Choroidal Blood Flow during Exercise-Induced Changes in the Ocular Perfusion Pressure

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Purpose. The high metabolic rate of the human retina is supported by the choroidal vasculature. Knowledge of the normal choroidal blood flow (ChBF) responses to various physiological stimuli is therefore highly important if the pathophysiology of ocular diseases involving the choroid is to be understood better. In the present study, the hemodynamic responses of the subfoveal ChBF were examined during and after an exercise-induced increase in the ocular perfusion pressure (OPP).

Methods. Twenty-six healthy volunteers, 19 to 55 years of age participated in this two-phase study. Each subject increased resting OPP through stationary biking at a heart rate (HR) of 140 beats per minute (bpm) over 20 minutes. The ChBF was measured by laser Doppler flowmetry (LDF), the systemic BP by electronic sphygmomanometry, and the resting intraocular pressure (IOP) by applanation tonometry.

Results. The OPP increased by approximately 43% at the onset of biking, and then decreased biphasically to approximately 12% above resting value by the end of biking. The ChBF remained within 10% of its basal value throughout biking. Immediately after biking, the OPP decreased twice as much as the ChBF in the same time frame.

Conclusions. The dissociation between the OPP and the ChBF during biking and recovery suggests that some mechanism keeps the ChBF close to its basal value, an observation that indicates blood flow regulation. (*Invest Ophthalmol Vis Sci.* 2003;44:2126–2132) DOI:10.1167/iovs.02-0825

The functional integrity of the retina relies on an adequate vascular perfusion level, an availability of vital metabolic nutrients and oxygen, and an efficient disposal of metabolic by-products. In humans, these factors are promoted by a dual vascular system, with the choroidal vasculature feeding the outer one third of the retina and the retinal vasculature nourishing the inner two thirds of the retina.

In the foveal avascular zone (FAZ), the retinal metabolism relies entirely on the choroidal vasculature. Legitimately, one can assume that inadequate blood perfusion in the foveal choriocapillaris, the innermost layer of the choroid, would lead to some impairment of central visual function. The observation by Linsenmeier and Steinberg[1] that the photoreceptors need virtually all the oxygen that a normal choroidal circulation can provide lends support to this hypothesis. Along this line of thought, Friedman[2] has hypothesized that age-related macular degeneration is a manifestation of a vascular problem resulting from an increased resistance to blood flow in the choroid underlying the FAZ.

The widely recognized importance of understanding the pathophysiology of diseases, such as glaucoma, diabetic retinopathy, age-related macular degeneration, and others, has recently led to the development of new techniques for the noninvasive measurement of retinal blood flow[3–7] and its response to various physiological stimuli.[3,8,9] These techniques have extended markedly our understanding of the vascular physiology of the retina and optic nerve in humans, and its implication in the pathogenesis of various sight-threatening ocular diseases. For the choroidal circulation, however, a parallel body of knowledge is not available, because of the absence of valid techniques for reliably quantifying choroidal hemodynamics.

The demonstrated feasibility of near-infrared (811 nm) laser Doppler flowmetry (LDF) to quantify the subfoveal choroidal blood flow (ChBF) response to physiological provocation has opened new avenues in the investigation of the physiology of the choroidal vascular system.[10] With this technique, recent studies have documented the regulatory response of the ChBF to acute reductions in the ocular perfusion pressure (OPP) caused by transient elevations in the intraocular pressure (IOP),[11] and increases in the OPP caused by static exercises, such as isometrics.[12] More recently, the ChBF has also been shown to decrease by almost 20% during dark adaptation of the retina[13] (Kergoat H, Lovasik JV, Bitton E, ARVO Abstract 3300, 2002) possibly in support of the dark current, or to meet the metabolic demands of the rod photoreceptors, in which sensitivity to light is increased by several log units during dark adaptation. Furthermore, LDF has been used to provide evidence for central neural control of ChBF in the human eye.[14]

Pursuing the study of the blood flow regulatory capabilities of the human choroid, the present work investigated the response of the subfoveal ChBF to significantly increased OPP induced by dynamic exercise consisting of stationary biking. To elaborate as fully as possible the hemodynamic reactivity of the choroid to ongoing changes in the OPP, the ChBF was measured continuously during the period of changing physiological stress, and during a recovery interval immediately after the dynamic exercise. The work reported herein was performed in two phases. In phase 1, which took place at the Institute of Research in Ophthalmology (Sion, Switzerland), the subfoveal ChBF was measured in naïve subjects only after biking, when fixation was stable enough for reliable continuous measurements of the ChBF to be made during a 10-minute recovery interval. The nature of the recovery data obtained in this phase encouraged us to measure the interaction between the ChBF and the OPP during biking, a clearly more difficult experimental task. In phase 2, which took place at the University of Montréal (Québec, Canada), intermittent measurements of the ChBF were made during biking from a selected trained

