Foveolar Choroidal Blood Flow in Age-Related Macular Degeneration

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Purpose. To compare measurements of the foveolar choroidal blood circulation in subjects with nonexudative, age-related macular degeneration (AMD) with those of control subjects.

Methods. Laser Doppler flowmetry was used to assess relative choroidal blood velocity (ChB_{Vel}), volume (ChB_{Vol}), and flow (ChB_{Flow}) in the center of the fovea. Measurements were obtained in 20 eyes of 20 subjects with 10 or more large drusen, visual acuity of 20/32 or better, and no evidence of choroidal neovascularization. Findings obtained in these subjects were compared with those of 10 eyes of 10 age- and blood pressure-matched control subjects with no large drusen. Foveolar choroidal blood flow measurements were obtained by asking the study participants to fixate on a probing laser beam.

Results. No significant differences in average age, blood pressure, or intraocular pressure were observed between subjects with AMD and control subjects. In subjects with AMD, average ChB_{Vol} was 0.24 ± 0.08 (± 1 SD) arbitrary units (AU); this value was 33% lower than that of control subjects (0.36 ± 0.11 AU; two-tailed, independent Student's *t*-test, $P = 0.005$). Average ChB_{Vel}, conversely, was not significantly different from normal (0.44 ± 0.07 AU) in subjects with AMD (0.44 ± 0.10 AU). Average ChB_{Flow} in subjects with AMD (8.7 ± 3.1 AU) was 37% lower than that of control subjects (13.7 ± 3.5 AU) ($P = 0.0005$). Average blood flow pulsatility was 6% higher in subjects with AMD (0.71 ± 0.15) than in control subjects (0.66 ± 0.14), but this difference was not statistically significant ($P = 0.42$).

Conclusions. Average ChB_{Flow} in the nonexudative stages of AMD is lower than that of age-matched controls, and the effect is caused mainly by a decrease in ChB_{Vol}. Further studies are needed to elucidate whether decreased ChB_{Flow} plays a role in the development of choroidal neovascularization, and whether ChB_{Flow} measurements may help identify subjects with AMD at risk for developing choroidal neovascularization. (*Invest Ophthalmol Vis Sci.* 1998;39:385-390)

Angiogenesis is the process by which new vessels are formed throughout the body. This phenomenon occurs normally during wound repair and after menstruation. In pathologic conditions, such as the late stages of age-related macular degeneration (AMD), one of the leading causes of blindness,[1-5] angiogenesis leads to the formation of choroidal neovascularization that can cause severe visual impairment by disrupting the normal macular function.

One of the factors that may trigger the development of angiogenesis is the presence of tissue ischemia and hypoxia. Several reports[6-14] have provided indirect and mostly qualitative evidence suggesting that choroidal blood flow is decreased in subjects with AMD. The choroidal circulation is of great importance for normal visual function, because it is responsible for all the supply and removal of metabolites to and from the outer retina.

The purpose of our study was to determine whether the foveolar choroidal circulation is abnormal in subjects with nonexudative AMD. The presence of an abnormal choroidal circulation in the earlier stages of the disease suggests an etiologic role of ischemia in the development of AMD. Assessment of the choroidal circulation was performed with the laser Doppler flowmetry technique.

Materials and Methods

Twenty eyes of 20 white subjects with nonexudative AMD and 10 or more large drusen (≥63 μm) and with visual acuity of 20/32 or better, no evidence of choroidal neovascularization, and otherwise normal external, slit-lamp, and funduscopic eye examination results were included in the study. None of the eyes studied had areas of geographic atrophy. Ages of these subjects ranged from 56 to 84 years (mean ± 1 SD, 69 ± 8 years). Other subject characteristics are summarized in Table 1. Five of the 20 subjects had exudative AMD with a choroidal neovascular membrane in the fellow eye. Eight subjects had a history of systemic hypertension, and six of them were receiving systemic medications. Two subjects were receiving hormone replacement therapy with conjugated estrogens (Premarin; Wyeth-Ayerst, Philadelphia, PA).

