of the emmetropic eye was about 300 μm. Relative ChBF was measured in both eyes by analyzing the Doppler signal with commercial skin blood flowmeters. The average pulsatile component of ChBF, ChBFp, was determined over the cardiac cycle, and its value was compared to the average total ChBF, ChBFAV. Responses of ChBF to various physiological stimuli, such as increased blood O2 and CO2 concentrations, rapid increases in intraocular pressure, and valsala maneuvers, were documented.

Results. Significant correlations were obtained between the ChBFAV values measured with both flowmeters (P < 0.001) and between the ChBFAV values measured in the right and left eye (P < 0.001). ChBFp represented less than 23% of ChBFAV. ChBFAV was not significantly affected by 5 minutes of breathing 100% oxygen. Raising end-tidal CO2 in one subject from 37 to 59 mm Hg increased ChBFAV by approximately 40%. Acute elevation of the intraocular pressure by suction cup or finger pressure on the globe reduced ChBFAV by as much as 90%. Valsalva maneuvers induced reproducible responses that were very different from those recorded from the skin microcirculation.


The lack of a noninvasive technique to measure choroidal blood flow (ChBF) in humans has compelled researchers to acquire information on this important hemodynamic parameter from studies in anesthetized animals. Numerous techniques have been used for this purpose, including calorimetry,1 direct measurement from choroidal veins,2 radioactive krypton desaturation,3 labeled microspheres,4 hydrogen clearance,5 indocyanine fluorescence angiography,6 and, more recently, laser Doppler flowmetry (LDF).7,8 LDF has been applied invasively in cats and rabbits in the investigation of the effect of various physiologic and pharmacologic conditions on ChBF.7,8 In these studies, surgery was performed to apply the fiberoptic probe of a commercial flowmeter against the exposed sclera of cats or to introduce the probe into rabbit eyes and to place it near the retinal surface, facing the choroid. Studies in minipigs7 and in cats10 have
demonstrated that LDF could also provide noninvasive measurements of ChBF. This article reports on the implementation of LDF in humans and provides examples of changes in ChBF induced by various physiologic challenges.

**MATERIALS AND METHODS**

**Subjects**

Measurements were performed in 16 healthy volunteers (7 women and 9 men) ranging in age from 20 to 64 years (35 ± 11 years, mean ± 1 SD). Subjects had no history of systemic or ocular disease, and results of their ocular examinations were normal. Refraction ranged between −4 and +0.75 diopters, but one subject had a refraction of −8 in both eyes. The study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after the nature and possible consequences of the study were explained. Approval by the institutional human experimentation committee was obtained. Intraocular pressure (IOP) at rest was recorded using a Langham or Goldmann tonometer and was < 21 mm Hg in all subjects. Brachial artery blood pressure was measured by sphygmomanometry. The systolic pressure, BP syst, ranged from 94 to 140 mm Hg, with a mean ± 1 SD of 116 ± 15 mm Hg. Diastolic pressure, BP diast, ranged from 46 to 82 mm Hg (65 ± 9 mm Hg). Perfusion pressure, defined as PP = 2/3 [BP diast + 1/3(BP syst − BP diast)] − IOP, ranged from 26 to 47 mm Hg (39 ± 5 mm Hg). The factor 2/3 accounts for the drop in blood pressure between the brachial and the ophthalmic arteries of seated subjects.

**ChBF Measurements**

ChBF was determined using a method based on the LDF technique. In brief, the frequency of laser light scattered by a moving particle is shifted by an amount ∆f = (1/2π)(Ki − Ks) · V, where Ki and Ks are the wave vectors of the incident and scattered light, respectively, and V is the velocity vector of the particle. Because |V| < c, |Ki| ≈ |Ks| = 2π n/λ, where n is the refractive index of the medium, c the speed of light, and λ the wavelength in vacuo of the laser light. When the laser beam impinges on red blood cells (RBCs) moving with different Vs, the spectrum of the scattered light, the so-called Doppler shift power spectrum (DSPS), has a width that is due not only to the multiplicity of Vs, but also to the effect of scattering of the laser light by the tissue itself. As a result, the RBCs do not receive light only in the direction of the incident beam but, rather, from numerous random directions. Furthermore, light scattered by an RBC can reach the detector along various directions because of additional scattering by the tissue or other RBCs. These processes give rise to a multiplicity in Ks and Ki, and, consequently, to a broader DSPS than would otherwise result solely from the various Vs.

