Reduced Foveolar Choroidal Blood Flow in Eyes with Increasing AMD Severity

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Purpose. In an earlier study, the authors reported that foveolar choroidal blood flow (ChBFlow) decreases in patients with AMD and drusen. To explore further the choroidal circulatory changes in patients with AMD, the relationship between ChBFlow and fundus features associated with increased risk of choroidal neovascularization (CNV) were investigated.

Methods. The study included 26 control eyes of 17 normal subjects and 163 eyes with early AMD characteristics of 123 patients with AMD. The AMD study eyes were divided into three groups according to increasing risk for development of CNV: (1) drusen ≥63 μm, no RPE hyperpigmentary changes in the study eye, and no CNV in the fellow eye; (2) drusen ≥63 μm, RPE hyperpigmentary changes in the study eye, and no CNV in the fellow eye; and (3) eyes with CNV in the fellow eye. Laser Doppler flowmetry was used to assess relative foveolar choroidal blood velocity (ChBVel), volume (ChBVol), and flow (ChBFlow). Differences in the mean circulatory parameters were assessed by analysis of variance (ANOVA) and test of linear trend.

Results. Mean ChBVel, ChBVol, and ChBFlow decreased with increased risk for CNV (linear trend, $P < 0.05$). The lowest circulatory parameters were observed in the eyes with the highest risk for CNV development. Trends for ChBVel and ChBFlow were still significant after adjustment for multiple factors.

Conclusions. There is a systematic decrease in choroidal circulatory parameters with an increase in the severity of AMD features associated with risk for the development of CNV, suggesting a role for ischemia in the development of CNV. (*Invest Ophthalmol Vis Sci.* 2005;46:1033–1038) DOI:10.1167/iovs.04-1050

Age-related macular degeneration (AMD) is the leading cause of blindness in people over the age of 65 in the United States and Western Europe.[1,2] One of the main events causing severe visual loss in AMD is the development of choroidal neovascularization (CNV) through an angiogenic process. Throughout the body, angiogenesis is often triggered by ischemia and hypoxia.[3,4]

A previous report from our group[5] has suggested that foveolar choroidal blood flow (ChBFlow) decreases in patients with AMD and large drusen. Although our preliminary measurements showed marked decreases in the circulation of the choroid but not necessarily ischemia and hypoxia, an intriguing possibility is that this reduction in ChBFlow could lead to ischemia and hypoxia and could play a role in the development of CNV. This hypothesis is supported by the deposition of materials and thickening of the RPE-Bruch's membrane complex that occur in AMD. This process, which may impede the diffusion of substances,[6] would increase the distance that oxygen must travel from the choriocapillaris to the photoreceptors, further reducing the availability of oxygen in the outer retina, as suggested by Linsenmeier and Padnick-Silver.[7]

Three recent studies in patients with asymmetric AMD disease have indeed suggested that ocular circulatory abnormalities (described in detail in the discussion section) may have a role in the development of CNV.[8–10] In addition, preliminary work of Ross et al.[11] has shown an association between the location of the macular choroidal watershed vascular filling zones detected by fluorescein angiography and choroidal neovascular membranes. During angiography, watershed filling zones correspond to the last areas of the choroid that fill with the dye. These areas correspond most probably to the boundaries between adjacent choroidal lobules. The presence of CNV in proximity to these areas that are the most prone to development of ischemia and hypoxia in case of a decrease in ChBFlow suggests indeed that ischemia may have a role in the development of AMD-related CNV.

The purpose of this investigation was to assess the association between ChBFlow and the presence of specific AMD fundus features that are known to be associated with an increased risk of CNV.

Methods

One hundred sixty-three eyes of 123 patients with AMD, visual acuity of 20/40 or better, intraocular pressure (IOP) of ≤21 mm Hg, and no other intraocular disease were included in the study. All study eyes had steady fixation, clear ocular media, and pupillary dilation of 5 mm or more. Patients with diabetes mellitus, high myopia (>7 D), previous periorbital or ocular radiation, and previous treatment with macular-toxic drugs were excluded from the study. All enrolled patients had ophthalmoscopic features typical of AMD. The ages of these patients ranged from 51 to 86 years (mean ± SD, 72 ± 8). Other characteristics are summarized in Table 1. Fifty-two patients had systemic hypertension (defined as having a history of elevated blood pressure). Forty-seven of these 52 were receiving antihypertensive therapy.