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group of subjects. These data were combined with continuous ChBF measurements made from a second cohort of trained subjects using new LDF technology that made possible moment-to-moment measurements of the ChBF during and after biking. This latter study also benefited from new technology that provides real-time noninvasive measurements of the systemic BP for calculating concomitant changes in the OPP.[15]

METHODS

Phase 1: Characterizing the Recovery Profiles for the OPP and the ChBF after 20 Minutes of Biking

Phase 1 involved 14 healthy volunteers with a group average age of 28.1 ± 11.3 years. Their general health status was ascertained by a standard hospital admission questionnaire regarding past or ongoing ocular disease, family history of vascular disorders of the eye or body, and medications taken. Before subjects were admitted to the study, an eye examination was performed to exclude individuals with ametropia exceeding ±7 D, manifest ocular disease, and an intraocular pressure (IOP) of 22 mm Hg or more. Furthermore, the subjects had to be ready to bike for 20 minutes at the specified heart rate, and to show steady fixation of the probing laser in a short trial in the LDF, so that reliable continuous measurements of the ChBF could be made during the recovery interval.

The research LDF used for measuring subfoveal ChBF has been described previously.[16] It uses a near-infrared (IR) laser diode incorporated into a standard fundus camera (TRC-50; Topcon, Tokyo, Japan). During measurements of the ChBF, subjects were asked to fixate the laser beam, which appeared as a faint red spot of shimmering light within a dark field. The diameter of the probing laser beam on the fundus of an emmetropic eye after slight defocusing was approximately 300 μm.[10] The relative changes in the subfoveal ChBF parameters that were measured included the ChBF velocity (ChBVel), the mean speed of the red blood cells in the volume of target tissue, the ChBF volume (ChBVol), the number of these cells in the sampled volume of tissue, and the ChBF, per se, defined as ChBVol × ChBVel. In this phase, the ChBF was measured continuously for 10 minutes just after 20 minutes of biking at a heart rate (HR) of 140 beats per minute (bpm). After only a brief description of what was needed to get valid measurements of the ChBF using the LDF, all volunteers were able to provide stable ChBF measurements. To measure the ChBF after 20 minutes of stationary biking, each subject quickly dismounted the bike at the end of the biking interval and positioned his or her head in the chin–forehead rest of the LDF system that had been adjusted for that individual before biking. The LDF recordings started as soon as the position of the light sensor atop the foveal laser reflex was assured by the examiner with an IR viewing system. The Doppler-shifted signals were fed into a computer (NeXT Computer Inc., Redwood, CA) that provided real-time fast Fourier transform (FFT) analysis of the incoming data. These signals were then analyzed in the time–amplitude domain with a special computer program written for this purpose. The systemic BP was measured at regular intervals during recovery to calculate the concomitant changes in the OPP.

Phase 2: Quantifying the Interactions between the OPP and the ChBF during and after Biking

Phase 2 of the research took place some 14 months later at the University of Montreal. Because of the large size of the LDF system and the time needed to perform standard sphygmomanometry, the first data set in phase 2 was based on intermittent measurements of the ChBF and BP during biking. Later, we were able to quantify these parameters continuously throughout the biking and recovery intervals. The intermittent measurements of ChBF were made with a fundus camera (Kowa-Pro, Kowa, Tokyo, Japan)–based LDF system[17] (Oculix Inc., Arbaz, Switzerland) for a group of five subjects (mean age, 28.0 ± 9.3 years). These subjects were trained to bike at the prerequisite HR of 140 bpm, stop biking every 3 minutes, lean forward into the LDF system abutting the front of the bike to allow sampling of the ChBF for approximately 15 seconds, and then resume biking at an HR of 140 bpm until the next LDF measurements some 3 minutes later. The brachial BP needed to calculate the OPP was taken while subjects were still biking, approximately 1 minute before each ChBF measurement. The second data set in Phase 2 was based on continuous measurements of the ChBF and the BP throughout the biking and recovery intervals in a cohort of seven trained volunteers (mean age, 29.0 ± 4.5 years). These real-time measurements were made possible by a new compact confocal LDF (cLDF),[18] conceived and built at the Institute of Research in Ophthalmology and a noninvasive BP acquisition system (model 7000 NIBP; Colin Medical Instruments Corp., San Antonio, TX). Throughout testing, the cLDF was held before a subject's test eye by a sturdy scaffold that vaulted the stationary bike close to the subject. The viewing port of the cLDF was embedded within a 1-inch layer of high-density foam to support the forehead and periorbital area of each subject and thereby improve fixation stability. To record the raw systemic BP without movement artifacts, the wrist with the piezoelectric sensor (model 7000 NIBP; Colin) placed atop the radial artery was suspended at heart level by Velcro elastic straps that were attached to a rigid stand that was independent of the bike and the cLDF support.