Results were compared with those of 10 eyes of 10 subjects with no large drusen, visual acuity of 20/25 or better, and

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Table 1. Characteristics of Control Subjects and Subjects with Age-Related Macular Degeneration

	Control		AMD		
Mean (SD)	*Mean*	*(SD)*	*Mean*	*(SD)*	*P Value**
Age (years)	65	(8)	69	(8)	0.20
Diastolic pressure (mm Hg)	81	(11)	78	(8)	0.45
Systolic pressure (mm Hg)	150	(16)	143	(13)	0.29
Intraocular pressure (mm Hg)	16.6	(2.2)	16.4	(2.1)	0.85
Perfusion pressure (mm Hg)	52	(6)	49	(6)	0.23
Refractive error (diopters)	0.3	(2.7)	0.03	(1.2)	0.80
Sex (M/F)	5/5		11/9		1.0†

*By independent Student's *t*-test.
†By Fisher's exact test.
AMD, age-related macular degeneration.

otherwise normal external, slit-lamp, and funduscopic eye examination results. Ages of the control subjects ranged from 55 to 76 years (65 ± 8 years). Other control subject characteristics are summarized in Table 1. Two of these subjects had a history of systemic hypertension and were receiving systemic medications. One of these two subjects, and an additional one, were receiving systemic therapy for elevated blood cholesterol levels. Another subject received hormone replacement therapy.

After a detailed explanation of the procedures, all subjects were asked to sign an appropriate consent form approved by the human experimental committee of our institution. The tenets of the Declaration of Helsinki were followed.

Before the measurements were made, pupils were dilated with 1% tropicamide (Alcon, Fort Worth, TX) and 10% phenylephrine hydrochloride (Sanofi Winthrop, New York, NY). Blood flow measurements were obtained in one eye of each subject. In most subjects, the right eye was measured. The exceptions were three subjects with AMD who had a choroidal neovascular membrane in the right eye, and one subject with AMD and one control subject who had less steady fixation in the right eye.

Determinations of relative foveolar choroidal blood velocity (ChB_{Vel}), volume (ChB_{Vol}), and flow (ChB_{Flow}) were obtained using a method based on the laser Doppler flowmetry technique. Detailed descriptions of the method have been previously published.[15-18] A diode laser beam (670 nm) with an intensity of 20 μW was delivered through a fundus camera (Model TRC; Topcon, Tokyo, Japan). The diameter of the probing laser beam was approximately 200 μm.

During blood flow measurements, an area of the posterior retina (30° in diameter) was illuminated at a wavelength of 570 μm with a retinal irradiance of approximately 0.03 mW/cm^2. With this light, we were able to observe the position of the laser on the foveola. Subjects were asked to fixate on the probing laser beam to determine the foveolar choroidal blood flow. Measurements obtained in this manner correspond primarily to determinations of choriocapillary flow, as discussed previously by Riva et al.[15]

During the measurements, proper fixation was ascertained by direct observation of the foveola through the fundus camera. All measurements were performed with the subjects seated in a darkened room.

In each subject, a continuous measurement of the choroidal circulation was obtained for approximately 30 seconds. Analysis of these data was performed by a masked observer using a computer (NeXT Computer, Redwood City, CA) with software specifically developed for the analysis of Doppler signals from ocular tissues.[17] The masked observer selected parts of the recording for analysis that showed stable circulatory parameters. In each subject, approximately 2 seconds of recording time were included in the analysis of the data. Figure 1 shows a typical recording in a 71-year-old subject with AMD. The highlighted section depicts a segment of approximately 2.5 seconds selected for analysis.

