Foveal Cone–Photoreceptor Integrity in Aging Macula Disorder

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PURPOSE. To establish the relation between AMD stage and a quantitative measure for the integrity of foveal cone photoreceptors related to the optical Stiles-Crawford effect.

METHODS. Fifty-six AMD eyes and 57 control eyes were included in the final analysis. AMD was graded in accordance with the International Classification System into five mutually exclusive stages. Stages 0 to 1 were labeled no AMD, stages 2 to 3 were labeled early AMD, and stage 4 was labeled late AMD. Fundus reflectometry, together with a model-fit procedure, provided information on directional cone reflectance (Rd), a quantitative measure for the integrity of foveal cone photoreceptors. Optical densities of macular pigment (MPOD) and melanin (MOD) were also obtained. A general linear model analysis was used to compare Rd, MPOD, and MOD among the AMD stages.

RESULTS. Mean Rd was lower in early AMD (0.92%, P < 0.001) and late AMD (0.86%, P < 0.001) compared with mean Rd in the no-AMD stage (1.01%). Mean MPOD was not different in early AMD (0.53, P = 0.05), but it was lower in late AMD (0.19, P < 0.001) compared with mean MPOD in the no-AMD stage (0.42). Mean MOD was lower in early (1.09, P = 0.001) and late (1.01, P = 0.004) AMD compared with mean MOD in the no-AMD stage (1.25).

CONCLUSIONS. Foveal cones show signs of misalignment and/or outer segment deterioration in early AMD. Melanin rather than macular pigment may play a protective role against AMD, although loss of these ocular pigments can also be caused by AMD. (Invest Ophtalmol Vis Sci. 2008;49:2077–2081) DOI: 10.1167/iovs.07-1181

In 1933, the British researchers Stiles and Crawford1 reported that light entering the human eye at the center of the pupil was several times more effective in producing the sensation of vision than light entering near the pupil margin. The physiological explanation of this phenomenon, later called the Stiles-Crawford Effect (SCE), is that cone photoreceptors yield a directional sensitivity. Waveguide properties of cone photoreceptor inner segments, guiding the light to the outer segment photopigments, optimize absorption of axial incident light rather than off-axis light. The optical equivalent of this physical effect is called the optical SCE. A small fraction of the incident light is reflected back toward the pupil. In a healthy retina, more light is reflected toward the middle of the pupil, where most cone photoreceptors are aimed. In disease, the optical SCE is a sensitive indicator of cone photoreceptor disturbances.2

Aging macula disorder (AMD), as we now prefer to call age-related macular degeneration,3 is a degenerative disease primarily affecting the macula and an increasingly prevalent cause of irreversible blindness in the industrialized world.4–9 Early AMD is characterized by drusen and pigmentary abnormalities with relatively few visual symptoms. However, in its late stage, AMD often leads to a disabling central scotoma.

It is known that drusen disturb the orderly alignment of overlying cone photoreceptors.4,10 The retinal pigment epithelium (RPE) supplies the photoreceptors with nutrients and maintains the integrity of the subretinal space.11 Thus, RPE changes may also affect the optical quality of the involved cone photoreceptors.12 Several electrophysiological and psychophysical studies aimed at examining visual function in early AMD have found disturbances in light sensitivity and in adaptation throughout the retina regarding both cone and rod photoreceptors.13–18

A recently developed device, the Foveal Reflection Analyzer,19 simultaneously measures cone photoreceptor directionality and foveal spectral reflectance in a few seconds. Directionally reflected light from the foveal cones relates to the optical quality of these photoreceptors. In addition, a procedure of model-fit to the spectral reflection20 provides information on the optical densities of ocular absorbers: lens, macular pigment (MPOD), melanin (MOD), and blood.

This study was primarily conducted to establish the relation between the stage of AMD and a quantitative measure for the integrity of foveal cones related to the optical SCE. Because MPOD and MOD were also available, we evaluated the distribution of the optical densities of these pigments in the different AMD stages, looking for possible protective effects.

METHODS

Participants

The AMD patients who participated in the study were obtained from two sources. One group (n = 25) was found by searching the fundus photography and fluorescence angiography database of the Ophthalmology Department of the University Medical Center (UMC) Utrecht for the presence of drusen and/or pigmentary alterations on fundus photographs or fluorescence angiograms (FAs) performed within the past 3 years. The other group (n = 15) consisted of patients with AMD who visited the outpatient clinic of the Ophthalmology Department of the UMC Utrecht. Only patients older than 55 years who were able to give informed consent were eligible. In addition, these patients had to have diagnosed AMD in at least one eye and/or the presence of drusen and/or RPE changes in at least one eye on earlier performed FA and/or fundus photograph, or the presence of atrophic or neovascular AMD in one eye on earlier performed FA and/or fundus photograph. Patients with known diabetes mellitus or an ophthalmic history other than...
AMD or intraocular lens (IOL) implantation were excluded. We also excluded eyes with a best corrected visual acuity (BCVA) lower than 0.2, to assure stable fixation. Eyes of which a newly made fundus photograph was ungradable (e.g., missing, low quality) or on which retinal disease other than AMD was seen were also excluded. Altogether, 73 eyes of 40 patients with AMD were included.