A diode laser beam (λ = 811 nm, 60 μW at the cornea) was delivered to the eye through the illumination pathway of a fundus camera (Model TRC, Topcon; Tokyo, Japan). The beam was defocused, and its diameter at the fundus was about 300 μm. The optical fiber aperture (nominal diameter 450 μm, imaged to approximately 200 μm at the fundus of an emmetropic eye) used to detect the scattered light was placed at the center of the illuminated site. The location of this aperture at the fundus, as well as the site illuminated by the laser beam, could be observed on the monitor of a television camera sensitive to near-infrared light. The photocurrent generated by the scattered light was analyzed by blood perfusion monitors that were adapted to process the same low light level signal obtained from the human eye (BPM403A, Vasamedics, Minneapolis, or PeriFlux PF3, Perimed, Stockholm, Sweden). These two instruments process the laser Doppler signal in different ways (digital versus analog), which required that different interfaces be built to adapt them to our needs. Both provide relative flow, F, derived from the product of velocity and volume of the RBCs within the illuminated volume of the tissue. Using the BPM403A, we also determined in a number of experiments the velocity of the RBCs. The measured quantities were recorded on a three-channel strip chart recorder. A spectrum analyzer also displayed the DSPS on the screen of an oscilloscope. During all measurements, the Doppler signal was fed into a loudspeaker, allowing the pulsating pitch of the signal to be heard.

**Baseline Measurements**

Baseline choroidal blood velocity (ChBVel) and ChBF were measured with identical laser beam power and divergence at the cornea in all subjects. The units of these quantities were mm of chart paper. The chart scales were set to provide nearly equal ChBF BPM403A and ChBF PF3 values in the first eye that was measured. They were left untouched from then on. The time constants of the flowmeters were 0.1 second for the BPM403A and 0.2 second for the PF3. A recording of at least 1 minute was obtained for each eye simultaneously with both instruments. The average of ChBF over the heart cycle (duration T), ChBF AV, is defined as

\[ ChBF_{AV} = ChBF_{diast} + ChBF_{p} \]  

(1) where

\[ ChBF_{p} = \frac{1}{T} \int_{0}^{T} (ChBF(t) - ChBF_{diast}) \, dt \]  

(2)
represents the time average of the pulsatile component of ChBF. This precise measure was approximated by

\[ ChBF_p = k \cdot (ChBF_{syst} - ChBF_{diast}) \]

where \( ChBF_{syst} \) and \( ChBF_{diast} \) are the systolic and diastolic values of ChBF, respectively, and \( k \) is a constant that was determined in a single subject by equating \( ChBF_p \) obtained after numeric integration of equation 2 and using the \( ChBF_{syst} \) and \( ChBF_{diast} \) values from the strip chart recordings. The value of \( k \) obtained from this procedure was used in all subjects.

ChBF was simultaneously measured with both flowmeters. In one subject, technical problems prevented measurements with the PF3. In each of the first 24 eyes measured, a DSPS was obtained from a 3-second, randomly chosen portion of the 1-minute recording, and the first moment of the DSPS, which represents \( ChBVel \), was calculated based on the spectral values above 40 Hz. Blood volume (ChBVol) was derived from the measured ChBF and ChBVel, using the formula \( ChBVol = ChBF / ChBVel \).

**Physiological Challenges**

ChBF was recorded under the following conditions: in four subjects, at baseline and while breathing 100% \( \text{O}_2 \) for 5 minutes; in one subject, while breathing 7.5% \( \text{CO}_2 \) for approximately 5 minutes; in 3 subjects before, during, and after valsalva maneuvers. In the latter situation, the ChBF response was compared with that of blood flow obtained from the finger tip with the PF3 flowmeter.

To stop blood flow in an attempt to determine the ChBF values corresponding to zero choroidal blood flow, measurements were performed in one subject before and during the application of a Langham suction cup (10 mm in diameter and a volume of 0.3 ml), to which a negative suction pressure of 400 mm Hg was rapidly applied, and also before and during the application of finger pressure on the globe.