In 12 subjects with AMD and 8 control subjects, the procedure described above was repeated, and three separate determinations were conducted during the same experimental session to assess the reproducibility of the measurements. From these three measurements, a coefficient of variability (*CV*) was calculated as follows:

$$CV = (\text{mean/standard deviation}) \times 100 \quad (1)$$

The average pulsatile component of ChB_{Flow} was determined over the cardiac cycle, which was recorded with an infrared pulse monitor. The pulsatile component of ChB_{Flow} was calculated as follows:

$$1 - ChB_{Flow(systolic)}/ChB_{Flow(diastolic)} \quad (2)$$

Brachial artery systolic and diastolic blood pressures (BP_s and BP_d, respectively) were determined by sphygmomanometry after blood flow measurements. Intraocular pressure (IOP) was measured by applanation tonometry. The mean brachial artery pressure (BP_m) was calculated according to the following formula:

$$BP_m = BP_d + 1/3(BP_s - BP_d) \quad (3)$$

The perfusion pressure (PP) for the study eye was estimated according to the following formula:

$$PP = 2/3BP_m - IOP \quad (4)$$

Independent, two-tailed Student's *t*-tests, linear regressions, Fisher's exact test, and correlation coefficients were used in the statistical analysis of the results. The assumption of normality of the data was assessed using the Shapiro-Wilk test.

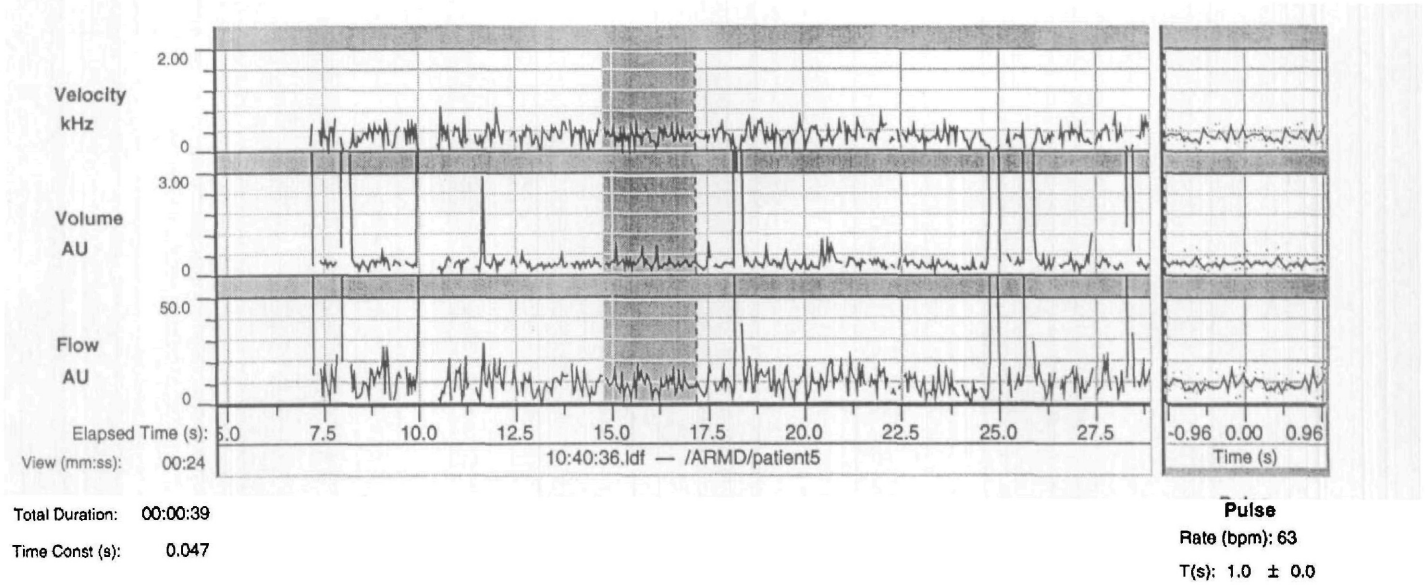

Figure 1. Typical recording of a 71-year-old male subject with age-related macular degeneration showing measurements of relative blood velocity, volume, and flow. The area *highlighted* corresponds to a segment of the recording chosen for analysis with relatively stable circulatory parameters.

Probability values less than 0.05 were considered statistically significant.