The new cLDF device has been described earlier.[18] Briefly, it uses a compact confocal laser optics delivery and light-detection arrangement, weighing approximately 5 pounds, to focus a 785-nm probing laser beam on a 15-μm diameter area atop the foveola. The intensity of the laser beam at the corneal plane is approximately 90 μW, well within the American National Standards Institute (ANSI) safety standards.[19] The light backscattered by the red blood cells and surrounding tissue in the subfoveal choriocapillaris is detected by a hexagonal array of fiber optics. The direct current (DC) which is proportional to the total amount of light reaching the light sensor is also measured continuously, and serves as an objective index of the fixation stability. The HR, detected by an IR ear clip sensor is used to calculate the pulsatility index of the ChBF parameters. A special artifact rejection algorithm in the analysis program automatically eliminated from analysis any portion of the ChBF record (approximately 0.5 second) that was disturbed by a blink.[13] Steady fixation of the probing laser produced a steady DC signal that appeared as a straight line in the separate data-acquisition window. Any eye movement away from the probing laser caused an abrupt shift in the DC trace that incited the experimenter to encourage the subject to maintain fixation of the laser spot. After data acquisition, the experimenter removed this well-demarcated portion of the record (typically only a few seconds) in the DC channel from analysis, a procedure that simultaneously removed the time-matched records for all other blood flow parameters and the heart rate. The continuously recorded ChBF during biking was analyzed using the computer-based analysis system described in conjunction with the camera-LDF instrument.[17] Because the digitizing rates for the cLDF and the blood pressure acquisition system (model 7000 NIBP; Colin) were each approximately 20 Hz, consecutive data points were grouped into 5-second bins to simplify data management and still provide high-resolution measurement of changes in the OPP and ChBF over time. Because the absolute ChBF and BP used to calculate the OPP differed across subjects, the ChBF and OPP were normalized to allow a comparison of their interaction on a common scale. Normalization of individual data consisted of assigning the first OPP and ChBF point in biking a value of 100% and subsequent points a percentage of that value.

Dynamic Exercise and Physiological Variables

The OPP was increased by biking on a computerized stationary exercise bicycle (Cateye EC-1500 Ergociser; Physio ERP, Ltd., Laval, Québec, Canada). Before testing of individual subjects, the bike was adjusted for the subject's ergonomic efficiency and comfort. The OPP was calculated according to the formula: OPP = 2/3 BPmean − IOP, where the mean blood pressure (BPmean) was calculated as: BPmean = BPdiast + 1/3 (BPsyst − BPdiast), where BPsyst and BPdiast

are systolic and diastolic blood pressure, respectively.[20] After each subject was accustomed to the laboratory environment, the IOP and the systemic BP were measured twice and averaged to derive their respective resting values. The pupil of the test eye was dilated with 1 drop of 1% tropicamide plus 1 drop of 2.5% phenylephrine HCl. Subjects were instructed to reach and maintain the target HR of 140 bpm as quickly as possible (typically achieved within 30 seconds) but without excessive exertion. Guided by the experimenter's reporting of the HR, a subject could request an adjustment of the pedaling cadence or resistance to maintain the target HR of 140 bpm throughout the biking interval.

Statistical Analyses and Ethical Considerations

Analyses to identify statistically significant trends in all data sets involved repeated measures ANOVA and linear regression analyses for an α level of $P = 0.05$. Any statements concerning parametric changes refer to differences that achieved statistical significance, qualified by the probability. In the following sections, all presentations of OPP and ChBF data trends over time refer to group-averaged results for normalized data.

All methods and procedures complied with the tenets of the Declaration of Helsinki. All testing performed in Switzerland complied with the ethics standards of the University of Lausanne, whereas all testing performed at the University of Montreal had approval from that institution's ethics committee for the use of humans in experimentation. All subjects were informed of the nature of the experiment before the study and the right to withdraw at any time for any reason of their choosing without prejudice. All subjects read and then signed the approved ethics form.