**RESULTS**

Figure 1 represents 11-minute recordings of relative ChBVel and ChBF obtained with the BPM 403A (time constant, \( \tau = 0.1 \) sec) and the PF3 (\( \tau = 0.2 \) sec) in a subject with good target fixation. The same signal was fed into both flowmeters. The recorder pens were raised (blank spaces) during regularly spaced periods of time during which the subject was asked to blink. Fast variations synchronous with the cardiac cycle were present in all three recordings (although they are not resolved in this graph) were observed to be in phase with the cardiac cycle.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933403/)

**FIGURE 1.** Recording (=11 minutes) of relative ChBF\(_{PF3}\), ChBF\(_{BPM403A}\), and ChBVel\(_{BPM403A}\) in a normal volunteer. Time constants of the PF3 and BPM403A were 0.2 and 0.1 second, respectively. The subject was asked to blink at regular intervals, during which the recorder pens were lifted. The large spikes at the beginning and end of some segments were due to blinking. The fast variations within each segment (not resolved in this graph) were observed to be in phase with the cardiac cycle.

**FIGURE 2.** ChBVel shown on an extended time scale demonstrates the pulsatile nature of choroidal blood flow. Inset: Average ChBVel based on 10 cardiac cycles. From the ChBVel\(_{syst}\) and ChBVel\(_{diast}\) values and the values of ChBVel\(_p\) derived as in equation 2, the constant, \( k \), was calculated as 0.51.

Figure 2 shows a portion of ChBVel in one subject and the corresponding average wave form based on 10 heart cycles. From the latter, we determined the constant \( k \) using the procedure described above, and a value of \( k = 0.51 \) was obtained. Estimating the diastolic and systolic values of ChBVel and ChBF by visual observation of the recordings in Figure 1, ChBF\(_{AV} \) was calculated from equations 1 and 3. The mean value

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933403/ on 11/08/2018
FIGURE 3. Correlation between the ChBF<sub>AV</sub>(OU), = mean of ChBF<sub>AV</sub>(OD) and ChBF<sub>AV</sub>(OS), measured simultaneously with the PF3 and BPM403A flowmeters in 15 subjects. ChBF<sub>AV</sub>(OU, BPM403A) = 0.77 × ChBF<sub>AV</sub>(OU, PF3) + 2.3. The same signal was delivered to each instrument. Flow units are relative. The same laser delivery and detection scheme was used in all subjects. Also shown is the 95% confidence interval.

of the two eyes, ChBF<sub>AV</sub>(OU), was obtained. In 15 subjects, ChBF<sub>AV</sub>(OU) measured with the BPM403A flowmeter correlated significantly with ChBF<sub>AV</sub>(OU) derived from measurements with the PF3 instrument (r = 0.81, P < 0.001) (Fig. 3).

Table 1 summarizes the data on ChBF<sub>AV</sub> and ChBVel<sub>AV</sub> obtained with both flowmeters from each eye and the mean values from both eyes. A significant correlation (r = 0.82, P < 0.001 with PF3 and r = 0.81, P < 0.001 with BPM403A) was obtained between the ChBF<sub>AV</sub>(OD) and ChBF<sub>AV</sub>(OS) (Fig. 4, top). ChBVel<sub>AV</sub> (OS) also correlated with ChBVel<sub>AV</sub> (OD) (r = 0.75, P < 0.01) (Fig. 4, bottom). When comparing subjects with blue (n = 7) and dark brown eyes (n = 4; 3 Asians, 1 black), no statistically significant difference was found between the ChBF<sub>AV</sub> (OU) values (16 ± 8 [blue] and 10 ± 5 [dark]), the ChBVel<sub>AV</sub> (OU) values (24 ± 5 and 23 ± 5) or the ChBVel<sub>AV</sub> (OU) values (0.67 ± 0.2 and 0.44 ± 0.2); all values are given in arbitrary units. There was no difference between the ChBF<sub>AV</sub>(OU) values in seven women (15 ± 10) and in nine men (16 ± 7). ChBF<sub>AV</sub>(OU) did not correlate with age, refraction, or perfusion pressure. There was no significant correlation between ChBF<sub>AV</sub>(OU) and ChBVel<sub>AV</sub>(OU) (r = 0.96, P > 0.05), and between ChBVol<sub>AV</sub> and ChBVel<sub>AV</sub> (r = 0.07, P < 0.001), as shown in Figure 5.