Results

No clinically or statistically significant differences in age, BP_d, BP_s, BP_m, IOP, or PP were observed between subjects with AMD and control subjects (Table 2).

In subjects with AMD, the average ChB_{Vol} was 0.24 arbitrary units (AU; Table 2), a value that was 33% lower than the average of 0.36 AU observed in control subjects (two-tailed, independent Student's *t*-test, $P = 0.005$; Fig. 2). Average ChB_{Vel}, however, was not significantly different from normal (0.44 AU) in subjects with AMD (0.44 AU, $P = 0.97$; Fig. 3).

The average ChB_{Flow} was 8.7 AU in subjects with AMD, a value that was 37% lower than the average of 13.7 AU in control subjects ($P = 0.0005$; Fig. 4).

The average blood flow pulsatility in subjects with AMD was 0.71, a value that was 6% higher than the average of 0.66 observed in control subjects, but this difference was not statistically significant ($P = 0.42$; Fig. 5).

Table 2. Relative Foveolar Choroidal Blood Volume, Velocity, Flow, and Flow Pulsatility in Control Subjects and Subjects with Age-Related Macular Degeneration

	Control		*AMD*		
	Mean	*(SD)*	*Mean*	*(SD)*	***P* Value***
ChB_{Vol} (AU)	0.36	(0.11)	0.24	(0.08)	0.005
ChB_{Vel} (AU)	0.44	(0.07)	0.44	(0.10)	0.97
ChB_{Flow} (AU)	13.7	(3.5)	8.7	(3.1)	0.0005
Flow Pulsatility	0.66	(0.14)	0.71	(0.15)	0.42

*By two-tailed, independent Student's *t*-test.

AMD, Age-related macular degeneration; ChB_{Vol}, relative foveolar choroidal blood volume; ChB_{Vel}, relative foveolar choroidal blood velocity; ChB_{Flow}, relative foveolar choroidal blood flow.

The coefficients of variability for ChB_{Vel}, ChB_{Vol}, and ChB_{Flow} were 14%, 25%, and 18%, respectively, in subjects with AMD, and 8%, 18%, and 13%, respectively, in normal controls. No statistically significant differences were observed between the coefficients of variation for subjects with AMD and those for normal controls.

An analysis of the data, excluding eight subjects with AMD and two control subjects with a history of systemic hypertension, showed a significant decrease in ChB_{Flow} in subjects with AMD, similar to that obtained in the analysis that included all subjects.

No significant differences in any of the circulatory parameters were observed between the five study eyes of five sub-

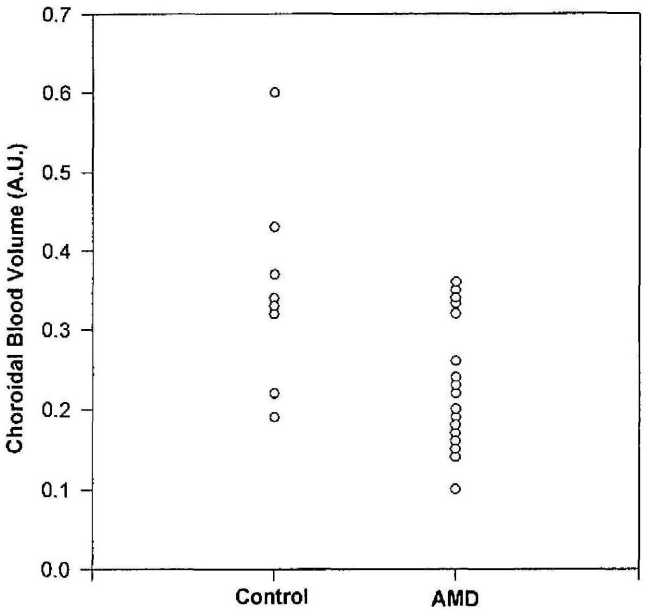

Figure 2. Relative foveolar choroidal blood volume (ChB_{Vol}) in arbitrary units (AU) for age-related macular degeneration (AMD) subjects and control subjects.