The eyes of patients with AMD were divided into three groups according to their ophthalmoscopic AMD features associated with increased risk of CNV (Table 2). Group 1 included 56 eyes of 43 patients who had drusen larger than 63 μm and no retinal pigment epithelium (RPE) hyperpigmentary changes in the study eye and no CNV in the fellow eye. Group 2 included 88 eyes of 61 patients with drusen larger than 63 μm and RPE hyperpigmentary changes in the study eye and no CNV in the fellow eye. Group 3 included 19 eyes of 19 patients who had drusen larger than 63 μm in the study eye and

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TABLE 1. Characteristics of Control Subjects and Patients with AMD

	Control	AMD 1	AMD 2	AMD 3	*P**
Age (y)	66.5 (9.70)	71.1 (8.58)	71.7 (8.22)	73.6 (7.62)	0.08
Mean blood pressure (mm Hg)	92.2 (12.4)	93.1 (14.6)	99.8 (13.1)	102.2 (18.1)	0.02
Intraocular pressure (mm Hg)	13.8 (2.94)	15.1 (3.17)	14.8 (3.03)	14.6 (2.94)	0.53†
Perfusion pressure (mm Hg)	46.9 (7.96)	46.8 (9.65)	51.2 (8.20)	54.7 (10.6)	0.01†
Refractive error (D)	−0.52 (2.28)	0.84 (2.68)	0.41 (2.13)	1.24 (2.13)	0.12†
Male/female ratio	7/10	21/22	30/31	6/13	0.55
Hypertensive status (yes/no) (*n*)	10/7	17/26	19/42	16/3	0.0003
Current cigarette smoking (yes/no) (*n*)	1/16	5/38	4/57	1/18	0.82

Data are expressed as the mean (SD).

* For comparisons between the four groups by one-way ANOVA for continuous measurements (age, blood pressure, intraocular pressure, perfusion pressure, and refractive error), and by Fisher exact test for categorical measurements (gender, hypertension status, and current smoking status). $P < 0.05$ suggests statistically significant differences between the groups.

† The GEE approach was used to adjust for the correlation in measurements between paired eyes of the same subject.

CNV in the fellow eye. Thirteen of these 19 study eyes also had RPE hyperpigmentary changes.

Grading of fundus photographs of control subjects and patients with AMD was performed in a masked fashion by the Fundus Photography Reading Center of the University of Pennsylvania. Grading of drusen characteristics and RPE changes in the study eyes was performed according to the Complications of Age-Related Macular Degeneration Trial protocol (unpublished Manual of Procedures, CAPT Coordinating Center, Philadelphia, PA). The presence of CNV in the fellow eye was determined from fundus photographs and/or clinical examination by a masked observer.

Results in these patients were compared with those of 26 eyes of 17 subjects with no drusen larger than 63 μm, visual acuity of 20/40 or better, IOP of 21 mm Hg or less, and otherwise normal findings in external, slit lamp, and ophthalmoscopic eye examinations. Ages of the control subjects ranged from 51 to 82 years (67 ± 10). Other control subject's characteristics are summarized in Table 1. Ten control subjects had systemic hypertension, and all of them were receiving antihypertensive therapy.

A detailed explanation of the study's purposes and protocol was given to each participant of the study. All subjects were asked to sign an appropriate consent form approved by the human experimental committee of our institution and in compliance with the tenets of the Declaration of Helsinki.

Before the measurements, pupils were dilated with tropicamide 1% (Alcon, Fort Worth, TX) and phenylephrine hydrochloride 10% (Sanofi Winthrop, New York, NY). According to the inclusion and exclusion criteria, blood flow measurements were obtained in one or both eyes of each subject.