ChBVol<sub>AV</sub>(OU) ranged from 0.12 to 1.14 arbitrary units (Table 1). There was a significant correlation between ChBVol<sub>AV</sub>(OU) and ChBF<sub>AV</sub>(OU) (r = 0.91, P < 0.001).

Table 2 shows the values of the pulsatile components ChBF<sub>p</sub> and ChBVel<sub>p</sub>. ChBF<sub>p</sub> in OD was significantly correlated with ChBF<sub>p</sub> in OS (r = 0.79, P < 0.01 with both flowmeters). The same was true for ChBVel<sub>p</sub> (r = 0.84, P < 0.001). Without the corrections described below, ChBF<sub>p</sub> amounted to >14% of ChBF<sub>AV</sub> and ChBVel<sub>p</sub> to >15% of ChBVel<sub>AV</sub> (Table 1). It is worth looking more closely at the ratio P<sub>ChBF</sub> = ChBF<sub>p</sub>/ChBF<sub>AV</sub>. It can be shown that if k = 0.5, which is similar to the value of 0.51 obtained in this study, P<sub>ChBF</sub> = (ChBF<sub>syst</sub> - ChBF<sub>diast</sub>)/(ChBF<sub>syst</sub> + ChBF<sub>diast</sub>), which corresponds to the pulsatility modulation parameter of ChBF. The same considerations apply for P<sub>ChBVel</sub>.

### Table 1. Time Average of Choroidal Blood Flow and Blood Velocity in the Foveal Region

<table>
<thead>
<tr>
<th></th>
<th>PF3†</th>
<th>BPM403A*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>ChBF&lt;sub&gt;AV&lt;/sub&gt;(OD)</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>ChBF&lt;sub&gt;AV&lt;/sub&gt;(OS)</td>
<td>4</td>
<td>32.5</td>
</tr>
<tr>
<td>ChBF&lt;sub&gt;AV&lt;/sub&gt;(OU)</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>ChBF&lt;sub&gt;AV&lt;/sub&gt;(OD - OS)(%)</td>
<td>-56</td>
<td>+50</td>
</tr>
<tr>
<td>ChBVel&lt;sub&gt;AV&lt;/sub&gt;(OD)</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>ChBVel&lt;sub&gt;AV&lt;/sub&gt;(OS)</td>
<td>17.5</td>
<td>32.5</td>
</tr>
<tr>
<td>ChBVel&lt;sub&gt;AV&lt;/sub&gt;(OU)</td>
<td>-75</td>
<td>50</td>
</tr>
</tbody>
</table>

All values are expressed in arbitrary units.

OD = right eye; OS = left eye; OU = both eyes.

ChBF<sub>AV</sub> = flow averaged over the cardiac cycle = ChBF<sub>diast</sub> + 0.51 × (ChBF<sub>syst</sub> - ChBF<sub>diast</sub>).

ChBF<sub>AV</sub>(OD - OS)(%) = percent difference between ChBF<sub>AV</sub>(OD) and ChBF<sub>AV</sub>(OS) = 100 × (ChBF<sub>AV</sub>(OD) - ChBF<sub>AV</sub>(OS))/(ChBF<sub>AV</sub>(OD) + ChBF<sub>AV</sub>(OS))/2.

ChBVel<sub>AV</sub> = ChBVel<sub>diast</sub> + 0.51 × (ChBVel<sub>syst</sub> - ChBVel<sub>diast</sub>).

ChBVel<sub>AV</sub>(OD - OS)(%) = percent difference between ChBVel<sub>AV</sub>(OD) and ChBVel<sub>AV</sub>(OS).