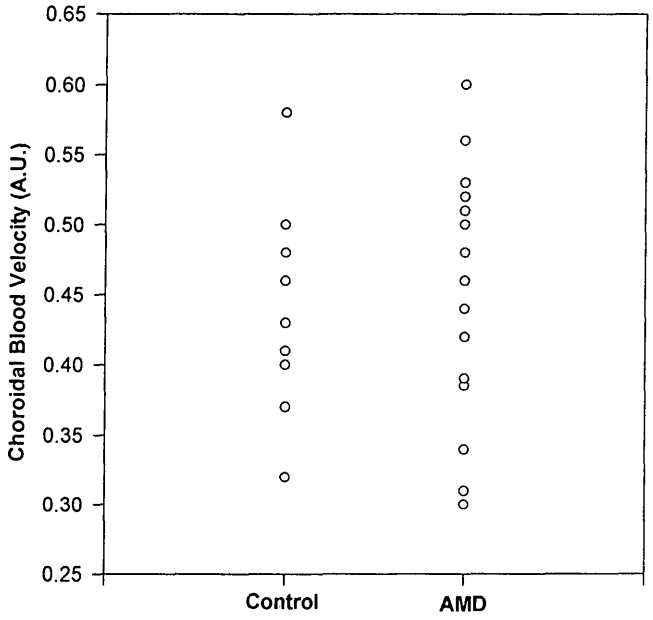

FIGURE 3. Relative foveolar choroidal blood velocity (ChB_{Vel}) in arbitrary units (AU) for age-related macular degeneration (AMD) subjects and control subjects.

jects who had exudative AMD in the fellow eye and those study eyes of subjects who did not have exudative AMD in the fellow eye. No strong conclusions regarding possible differences between these two subgroups can be made, however, because the number of subjects is small.

No significant correlations were observed between systemic blood pressure, IOP, or PP, and any of the circulatory parameters. A negative correlation of borderline significance was observed between ChB_{Flow} and age in normal subjects ($R = -0.59$; $P = 0.07$) but not in subjects with AMD ($R = 0.05$; $P = 0.84$).

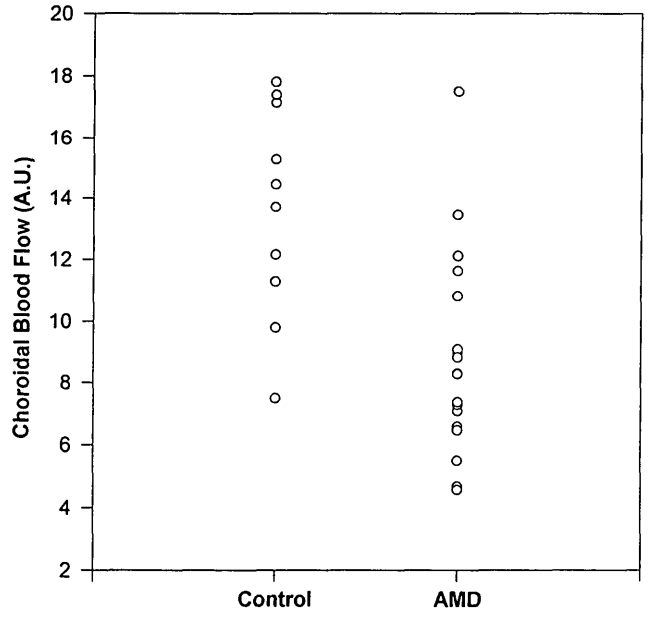

FIGURE 4. Relative foveolar choroidal blood flow (ChB_{Flow}) in arbitrary units (AU) for subjects with age-related macular degeneration (AMD) and control subjects.