Results

Phase 1

In the 14 subjects who participated in Phase 1 of the study, the resting IOP and the resting systemic BP were 13.9 ± 1.9 mm Hg and (systolic/diastolic) 121.2 ± 15.1/77.6 ± 11 mm Hg, respectively. The upper right hand pair of curves in Figure 1A illustrate the percentage change in the normalized ChBF and OPP as a function of time immediately after the 20-minute biking interval. Most notably, the ChBF and the OPP decreased by approximately 12% ($P = 0.0001$) and 22% ($P = 0.0024$) at a rate of approximately 0.06% per minute and 0.11% per minute, respectively, within the initial 250 seconds and thereafter remained unchanged. The ChBVel and ChBVol (not presented graphically) also demonstrated biphasic recovery profiles. The ChBVel decreased by approximately 6% ($P = 0.0001$) within the first 200 seconds and thereafter decreased more slowly to a maximum reduction of approximately 10% by the end of the recovery interval. The ChBVol decreased by approximately 5% in the first 200 seconds ($P = 0.0034$) but then increased to return to baseline within 300 seconds. The HR at the end of biking (136 ± 4 bpm) decreased by approximately 20% in the first 100 seconds ($P = 0.0001$) and then decreased by an additional 6% over the remaining time in recovery.

Phase 2

The resting IOP and systemic BP for the 12 subjects who participated in phase 2 were 13.3 ± 2.1 mm Hg and 120.2 ± 6.7/74.6 ± 8.9 mm Hg, respectively. The interaction between the OPP and the ChBF derived in Phase 2 is shown in Figure 1B. The larger empty circles and squares indicate the changes in the OPP and the ChBF respectively in the group of five subjects trained to have their systemic BP and ChBF measured at regular intervals during biking. The smaller shaded circles and squares show the changes in the OPP and the ChBF with time into biking and recovery, derived from continuous measurements of these variables in a different cohort of seven subjects. Collectively, these data revealed a clear dissociation between the OPP and the ChBF—that is, the ChBF was not passively driven by changes in the OPP.

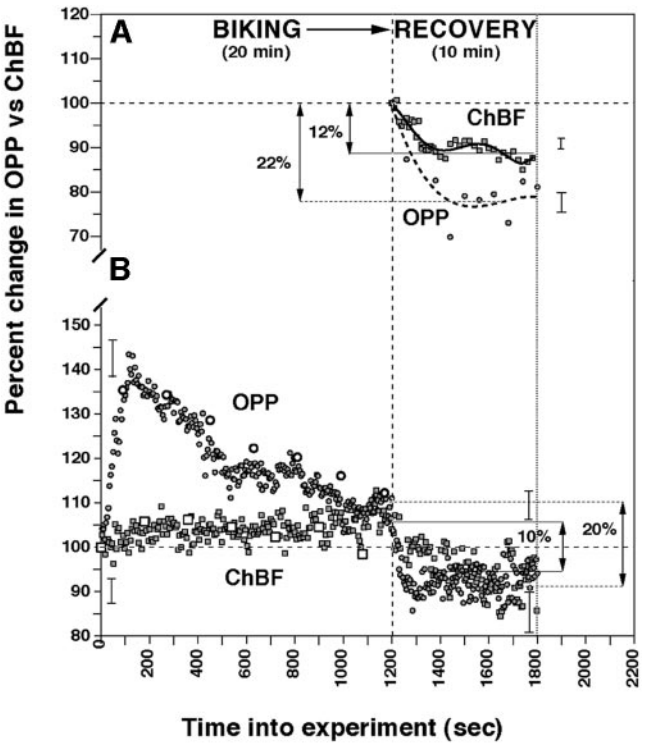

Figure 1. (**A**) Normalized, group-averaged ($n = 14$) ChBF and OPP, as a function of time into the recovery period after a 20-minute interval of continuous biking. The plateaus achieved by the ChBF and the OPP after 200 seconds into recovery differed significantly ($P = 0.0001$). (**B**) Normalized, group-averaged data illustrating the interaction between the OPP and ChBF during 20 minutes of biking at an HR of 140 bpm and immediately afterward. Although the stationary biking caused the OPP to increase by a maximum of approximately 43% ($P = 0.0001$) and subsequently decrease to a level some 10% above resting, the ChBF remained within 10% of basal ($P = 0.0001$), indicating an independent control of the degree of blood flow in the choroid. *Open circles* and *squares*: data points for the OPP and the ChBF obtained from a cohort of five subjects in whom the ChBF was sampled at regular intervals during biking. *Smaller shaded circles* and *squares*: continuously recorded data points for the OPP and ChBF, respectively, during biking and during the recovery interval, in a different group of seven subjects. During the recovery interval, the OPP decreased by approximately 20% ($P = 0.0001$), whereas the ChBF decreased by only 10% ($P = 0.0095$). (**A**, **B**, *vertical bars*) Group-averaged SEM for the data points comprising a response profile.