* n = 16; † n = 15.
FIGURE 4. ChBF <sub>AV</sub> (top) and ChBVel<sub>AV</sub> (bottom) measured with the BPM403A from the right eye (OD) versus the same quantities from the left eye (OS) in 16 subjects. Flow units are relative. The same laser delivery and detection scheme was used in all subjects. The regression lines are ChBF<sub>AV</sub> (OD) = 0.73 × ChBF<sub>AV</sub> (OS) + 4.5 and ChBVel<sub>AV</sub> (OD) = 0.65 × ChBVel<sub>AV</sub> (OS) + 8.5. Also represented is the 95% confidence interval.

The first moment of the DSPS (Fig. 6), which is equal to ChBVel (expressed in Hz), ranged from 411 to 670 Hz (560 ± 108 Hz, mean ± SD) in the first 24 eyes studied. From recordings such as those in Figure 1, assuming that about 10 segments of 15 seconds are recorded for 4 minutes, the 95% confidence interval for mean ChBF is approximately ±4%. This means that if another 10 segments of 15 seconds are obtained, for example, after a physiological challenge or after the administration of a pharmacologic agent, a change in ChBF of >8% from the baseline condition would be considered significant at <i>P</i> < 0.05, assuming that the variance is not changed by the challenge or the drug.

Five minutes of 100% O<sub>2</sub> breathing did not significantly affect ChBVel<sub>AV</sub> or ChBF<sub>AV</sub> because the average difference between the values at baseline and at 5 minutes of O<sub>2</sub> was 0.5 ± 6% for ChBVel<sub>AV</sub> and 2 ± 7% for ChBF<sub>AV</sub> (<i>P</i> > 0.05 for both). Comparing 1 minute of baseline recording with the recording between 4 and 5 minutes of 100% O<sub>2</sub> in the four subjects measured, we calculated that a change in ChBF<sub>AV</sub> of more than ±9% would have been needed for it to have been considered significantly different from baseline.

In response to the breathing of 7.5% CO<sub>2</sub>, causing an increase in end-tidal P<sub>CO</sub><sub>2</sub> from 38 to 57 mm Hg, ChBF<sub>AV</sub> increased by about 40% (Fig. 7). Heart rate increased by 4% (from 74 to 77 beats/minute), and mean brachial artery blood pressure increased by 13% (from 96 to 108 mm Hg).

The changes in ChBF induced by valsala maneuvers were similar in the three subjects and in the eight repeated trials performed in one subject, although the magnitude of the changes showed some variation. During the time of increased intrathoracic pressure, ChBF<sub>AV</sub> remained similar to baseline (<i>P</i> > 0.05) (Fig. 8, bottom). ChBF<sub>AV</sub> dropped markedly on release of the intrathoracic pressure, reaching a minimum value about 5 seconds later and then returning to baseline. In the eight repeated measurements, ChBF<sub>AV</sub> at 5 seconds after the release of the pressure was 41% ± 14% (<i>P</i> < 0.001) below baseline. Similarly, in the other two subjects, ChBF<sub>AV</sub> decreased by 45% and 54%. For comparison, Figure 8 (top) shows responses to valsala of skin blood flow in the finger, measured with the PF3.
TABLE 2. Time Average of Pulsatile Component of Choroidal Blood Flow and Blood Velocity in the Foveal Region

<table>
<thead>
<tr>
<th></th>
<th>PF3</th>
<th></th>
<th>BPM403A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean ± SD</td>
<td>Minimum</td>
</tr>
<tr>
<td>ChBFp(OD)</td>
<td>0.5</td>
<td>4.5</td>
<td>2.2 ± 1.2</td>
<td>0.75</td>
</tr>
<tr>
<td>ChBFp(OS)</td>
<td>0.5</td>
<td>5</td>
<td>2.1 ± 1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>ChBFp(OU)</td>
<td>0.5</td>
<td>4.5</td>
<td>2.2 ± 1.1</td>
<td>0.75</td>
</tr>
<tr>
<td>ChBFp/ChBFav(OU)</td>
<td>0.08</td>
<td>0.3</td>
<td>0.14 ± 0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>ChBVelp(OD)</td>
<td>2</td>
<td>6</td>
<td>3.5 ± 2</td>
<td>2</td>
</tr>
<tr>
<td>ChBVelp(OS)</td>
<td>2</td>
<td>6</td>
<td>3.6 ± 2</td>
<td></td>
</tr>
<tr>
<td>ChBVelp(OU)</td>
<td>2.3</td>
<td>6</td>
<td>3.6 ± 2</td>
<td></td>
</tr>
<tr>
<td>ChBVelp/ChBVelav(OU)</td>
<td>0.08</td>
<td>0.35</td>
<td>0.15 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed in arbitrary units.
OD = right eye; OS = left eye; OU = both eyes.
ChBFp = pulsatile choroidal blood flow = 0.51 × (ChBF^p - ChBF_diastate).
ChBVel_p = blood velocity = 0.51 × (ChBVel^p - ChBVel_diastate).