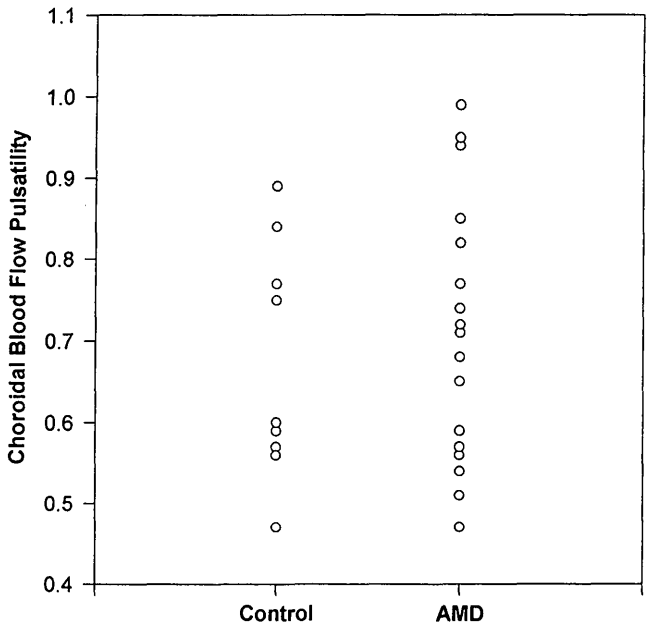

FIGURE 5. Blood flow pulsatility for subjects with age-related macular degeneration (AMD) and control subjects.

DISCUSSION

Our results suggest that ChB_{Flow} is reduced 37% in subjects with the nonexudative stages of AMD. This reduction is primarily caused by a 33% decrease in ChB_{Vol}, because ChB_{Vel} does not seem to be affected. These results are in agreement with those of the following studies that suggest AMD related choroidal blood flow abnormalities in a mostly qualitative and indirect way.

Pauleikhoff et al.[6] and Boker et al.[7] reported choroidal perfusion abnormalities on fluorescein angiograms of subjects with AMD. These abnormalities may represent decreased blood flow or increased fluorescein blockage from AMD-related conditions. Sarks et al.[8,9] reported a reduction in the cross-sectional area of choriocapillaries in AMD. Chen et al.[10] described areas of delayed choroidal perfusion on fluorescein angiography that were associated with decreased visual function, suggesting a role for choroidal vascular impairment in the development of AMD.

Holz et al.[11] reported that slow choroidal filling on fluorescein angiography is a significant risk factor for the development of geographic atrophy of the retinal pigment epithelium in subjects with AMD, suggesting that ischemia plays a role in the etiology of this condition. Prunte and Niesel[12] performed indocyanine green videoangiography in five subjects with dry AMD and found increases in arterial filling time that suggested choroidal blood flow decreases.

Friedman et al.[13] reported that subjects with AMD have more scleral rigidity than age-matched subjects without this condition. In a more recent article, Friedman et al.[14] also found, using color Doppler imaging, that blood velocity is decreased and blood pulsatility is increased in the central retinal artery and short posterior ciliary arteries of subjects with AMD. Based on these findings, they proposed that AMD is associated with an increase in the resistance of the choroidal vasculature caused by a decrease in compliance of the sclera and the choroidal vessels.

Although caution must be exercised when extrapolating inferences about choroidal vascular resistance and blood flow

from extraocular blood velocity and pulsatility measurements, these results raise the possibility of a decreased choroidal blood flow that could hinder the normal metabolism of the macula by impeding a normal supply of metabolites and the removal of waste products. Our study provides, for the first time, quantitative evidence of a decrease in foveolar choroidal blood volume and flow that is similar to that of the reports mentioned above.

Results suggesting decreases of the retinal[19,20] and choroidal[21,22] macular circulations with increasing age have also been reported in normal eyes. These decreases in flow are consistent with morphologic changes that occur in the normal aging retina and choroid.

Ramrattan et al.,[23] for example, showed that there is a 45% decrease in the density and a 34% decrease in the lumen of the normal human macular choriocapillaries between the first and the tenth decades. In addition, a number of age-related changes also occur in the retina, such as decreases in photoreceptor density,[24] cytoplasm volume in retinal pigment epithelial cells,[25] and the number of cells in the ganglion cell layer.[26]

It is impossible to determine, at this time, whether these age-related decreases in the retinal and choroidal cellular components result in a decreased metabolic demand and, consequently, a decrease in blood flow or whether the decreased blood flow that occurs with aging is the primary factor that leads to the decrease in retinal and choroidal cellular components.