Determinations of relative foveolar choroidal blood velocity (ChBVel), volume (ChBVol), and ChBFlow were obtained with the laser Doppler flowmetry (LDF) technique (Oculix Sarl, Arbaz, Switzerland). ChBVel, which is proportional to the mean velocity of the RBCs within the volume sampled by the laser light and ChBVol, which is proportional to the number of RBCs, are independent measurements. ChBFlow is calculated by the instrument from these two parameters according to the following formula: ChBFlow = Constant × ChBVel × ChBVol.[12] Detailed descriptions of the method have been published.[13–16] A diode laser beam (670 nm) with an intensity of 20 mW 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 nm with a retinal irradiance of approximately 0.03 mW/cm^2. This light enabled the observation of the position of the laser on the foveola. Subjects were asked to fixate on the probing laser beam to determine foveolar ChBFlow. Measurements obtained in this fashion correspond mainly to determinations of choriocapillaris flow, as discussed previously by Riva et al.[13]

Proper fixation during the measurements 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, three continuous 30-second measurements of the choroidal circulation were obtained. Analysis of these data was performed by a masked observer using a NeXT computer (Redwood, CA) with software specifically developed for the analysis of Doppler signals from ocular tissues.[14] The masked observer selected for analysis parts of the recordings that showed stable circulatory parameters. On average, approximately 12 seconds of stable measurements were selected for analysis in each eye.

To assess the reproducibility of the blood flow data, we calculated a coefficient of variability (CV) for each study eye derived from three subsequent measurements. CV was calculated using the following formula: CV = (SD/mean) × 100. CV was 10.3% ± 7.2% for ChBFlow.

Brachial artery systolic and diastolic blood pressures (BP_s and BP_d, respectively) were determined by sphygmomanometry (Accutorr 1A; Datascope, Paramus, NJ) after blood flow measurements. IOP was measured by applanation tonometry. The mean brachial artery pressure (BP_m) was calculated according to the following formula: $BP_m = BP_d + \frac{1}{3}(BP_s - BP_d)$. Perfusion pressure (PP) for the study eye was

TABLE 2. Fundus Characteristics of Study and Fellow Eyes in the Control and AMD Groups

	Patients (*n*)	Eyes (*n*)	Fundus Features	
			Study Eye	Fellow Eye
Control	17	26	No drusen > 63 μm	No drusen > 63 μm
AMD 1	43	56	Drusen ≥ 63 μm	No CNV
AMD 2	61	88	Drusen ≥ 63 μm, RPE hyperpigmentation	No CNV
AMD 3	19	19	Drusen ≥ 63 μm, RPE hyperpigmentation	CNV

Bilateral measurements were made in all patients in whom both eyes met the inclusion and exclusion criteria. In approximately half of the subjects, bilateral measurements were obtained, except for Group 3, which had low visual acuity in the fellow eye because of the presence of CNV.

TABLE 3. Mean ChBVel, ChBVol, and ChBFlow in Control Subjects and Patients with AMD

	Control	AMD 1	AMD 2	AMD 3	*P* for linear trend†
ChBVel	0.43 ± 0.02	0.38 ± 0.01	0.38 ± 0.01	0.37 ± 0.02	0.047
ChBVol	0.26 ± 0.02	0.25 ± 0.02	0.23 ± 0.01	0.20 ± 0.01	0.02
ChBFlow	9.80 ± 0.84	7.78 ± 0.56	7.49 ± 0.40	6.54 ± 0.47	0.003

Data are expressed as the mean ± SE in arbitrary units.

† The GEE approach was used to adjust for the correlation between eyes of the same subject. $P < 0.05$ suggests that the circulatory measurements decrease linearly as AMD severity increases.

estimated according to the following formula $PP = \frac{2}{3}BP_m - IOP$. Both of these formulas are commonly used in the microvascular literature.

The average of the three replicates for each circulatory parameter was used in all data analyses. Comparisons of the circulatory parameters among normal control subjects and three AMD groups were performed by using the generalized estimating equation (GEE) approach to linear regression to adjust for the correlation between eyes of the same patient.[17] Calculations were executed on computer (PROC GENMOD; SAS ver. 8.2; SAS, Cary, NC) and specified an exchangeable working correlation structure to describe the correlation in circulatory measurements between eyes.[18] Data from each patient are identified to the computing algorithm by specifying a unique identification number for each patient. For patients with measurements on both eyes, the correlation between eyes is involved in the calculation of standard errors. To test whether the mean circulatory parameters decrease with increasing severity of AMD, a contrast with equally spaced coefficients for the means from the four groups (test of linear trend) was calculated using a contrast statement in PROC GENMOD (SAS) for both univariate analysis (without adjustment of any other covariates) and multivariate analysis to adjust for the effect of other covariates. The covariates were included in the multivariate model when they were found to be significantly associated with circulatory parameters in the univariate model. $P < 0.05$ was considered statistically significant.