Within 100 seconds after biking, the OPP exceeded the resting value by approximately 43% ($P = 0.0001$), whereas the ChBF remained unchanged from its resting level at that point. The OPP then decreased biphasically from its peak. The initial decrease was approximately four times faster (0.08% per second) in the first 500 seconds than during the remainder of the biking interval (0.02% per second). Immediately after biking was stopped, the OPP decreased by approximately 20% within 100 seconds ($P = 0.0001$) and remained at that level throughout the recovery interval.

In contrast to the large decrease in the OPP after its peak at the 150-second mark, the ChBF increased linearly above its resting level, but by only 5% to 6%, from the start to the end of biking ($P = 0.0001$). Subsequently, the ChBF decreased abruptly at the end of biking ($P = 0.0095$) and remained close to its resting value during the remaining recovery interval. The

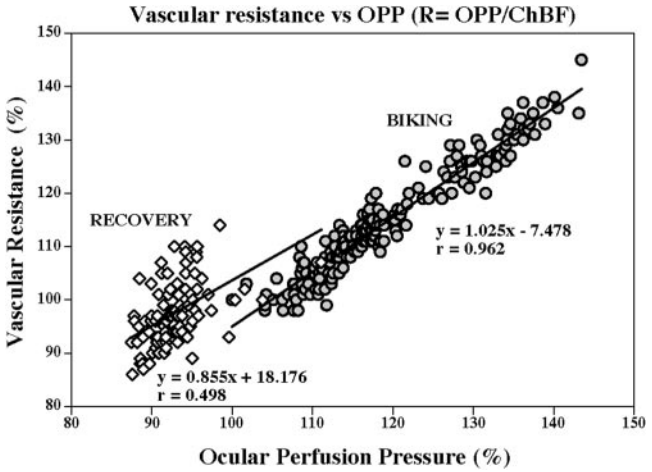

FIGURE 2. Graph showing the influence of changes in the OPP on the vascular resistance during the biking and recovery intervals for the continuous ChBF measurement data shown in Figure 1B. The vascular resistance was calculated as R = OPP/ChBF. During biking, the significant correlation between the OPP and the ChBF is best described by a linear model defined by $y = 1.025x - 7.478$ ($r = 0.962$, $P = 0.0001$). During recovery, the OPP and ChBF remained linearly correlated and defined by $y = 0.855x + 18.176$ ($r = 0.498$, $P = 0.0001$).

relative differences between the changes in the OPP and the ChBF during the recovery intervals were remarkably similar in shape and magnitude in both phases of the study (Fig. 1A vs. 1B). However, the OPP decreased approximately twice as fast and twice as much as did the ChBF.

Figure 2 presents the interaction between the normalized OPP and the normalized vascular resistance (R) which was calculated as R = OPP/ChBF, during stationary biking and the recovery interval. A linear fit for both data sets showed a significant correlation between the vascular resistance and the OPP. During biking (Fig. 2, data set at top right) the slope of the linear regression line through these data points was +1.025 and showed a highly significant correlation of $r = 0.962$ ($P = 0.0001$). During the recovery interval (Fig. 2, data set at bottom left), the slope of the linear fit through the data points illustrating the interaction between the vascular resistance and the OPP was also high (+0.855) but the data points showed less correlation ($r = 0.498$; $P = 0.0001$). A summary of the statistical analyses for each data set derived in this study is presented in Table 1.

DISCUSSION

The present study focused on the interactions between the ChBF and the OPP during and after 20 minutes of stationary biking at an HR of 140 bpm, which significantly increased the OPP above resting levels. The change in OPP was calculated from changes in the BPmean, assuming that the IOP remained close to resting level. The IOP was not measured continuously during the test routines, because repeated applanation of the cornea would ultimately cause some epithelial abrasion and corneal edema, which could seriously degrade the quality of LDF measurements. Although Marcus et al.[21] reported a 6-mm Hg decrease in IOP after just 4 minutes of moderate treadmill exercise, biking experiments in our laboratory did not reveal a large change in the resting IOP after 20 minutes of stationary biking.[22,23] Other studies evaluating the IOP and using a variety of exercise modalities also showed minimal or no change in the IOP.[12,24,25] In our laboratory, measurements of the IOP (n = 62) by applanation tonometry before and after stationary biking, as described in the present study indicated a group-averaged, statistically significant ($P = 0.0001$) decrease in the IOP of only 0.75 mm Hg. The error introduced into the calculation of the OPP by assuming that the resting IOP did not change was an underestimation of only 1.42% averaged over the biking and recovery intervals.