(time constant of 0.2 seconds), and ChBFav, measured with the BPM403 (time constant of 0.1 seconds). Both responses were simultaneously recorded on three separate occasions, and each time both responses differed in the same way. During increased intrathoracic pressure, skin blood flow started to decrease earlier (≈6 seconds) than ChBF. However, both flows showed a similar time course after the release of pressure.

Rapid increases of the IOP by suction cup to 400 mm Hg reduced ChBVel and ChBF (Fig. 9a) by as much as 80% and 70%, respectively. Even at this pressure, some pulsating pitch was perceptible in the Doppler sound, indicating that the flow of blood had not been completely stopped. Similarly, attempts to reduce ChBF to zero by finger pressure on the eye until the central visual field faded were not completely successful because the decrease was only approximately 90% (Fig. 9b).

DISCUSSION
After previous studies that demonstrated the validity of LDF for the investigation of choroidal hemodynamics in animals,7-10 this study represents a first attempt to measure local, relative ChBF in humans in an noninvasive way. The foveal region of the fundus was chosen as the measuring site for the following reasons: It is the site of a number of pathologic conditions, such as macular degeneration and macular dystrophy, that may have a choroidal vascular etiology; it is free of retinal vessels; its localization is straightforward because the subject fixates directly at the laser beam; and functionally it is the most important site of the fundus.

Because choroidal blood flow has been shown to

![FIGURE 6. Typical Doppler shift power spectrum of the Doppler signal analyzed by the flowmeters. m1 is the first moment of the distribution based on spectral values above 40 Hz.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933403/)  

![FIGURE 7. Effect of breathing 7.5% CO2 on ChBFav (mean values ± SD, based on five successive data points).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933403/)
Choroidal Blood Flow in Humans by LDF

FIGURE 8. Effect of valsalva on ChBF (bottom) and skin blood flow measured from the finger (top). ChBF (relative units) was measured with the BPM403A (time constant, \( \tau = 0.1 \) second) and skin blood flow (also relative units) was recorded with the PF3 (\( \tau = 0.2 \) second). During recording, the strip chart pen of the ChBF trace has been lifted at the start and stop of the maneuver, when the subject was temporarily unable to keep his eye aligned with the fundus camera.

be insensitive to increased arterial O\(_2\) concentration,\(^{16,14,15}\) the lack of a response of ChBF to 100% O\(_2\) breathing confirms that our method measures choroidal rather than retinal blood flow. If retinal blood flow were measured, one would have found a decrease of at least 50%, as shown by previous blue field simulation studies of the velocity of leukocytes in retinal macular capillaries during increased arterial O\(_2\) concentra-
tion.\(^{16-18}\) Furthermore, the quasi-exponential shape and frequency range (<700 Hz) of the DSPS obtained in this study, which are typical of those obtained in LDF of tissue microcirculation,\(^{10}\) strongly suggest that the Doppler signal from the foveal region is predominantly caused by RBCs moving in the choriocapillaries rather than in large choroidal vessels behind it. In contrast, DSPS recorded from individual vessels, such as retinal arteries and veins, have a rectangular shape with Doppler frequencies typically up to 10 to 50 kHz throughout the heart cycle.