Our results suggest that choroidal blood flow in nonexudative AMD is decreased below normal, primarily because of a decrease in blood volume. In searching the literature for AMD-related structural, choroidal vascular changes that could explain these results, we have found discrepant reports. In addition, we have realized that, in most of these reports, it is difficult to separate the effects of normal aging from the effects of AMD, and it is difficult to differentiate the early AMD vascular changes from the late changes caused by geographic atrophy and disciform scarring.

Reports of obliteration of choriocapillaries in the macular area,[27] narrowing of the lumen and loss of the cellularity of choriocapillaries,[28] and thinning of the choroid, especially the choriocapillaries layer,[8] provide evidence of AMD-related morphologic changes that could explain the decreases in choroidal blood volume and flow observed in our study.

On the other hand, Spraul et al.[29] reported an increased choriocapillary density in the submacular area of eyes with advanced AMD, a finding that is inconsistent with that of other studies and our results. In the same study, however, they found that eyes with AMD display fewer large choroidal vessels than eyes without AMD, which is consistent with our results and which suggests that choroidal blood flow is decreased in AMD.

Our results showing a negative correlation of borderline statistical significance between ChB_{Flow} and age in normal subjects ($R = -0.59$; $P = 0.07$) are similar to those of a previous study.[21] The lack of such a correlation in subjects with nonexudative AMD could be a result of disease-related alterations in ChB_{Flow} that are greater than those produced by age.

In our study, we have included subjects with systemic hypertension because this condition is an important risk factor for AMD and is prevalent in subjects with AMD.[1,30] To exclude any confounding effect of systemic hypertension on our measurements, however, we also performed a separate analysis that excluded subjects with AMD and control subjects with a history of hypertension. This analysis showed a decrease in ChB_{Flow} in subjects with AMD similar to that seen in the analysis of all subjects.

Laser Doppler flowmetry provides measurements of relative blood velocity, volume, and flow. When comparing measurements obtained among different persons, there are factors that may introduce measurement variability. It is unknown, for example, how structural changes, such as the thickening of the Bruch's membrane[8] and the increase in basal laminar deposits,[29] may affect the intensity and coherence of the laser light reaching the choroidal vasculature.

One hypothetical source of variability could result from an AMD-related change that would reduce the intensity of the laser light reaching the choriocapillaries and, therefore, would decrease the penetration of light into the choroid, perhaps yielding a smaller blood volume and flow. Our results, showing a tendency toward an increase in flow pulsatility in AMD, suggest that this scenario is unlikely. A more shallow penetration of light would not reach as far into the deeper and larger choroidal vessels that have greater pulsatility. This would result in a smaller, not greater, pulsatility in AMD.

Another factor that could influence our measurements is the amount of pigmentation in the fundus. A previous study by Riva et al.[15] in normal subjects did not show any association between pigmentation and ChB_{Flow}. Furthermore, because all our study subjects were white, the variation in the amount of pigmentation was not large.

Possibly, a decrease of pigment at the site of measurement, resulting from a thinned retinal pigment epithelium over drusen, could affect our measurements. A comparison of the data of 11 eyes that had large drusen within the foveola with those of 9 eyes that did not have such drusen did not show significant differences in any of the circulatory parameters. This suggests that the presence of drusen material, changes in the amount of pigmentation at the site of measurement, or both, does not have a major effect on our flow determinations.

Previous studies[8,27-29] have described AMD-related morphologic changes in the choroidal vasculature that provide a structural basis for our findings of decreased blood volume and blood flow in this disease and support our conclusion that abnormal blood flow is present in the nonexudative stages of AMD. Further studies are needed to elucidate whether this abnormal choroidal blood flow results in ischemia of the macula, which may play a role in the etiology of AMD, and whether quantitative measurements of choroidal blood flow may help identify subjects who have an increased risk for choroidal neovascularization.

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