Because near-IR light can penetrate the retinal pigment epithelium,[26] and because the fovea is devoid of retinal vessels in the FAZ, our LDF measurements reflected the subfoveal ChBF exclusively. Geiser et al.[27] have demonstrated that near-IR LDF measures the ChBF exclusively, by showing that healthy subjects breathing 100% oxygen did not manifest any changes in the subfoveal ChBF. Because oxygen induces vasoconstriction in retinal vessels, the ChBF would have decreased had the LDF procedure included any significant contribution from the retinal vasculature. Furthermore, previous considerations strongly suggest that the LDF signal mainly originates from the choriocapillaris layer of the choroid.[10] Thus, the conclusions of the present study unequivocally apply to the choroid, but must be restricted to the subfoveal choroid,

TABLE 1. Statistical Analyses of Group Averaged Data

Study Sections*	Variables Compared	% Change	Significance Level ($\alpha = 0.05$)	P
Phase 1				
Biking vs. recovery	ChBF	−12 (in 250 sec)	ANOVA	=0.0001
	OPP	−22 (in 250 sec)		=0.0024
Other flow parameters	ChB Vel	−6 (in 200 sec)	ANOVA	=0.0001
	ChB Vol	−5 (in 200 sec)		=0.0034
HR: Biking vs. recovery	HR: biking (140 bpm)	HR: recovery −20 (in 100 sec)	ANOVA	=0.0001
Phase 2 (continuous record)				
Rest. vs. biking	OPP	+43 (in 100 sec)	ANOVA (data across biking-recovery intervals)	=0.0001
	ChBF	+5–6 (over 20 min)	Linear regression	=0.0001
End of biking vs. recovery	OPP	−20 (in 100 sec)	ANOVA	=0.0001
	ChBF	−10 (in 160 sec)		=0.0095
R vs. OPP				
Biking	R, OPP	Slope: +1.025 $r = 0.962$	Linear regression	=0.0001
Recovery	R, OPP	Slope: +0.855 $r = 0.498$	Linear regression	=0.0001

* Phase 1 corresponds to Figure 1A, Phase 2 to Figure 1B, and R vs. OPP to Figure 2.

which exclusively perfuses the cone photoreceptors. Measurements with an LDF system that can measure the ChBF response profiles in regions other than the FAZ are needed, to demonstrate any similarities or differences in the choroidal hemodynamics, as reported herein. From an anatomic point of view, the greater vascular density in the central than the more peripheral choriocapillaris, and the segregation of the cone and rod photoreceptors in the foveal versus peripheral fundus, it can be argued that vascular regulation in the peripheral choroid may differ from that reported in this study for the subfoveal choriocapillaris. The possibility that vascular hemodynamics in the human choroid are determined, at least in part, by its anatomic features and the density of the photoreceptor type it perfuses awaits experimental validation.

The validity of the noninvasive LDF technique for real-time noninvasive measurements of the subfoveal ChBF was established empirically by Riva and Petrig[28] when they first reported this method. They simultaneously measured the mean arterial pressure and the ChBF in cats after a lethal injection of pentobarbital sodium. Both the ChBF and the BP decreased linearly as the test animals died. In the same study, they demonstrated that the 95% confidence limits for the average of repeated ChBF measurements for a human observer with good fixation was low, ±4%. In a companion article, Riva et al.[10] provided additional data confirming the effectiveness of the LDF technique for measuring relative changes in the ocular blood flow in the human eye noninvasively. To confirm experimentally that our LDF system accurately reflected dynamic changes in blood flow velocity, we assessed the relationship between the LDF velocity readings and the actual changes in the velocity of particles in a special light-scattering apparatus developed specifically for this purpose. The regression line through the data points for these variables was defined by $y = 1.000x - 0.000$, and a statistical analysis indicated a statistical significance and a high correlation ($r = 1.00$, $P = 0.0001$). Measurements of the LDF velocity output for a fixed particle velocity over a 30-minute interval indicated a negligible variation of ±0.72%. By projection of these principles and findings to perfused living tissue, these latter observations confirmed a high stability and sensitivity of the LDF system to measure changes in the velocity of moving blood cells, and hence the calculation of the ChBF. Given this demonstration that the LDF was capable of making rapid and precise measurements of changes in the ChBF, the temporal and amplitude interactions between the ChBF and the OPP shown in Figure 1 reveal the rapid nature of vascular regulation in the human choroid during aerobic exercise. The near immediate choroidal reaction to dynamic changes in the OPP could not be measured previously, because suitable technology to measure real-time changes in the ChBF did not exist.