In all subjects, velocity and flow varied in synchrony with the cardiac cycle. The pulsatile components ChBVel\(_p\) and ChBF\(_p\) represented <15% of ChBVel\(_{AV}\) and ChBF\(_{AV}\), respectively. To allow comparison of our results on the choroidal circulation with the retinal circulation, we calculated the average and pulsatile components of V\(_{max}\), the centerline RBC velocity in main retinal arteries, for published data in normal subjects\(^{19,20}\) using the same definition as above—that is, \( V_{\text{max,AV}} = V_{\text{max,diast}} + 0.5 \left( V_{\text{max,syst}} - V_{\text{max,diast}} \right) \). In the retina, the pulsatile component was approximately 50% of \( V_{\text{max,AV}} \) in both independent studies. Our finding of much smaller choroidal pulsatility provides further evidence that our method measures blood flow in the choriocapillaris and not in large vessels because damping of pulsatility normally takes place as flow progresses from larger to smaller arterioles.

The determination of the magnitude of pulsatile flow are based on two assumptions. The first is that equation (3) provides a valid approximation to equation (2) for measuring ChBF\(_p\). Equation (3) is used by analogy to the formula commonly applied to deter-

FIGURE 9. (A) Effect on relative ChBF of negative pressure up to 400 mm Hg produced by a Langham suction cup applied at the limbus. A suction of 50 mm Hg was first applied to affix the cup to the globe. The suction was then gradually increased to 400 mm Hg in about 13 seconds and was kept at this value for 3 to 4 seconds. The interruptions in the recordings correspond to periods of blinking. The units of ChBF are relative. (B) Effect of finger pressure on the globe trying to stop ChBF to the eye. After about 15 seconds of recording, finger pressure was gradually increased for about 20 seconds and then quickly removed. The interruption in the recording was due to blinking. The units of ChBF are relative.
mine the pulsatile component of the blood pressure, \( \text{BP}_p \), from the diastolic and systolic values—that is, \( \text{BP}_p = \left(1/2\right) (\text{BP}_{\text{syst}} - \text{BP}_{\text{diast}}) \). Tested in one subject who showed a highly regular pulsatility (Fig. 2), and based on an average of 10 consecutive cardiac cycles, \( k \) was found to be equal to 0.51, which is not significantly different from the value of 0.48 ± 0.04 obtained by Feke et al.\(^{20}\) from retinal arterioles in humans. The second assumption is that the electrical “zero” on the chart recorder corresponds to the physiological zero flow (or zero velocity). Measurements of ChBF in cats after a lethal injection of pentobarbital (Fig. 7)\(^{10}\) showed that this assumption is justified. To determine the location of zero ChBF in humans, we applied strong enough pressure to the globe to produce fading of the central visual field. Although this maneuver did not stop the flow entirely, as evidenced by the still audible pitch of the Doppler sound during the cardiac cycle, flow was decreased by about 90% (Fig. 9). This means that the difference between the zero flow and the zero chart level is <10% of baseline ChBF. Using three units above the zero chart value for zero ChBF\(_{PF3}\) in Figure 1, a value that represents about 9% of the baseline of the highest ChBF measured among all subjects with the PF3 flowmeter, we recalculated the contribution of ChBF\(_p\) to ChBF\(_{AV}\) in all subjects and obtained a mean value of 0.23 ± 0.18 SD instead of the 0.14 (±0.07) previously obtained under the assumption that both zero levels coincide. Inspection of the data shows that the large standard deviation of 0.18 was mainly attributed to a subject with high myopia with a refraction of −8 in both eyes, whose ratio of ChBF\(_p\):ChBF\(_{AV}\) was about 4 SD away from the group mean value. Disregarding this subject, the mean value of ChBF\(_p\):ChBF\(_{AV}\) becomes 0.20 ± 12. This value represents an upper limit for two reasons: First, we took as zero ChBF a level based on the highest baseline ChBF\(_{AV}\) measured, and second, ChBF was not completely stopped. Because the true zero level is unknown and the maximum error is <10%, it seems warranted to use the chart’s zero level as the reference in measuring flow changes.

The ChBF\(_{AV}\) and ChBF values did not correlate with age, ocular refraction, or ocular perfusion pressure. This is most probably due to the small range of these parameters in our present population. We also did not find a significant influence of iris color, although ChBF\(_{AV}\) and ChBF\(_{VolAV}\) were about 35% lower in subjects with dark pigmentation. ChBF\(_{VelAV}\), however, was practically identical in both types of subjects. Clearly, more subjects are needed to establish the role of fundus pigmentation in ChBF measurements.