RESULTS

Table 3 shows average ChBVel, ChBVol, and ChBFlow in control subjects and in each of the AMD groups. Average ChBVel was 0.43 ± 0.02 arbitrary units (AU), 0.38 ± 0.01, 0.38 ± 0.01, and 0.37 ± 0.02, respectively, in control subjects and AMD groups 1, 2, and 3. A systematic decrease in ChBVel with increasing risk of CNV development was detected by linear trend test ($P = 0.047$; Fig. 1). This systematic trend was still significant ($P = 0.02$) for ChBVel after adjustment for age, IOP, perfusion pressure, and refractive error.

Average ChBVol was 0.26 ± 0.02, 0.25 ± 0.02, 0.23 ± 0.01, and 0.20 ± 0.01 AU, respectively in control subjects and AMD groups 1, 2, and 3. A systematic decrease in ChBVol with increasing risk of CNV development was detected by linear trend analysis ($P = 0.02$; Fig. 2). This systematic trend was not significant ($P = 0.11$) for ChBVol after adjustments for age and IOP.

Average ChBFlow was 9.80 ± 0.84, 7.78 ± 0.56, 7.49 ± 0.40, and 6.54 ± 0.47 AU, respectively in control subjects and AMD groups 1, 2, and 3. A systematic decrease in ChBFlow with increasing risk of development of CNV was detected by linear trend analysis ($P = 0.003$; Fig. 3). This systematic trend was still significant ($P = 0.02$) for ChBFlow after adjustment for age and hypertensive status.

DISCUSSION

Our results suggest that foveolar ChBFlow is lower than normal in eyes with AMD, confirming the findings in our previous study.[5] In addition, eyes with more AMD fundus features associated with risk for the development of CNV tend to show more pronounced decreases in ChBFlow. All three circulatory parameters—ChBVel, ChBVol, and ChBFlow— decrease progressively with an increase in the severity of AMD features (linear trend test, $P = 0.047$, 0.02, and 0.003, respectively). The linear trends remain statistically significant for ChBVel after adjustment for age, and for ChBFlow after adjustment for age and hypertension. Such adjustments are important, because increasing age has been shown to be associated with decreased ChBFlow in normal subjects.[19,20] patients with AMD show ChBFlow decreases that are larger than those produced by aging in normal eyes.[5] In addition, within this group, subjects with a history of hypertension have lower choroidal flow than subjects without a history of hypertension.

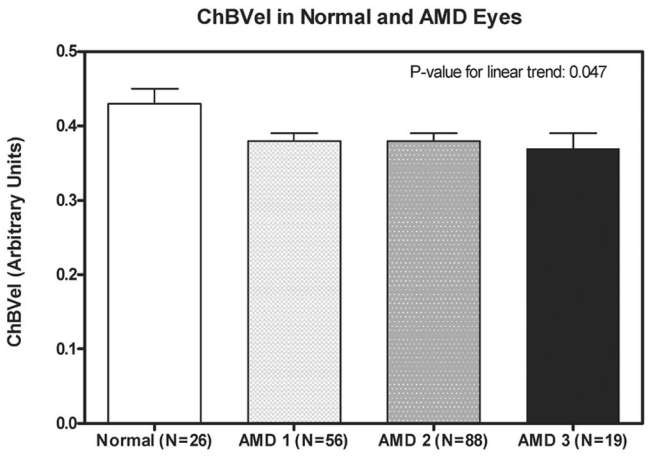

FIGURE 1. Relative ChBVel in the control and AMD groups.

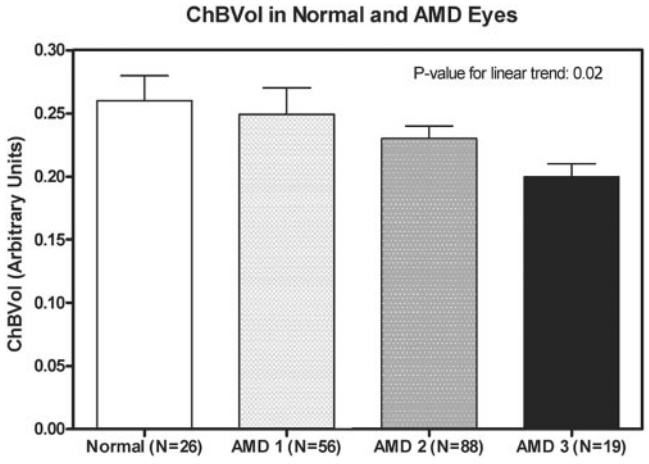

FIGURE 2. Relative ChBVol in the control and AMD groups.