To glean the degree of variability of the LDF system for measuring the ChBF, one of the original test subjects who demonstrated excellent fixation stability was asked to fixate the probing laser over a 30-minute interval. We determined the 95% confidence limit for the resultant mean ChBF of 35.36 AU to be approximately ±5% which was attributable in large part to the pulsatile nature of the ChBF (averaged pulse amplitude = 3.8 AU), because fixation was very steady (DC = 2.6 V ± 0.47%). It can therefore be concluded that the steadiness of any ChBF recording is a function of both the fixation stability and the pulse amplitude.

To illustrate further the responsivity and sensitivity of the LDF system and to counter any criticism of inadequate sensitivity or saturation of the LDF during biking, this subject was also asked to perform a Valsalva-like maneuver at the end of the 30-minute fixation interval. This procedure involved forced expiration against a closed glottis. Lovasik et al. (Lovasik JV, Kergoat H, Riva CE, Geiser M, Petrig BL, ARVO Abstract 3315, 2002) reported earlier that at a high level of forced expiration, the ChBF increased above resting value because of a large increase in the diastolic BP and a consequent backup of blood into the choroid. In this subject, high forced expiration caused a very rapid increase in the ChBF of approximately 24%, a continued increase in the ChBF during sustained expiration, and an immediate return to the resting level as soon as the forced expiration stopped. These findings demonstrated the ability of our LDF system to record accurately either a prolonged steady state ChBF or an abrupt increase or decrease in the ChBF. Furthermore, the results from a previous study[10] showed that the Doppler frequency spectrum of the light scattered from the choroid in the FAZ extended to frequencies well below the high cutoff frequency of 40 kHz for the LDF analysis system.

The large difference between the ChBF and the exercise-induced increase in the OPP illustrated in Figure 1B is the fundamental reason for our conclusion that a vascular regulatory mechanism for blood flow exists in the human choroid. During biking, the ChBF showed a small but significant ($P = 0.0001$) trend to increase by 5% to 6% above basal value. Regardless of the absolute magnitude of the increase in the ChBF during biking, it is clear that it increased by a small amount (5%–6%), whereas the OPP increased by 43% and then decreased by as much as 33% from its peak at the 150-second mark. Therefore, the ChBF did not passively parallel the ongoing changes in the OPP as would likely be the case if the ChBF was not regulated.

It could be argued that the pattern of changes in the ChBF in the present study were linked to exercise-induced changes in the CO_2. Geiser et al.[27] showed that breathing various mixtures of O_2 and CO_2, in humans, resulted in a change in the ChBF of 1.5% per 1 mm Hg increase in arterial pCO_2. However, their data were based on inhalation of gas at rest and therefore are not likely to apply to aerobic exercise. Because studies in respiratory physiology have constantly shown that the arterial pCO_2 remains unchanged or changes minimally during biking,[29] it is legitimate to assume that there was no change or only a minimal change in pCO_2 during our biking protocol. As such, we can conclude that the CO_2 could not have been the sole or dominant driving force for changes in the ChBF. If our objective had been to analyze O_2 consumption and CO_2 production in the lungs and arterial blood supply, there is no reason to assume that data from our subjects would have differed from that of other studies on this issue.

The seminal work by Bill and Sperber[30] concluded that the ChBF is passively driven by the OPP in humans. From this, it would be reasonable to conclude that the increase in ChBF measured in this study during biking was driven by the increase in the OPP. However, the large difference between these normalized parameters as shown in Figure 1B indicates that some mechanism kept the ChBF close to its resting level during biking. The data presented in Figure 2 shed some light on how this difference may have occurred. The one-to-one correlation between the OPP and the vascular resistance during biking suggests that a modulation of vascular resistance could account for the measured trends in the ChBF during biking. Because Poiseuille's law[31] stipulates that blood flow in a vessel is primarily determined by the radius of the vessel, the most plausible mechanism controlling blood flow in the choroid during biking is sympathetically mediated vasoconstriction. The slope of the linear regression line through the OPP and vascular resistance data points during recovery was interpreted as indicating that some sympathetic vasoconstriction may have persisted after biking, at least during the early phases of the recovery interval. Without such vasoconstriction, a positive rebound of the ChBF would be expected, but none occurred. The rapid decrease in the OPP at the end of biking,

together with the rapid decrease in the HR and ChBVol, also undoubtedly played important roles in decreasing the ChBF.