Because iris color is indicative of choroidal melanin pigmentation,\(^{21}\) the lack of influence of this parameter on blood velocity appears to support our conclusion that the Doppler signal predominantly originates from RBCs in the choriocapillaris. Had RBCs in the deeper, high-flow choroidal vessels been the main scatterers of light, we would have found higher velocities in subjects with blue eyes, in whom the relatively reduced amount of choroidal melanin pigment\(^{22}\) would have allowed more of the incident laser light to reach and return from the large choroidal vessels. This was not the case, however.

The large intersubject spread in ChBF\(_{AV}(OU)\) (a factor of 6.5 if the 16 subjects are considered, or 4.4 if the subject with high myopia is disregarded) was mainly attributable to the spread of the ChBF\(_{VolAV}(OU)\) (factors of about 6.4 or 3.6), as revealed by the high correlation between ChBF\(_{VolAV}(OU)\) and ChBF\(_{AV}(OU)\) (Fig. 5, top) and the lack of such a correlation between ChBF\(_{VelAV}(OU)\) and ChBF\(_{AV}(OU)\) (Fig. 5, bottom).

The large intersubject variability of ChBF\(_{AV}\) and ChBF\(_{VolAV}\) and the smaller values of these quantities in subjects with dark irises, although not statistically significant, suggest an intersubject variability in the sampling depth in the choriocapillaris that may be influenced by pigmentation. Nevertheless, this depth must be similar in both eyes and must exclude deep choroidal vessels as suggested by comparable velocities (Fig. 4).

The significant correlation between ChBF\(_{AV}(OD)\) and ChBF\(_{AV}(OS)\) and between ChBF\(_{VolAV}(OD)\) and ChBF\(_{VolAV}(OS)\) (Fig. 4) may provide the basis for a diagnostic test of unilateral macular diseases. As a measure of the sensitivity of the technique to diagnose an abnormal difference in ChBF between eyes, we have determined the minimum percentage difference between the ChBF\(_{AV}\) values in OD and OS that would be required for a significant difference from normal at the 95% confidence level (2 SD). Our results in Table 1 (\(\text{Delta} \text{ChBF}_{AV}(\text{OD-OS})\%\)) show such a minimum difference to be 60% for ChBF\(_{AV}\) and 62% for ChBF\(_{VolAV}\).

Unexpectedly, the response of ChBF to the valsalva maneuver (Fig. 8) was different from that of skin blood flow; the latter was similar to previously reported results.\(^{23}\) Contrary to what occurs in the skin circulation, ChBF did not decrease while intrathoracic pressure was elevated. Rather, ChBF remained practically constant during this phase, most probably because of a protective mechanism provided by the autonomic nervous system.\(^{24}\) The rapid transient drop in ChBF on release of the intrathoracic pressure may be attributed to the sudden, transient drop in arterial pressure and, consequently, in ocular blood perfusion pressure.

The goal of recording ChBF during the breathing of 7.5% CO\(_2\) (Fig. 7) was to verify that the technique can demonstrate increases in ChBF. Our finding of a 40% increase in ChBF is in close agreement with choroidal blood flow measurements in animals.\(^{15}\)
Choroidal Blood Flow in Humans by LDF

The ability to record relative ChBF in the foveal region in a continuous way, without the need to dilate the pupil, opens new avenues in the study of the choroidal circulation at this important site of the fundus. Future investigations should be devoted to reducing the instrument-related variability (laser wavelength, beam size at the fundus, diameter of detecting fiber) between subjects and between eyes of the same subject of the flow and volume measurements. To evaluate whether the technique, in its present stage, has clinical potential, measurements should be made in patients with unilateral macular disease to determine the extent of flow asymmetry that may occur. The technique, however, already has the sensitivity needed for testing the relative effect of various physiological stimuli and pharmacologic agents on the choroidal circulation in the foveal region of the fundus.

Key Words
choroidal blood flow, blood flow regulation, laser Doppler flowmetry, blood flow pulsatility, valsava maneuver

Acknowledgments
The authors thank Joan DuPont for her expert technical help.

References