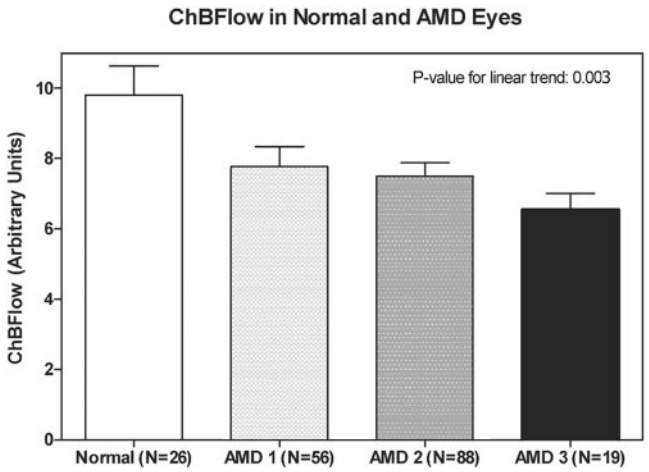

FIGURE 3. Relative ChBFlow in the control and AMD groups.

Multiple studies have investigated the association between various AMD fundus features and the risk of development of CNV. Large drusen, RPE hyperpigmentary changes in the study eye and the presence of CNV in the fellow eye have been shown to be associated with increased risk. Based on these studies we divided our AMD eyes into three groups, according to increasing risk of CNV.

Several studies have assessed the prognostic information of drusen regarding the development of CNV by looking at drusen size, type, confluence, and drusen area.[21–23] Bilateral drusen have been reported to be associated with an increased risk of the development of CNV.[23–26] Patients with bilateral drusen had cumulative risks of CNV development over the period of 5 years that ranged from 14% to 27%.[21,23,25,26]

Smiddy and Fine[25] also reported that the additional presence of focal RPE hyperpigmentation was associated with a 23-fold greater risk of development of an exudative process. Similarly, Leeuwen et al.[27] reported that hyperpigmentation tripled the risk of AMD in subjects with drusen within a 5-year period. In other studies, the 5-year cumulative risk for development of CNV in eyes with both large drusen and RPE changes ranged between 7.1% and 48%.[21,26,28]

Several studies have also shown that patients with CNV in one eye have the highest risk for development of CNV in the second eye.[23,27,30] The annual rates vary from 0.6% in a Japanese study[29] to 18% among Americans.[31] This is indeed the type of patient included in group 3 of our study. These patients with the highest risk for CNV showed the lowest ChBFlow of all groups studied, a finding that strongly suggests that decreased flow and ischemia may play a role in the development of CNV in AMD, in the same way that ischemia may trigger neovascularization in other tissues of the body.[3,4]

Our results showing the lowest flow in patients with CNV in the fellow eye are in accord with several studies that have suggested lower ChBFlow in AMD eyes with CNV. Mori et al.[9] reported that pulse amplitude in patients with exudative AMD is lower than in patients with nonexudative AMD and in age-matched control subjects. Chen et al.[8] showed that pulse amplitude was significantly decreased in eyes with disciform scarring in comparison to the contralateral eyes with drusen. Although the relationship between pulse amplitude and ChBFlow has not been clearly established, these results suggest that perhaps ChBFlow decreases in AMD eyes with CNV.

Using color Doppler imaging, Uretmen et al.,[10] reported decreased ophthalmic artery and temporal posterior ciliary artery velocities in eyes with CNV in comparison to eyes with nonexudative AMD, and Rigas et al. (*IOVS* 2004;45:ARVO E-Abstract 3110) found an increased resistance to blood flow in eyes with neovascular AMD, suggesting decreased ChBFlow. Dimitrova,[32] however, compared both eyes of patients with unilateral neovascular AMD and did not find any significant differences in resistance between neovascular and non-neovascular AMD.