In both phases of the study, it was observed that during the recovery interval, the OPP decreased faster and by approximately twice the amount as did the ChBF. The large decrease in the OPP is a mathematical consequence of a large reduction in the systolic arterial pressure, a variable in the equation used to calculate the OPP. Physiologically, the reduction in the systolic arterial pressure is attributed to the phenomenon of "arterial hypotension" wherein the arterial pressure decreases after a period of submaximal exercise, such as that used in the present study.[32] The differential decrease in the OPP and the ChBF indicated that the ChBF did not passively follow the decrease in the OPP, but rather was regulated by some active mechanism that was apparently activated to keep ChBF close to its basal value during the abrupt decrease in the OPP when biking stopped. The overall behavior of the ChBF during the biking and recovery intervals strongly suggests the existence of a blood flow regulation mechanism that is activated when the OPP either exceeds or declines below some critical value.

The site of vascular regulation could be before, at, or after the site of measurement. The fact that we found a decrease in ChBVol during recovery suggests some constriction at the level of the choriocapillaris. However, most of the vasoconstriction probably occurred at the arteriolar level—vessels known to control vascular resistance. The possible involvement of the ophthalmic artery and/or the short posterior ciliary arteries in the choroidal regulatory process cannot be ignored. Only small changes in the diameter of the internal carotid artery or its offshoot, the ophthalmic artery, could buffer most of the exercise-induced increase in the OPP through increased vascular resistance.[33] Finer levels of blood flow regulation may be a function of the larger vessels in Haller's and Sattler's layers of the choroid or in the choriocapillaris proper. Transcranial Doppler monitoring of changes in blood flow in the internal carotid and the ophthalmic artery during stationary biking are planned, to derive a more global perspective of the vascular mechanisms controlling ChBF.

It is interesting to note that the OPPs obtained from the first cohort of subjects ($n = 5$) used in phase 2 tended to be slightly higher than those obtained from the second group of subjects ($n = 7$), most notably midway into the biking interval. Although these differences were not statistically significant, they may have reflected differences in the absolute physiological workload across test groups during biking. The physiological workload may have been greater in those subjects who were required to stop biking for measurements of the ChBF and then bike rapidly to regain quickly the target heart rate of 140 bpm. This would have raised the systemic BP, and thus the calculated OPP, more compared to continuous biking. Given that the ChBF remained equally close to baseline during intermittent and continuous biking, it is reasonable to conclude that the human choroid can promote hemodynamic homeostasis throughout gradual or acute changes in the OPP.

The presence of a mechanism that limits the increase in ChBF during exercise, at least for the increases in OPP reached in this study, suggests that the choroidal circulation differs from that of the brain, because in the latter case, the increase in blood flow is nearly identical with the increase in systemic blood pressure. One of the differences between these vascular systems could be that the cerebral blood flow increase during exercise is due not only to an increase in the BP, but also to an increase in metabolism,[34] the latter component being absent in the choroidal system.

It is noteworthy that regulation of the ChBF may be equally effective during isometric and dynamic exercise. In a recent study, Riva et al.[12] reported that the ChBF was increased by only 12% even though isometric exercise (squatting) raised the OPP by as much as 60%. Beyond this level, the ChBF increased rapidly. Shunting of blood to where it is needed the most, specifically the large muscle groups in the legs, by itself may be a major factor determining the amount of blood perfusing the brain and the eye.

Finally, we must address the question of the physiological need for regulation of blood flow in the choroid. At present, a logical explanation for the need to regulate ChBF relates to preserving the function of the different neurons that constitute the delicate three-dimensional cytoarchitecture of the human retina. Restricting the degree of ChBF during elevated levels of OPP may represent a protective mechanism for the retina, which would otherwise incur considerable compressive forces across all areas of the fundus. An absence of blood flow regulation in the choroid would result in a parallel increase in the ChBF, with a concomitant increase in the IOP secondary to the increased choroidal blood volume within a minimally distensible scleral shell. The consequence of this would be large compressive forces exerted inward on the outer retina by the retinal pigment epithelium through its displacement by the engorged choroid, as well as an increased outward force on the inner retina by the vitreous because fluids are noncompressible. This notion is supported by the earlier observations by Kergoat and Lovasik[35] and Kothe and Lovasik[36] who demonstrated deleterious effects of even transient elevations of the IOP on visually evoked retinal potentials from the amacrine cells,[35] and the most vitread ganglion cell axons that relay retinal signals to the visual cortex.[36] Controlling the degree of ChBF may also be a protective mechanism against aneurysms or vessel leakage in smaller vessels downstream.

In summary, although our present study cannot comprehensively address the nature of the mechanisms controlling the ChBF, it is nonetheless evident from our findings that a large increase in the OPP is not accompanied by an equivalent increase in the ChBF. These data strongly support the notion of a regulatory mechanism for blood flow in the human choroid. The principal site of regulation of the ChBF subsequent to a physiologically significant increase in the OPP is possibly the larger vessels perfusing the eye, with a finer regulation occurring in the choriocapillaris.

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