Our findings showing decreased ChBFlow in AMD eyes also fit well with previous histopathologic studies. Sarks[33] and Sarks et al.[34] have shown reduction in the cross-sectional area of the choriocapillaris in AMD. These data are also in agreement with Ramrattan et al.,[35] who demonstrated that the density and diameter of the macular choriocapillaris decreases in AMD in comparison to normal control subjects, the report of Korenzweig[36] showing narrowing of the lumen and loss of cellularity of the choriocapillaris in AMD, and the data of Arnold et al.[37] and Spraul et al.,[38] suggesting the loss of choroidal veins in "reticular pseudodrusen."

Our results and these histopathologic findings are also in accord with evidence of choroidal perfusion abnormalities in AMD observed by investigators using different techniques. Pauleikhoff et al.[39] and Boker et al.,[40] reported choroidal perfusion abnormalities on fluorescein angiograms of patients with AMD. Chen et al.[41] described areas of delayed choroidal perfusion that were associated with decreased visual function in patients with AMD. Holz et al.[24] showed that slow choroidal filling is a significant risk factor for the development of geographic atrophy.

More recently, Pauleikhoff et al.[42] reported that prolonged filling of the choroidal lobules in the early phases of fluorescein angiography, and reduced choroidal fluorescence on indocyanine angiography are common features in eyes with early AMD. Using color Doppler imaging, Friedman et al.,[43] showed that blood velocity decreases and blood velocity pulsatility increases in the central retinal artery and short posterior ciliary arteries in AMD. Based on some of these findings, they propose that AMD may be associated with an increase in the resistance of the choroidal vasculature caused by a decrease in the compliance of the sclera and the choroidal vessels.[43] Using color Doppler imaging, Ciulla et al.[44] have also shown decreased blood velocities in the retrobulbar vasculature. In a different study using scanning laser ophthalmoscopy and indocyanine green angiography, Ciulla et al.[45] reported increased heterogeneity of choroidal filling time in patients with nonexudative AMD.[45] All these findings, which are in agreement with our results, strongly suggest that vascular impairment and ischemia play an central role in the etiology of AMD.

Disturbances in the choroidal circulation just mentioned could hinder the normal diffusion of substances and gasses across the RPE-Bruch's membrane complex, which has a crucial role in visual function. This disruption could lead to a situation in which waste materials may not be readily removed and crucial metabolites and gasses may not be adequately supplied to the neural retina. Because the choroidal circulation is the only source of nourishment and waste removal for the outer retina, particularly in the foveola, any alterations in choroidal circulation could be very deleterious.

Because the choroidal circulation decreases with age[19] in the normal eye and because this decrease is further exacerbated by AMD,[5] it is possible that the removal of waste products from the RPE-Bruch's membrane complex may become impaired in AMD. This process could be part of the mechanism that leads to drusen accumulation in this disease.

Our results show an association between decreased ChBFlow and increased severity of AMD. From our results, however, we cannot conclude whether these decreases have a role in the development of AMD. We also cannot exclude the possibility that the decreases in choroidal flow may be related to the loss of photoreceptors that occurs in AMD. As photore-

ceptors are lost, the declining demand for oxygen from the choroid could result in decreased blood flow.

The laser Doppler flowmetry technique provides measurements of relative blood velocity, volume, and flow. Because changes in the intensity and coherence of the laser light produced by AMD can theoretically affect the hemodynamic measurements, the comparison of LDF relative blood flow measurements between normal and AMD eyes is open to question.[5] Our results showing that ChBVel decreases with increased risk of CNV support our contention that ChBFlow is decreased in this disease because ChBVel measurement are less affected by changes in the media than ChBVol and ChBFlow measurements. That other histopathologic and circulatory studies have also shown evidence of decreased circulation in AMD further supports our findings.

In summary, our present study suggests that ChBFlow decreases with increase in the severity of AMD features, pointing to a potential role for ischemia in the development of CNV. We cannot conclude from our study, however, whether this decrease in flow triggers the development of CNV. Further studies are necessary to reach a strong conclusion as to whether this association between AMD fundus features and degree decrease in ChBFlow may help identify patients with AMD at risk for visual loss.

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