A group of participants with eyes with no AMD were recruited through an advertisement in a local newspaper. Only persons older than 18 years who were able to give informed consent and who had no diabetes mellitus or any ocular history except IOL implantation were eligible for inclusion. These persons were excluded if BCVA was lower than 0.8 and if the newly made fundus photograph was ungradable or any retinal disease was seen on it. As a routine, only the right eye was measured in these participants. Finally, data from 45 right eyes of 45 healthy subjects were available. Thus, 118 eyes of 85 subjects were measured in these participants. Finally, data from 45 right eyes of 45 patients with AMD were subsequently excluded (see Measurement of Optical SCE, MPOD, and MOD in this section).

In patients with AMD and healthy subjects, refractional status of the study eye(s) was obtained with an autorefractometer (Auto Refractometer Speedy-K; Nikon Corp., Tokyo, Japan). With these data, BCVA was determined with an ETDRS (Early Treatment of Diabetic Retinopathy Study) chart at 4 m. Pupils were dilated with tropicamide 0.5% and phenylephrine 5%. In mydriasis, five fundus reflectance measurements were made in the study eye(s) at the specific pupil plane position with highest reflectance (i.e., the position of the optical SCE maximum). Digital stereoscopic 30° color fundus photographs were then made (FF 450 Plus fundus camera; Carl Zeiss Meditec AG, Oberkochen, Germany).

The study protocol adhered to the tenets of the declaration of Helsinki and was approved by the local medical ethics committee. Written informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

### AMD Definition

Digital fundus photographs of the macular area were graded according to the International Classification System for ARM and AMD (ICSMAD) by one of the two professional graders from the Rotterdam Study as having disease severity of one of five mutually exclusive stages, 0 to 4.

In the present study, we deviated from the ICSMAD: all age-related maculopathy (ARM) was called AMD. Because the limited number of eyes in each stage, AMD was divided into early and late AMD: stages 0 and 1 were combined as no AMD, stages 2 and 3 were early AMD, and stage 4 was late AMD. A similar AMD classification has been used in other studies. Note, that grading was the only criterion used for classification. Thus, 14 eyes of the patient group ended up in the no-AMD group; none of the recruited healthy eyes had AMD. Because the optical model fitted the data very badly in all measurements of two eyes with no AMD, in one eye with early AMD, and in two eyes with late AMD (see Measurement of Optical SCE, MPOD, and MOD in this section), these eyes were finally excluded. AMD stage definitions, the currently used classifications, the final number of eyes/subjects, and mean ages have been summarized in Table 1.

### Measurement of Optical SCE, MPOD, and MOD

A prototype Foveal Reflection Analyzer (FRA) was detailed by Zagers et al. The present version was recently described by van de Kraats and Van Norren. Briefly, a halogen lamp (12V, 30 W, Woton 64260; Osram, Munich, Germany) illuminated a 1.8° spot on the fovea. Refraction errors were compensated by adjusting a Badal type front lens system. The light was spectrally filtered (6 mm BG26 filter; Schott AG, Mainz, Germany) and compensated by adjusting a Badal type front lens system. The light was then filtered and used for the foveal retina. Spectral data were evaluated with an updated version of the original Van de Kraats fundus reflectance model. The model describes the spectral aspects of light reflected from the fundus for all positions in the pupil profile (corresponding to angles at the retina), using a limited number of absorbing and reflecting layers. In short, the incoming light is thought to be reflected at the inner limiting membrane, at the discs and the outer segments of the cone photoreceptors (Rd), at the pigment epithelium, and at the choroid. Known spectral characteristics of the different absorbers in the eye (lens, MP, melanin, and blood) were used to optimize the density of the absorbers and the reflectance at the interfaces to fit the measured data. The recent version of the model simultaneously fits the optical SCE from the cone photoreceptors and the non-diffused reflection from more posterior layers in an extended wavelength range of 400 to 950 nm. In addition, the model incorporates the most recent spectral shapes of lens absorption templates. MPOD is fitted by using the absorption curves for zeaxanthin and lutein published by Handelman et al. The relative contributions of zeaxanthin and lutein to central MP are set at 70% and 30%, respectively. For the blood layer, a linear thickness gradient is used.
FiguRe 1. Typical spatial and spectral reflection curves of a healthy subject and a patient with early AMD. (A) Pupil profile at 540 nm of a 64-year-old healthy male (triangles) compared to a patient profile of a 66-year-old woman with early AMD (squares). The pupil profile of the healthy subject shows a Gaussian-shaped reflection originating from the foveal cone photoreceptors (~optical SCE), on a diffuse background. In the patient with early AMD, only a nondirectional diffuse background reflection was seen. (B) Spectral reflection curves from the same subjects as described in (A), measured on top of the optical SCE. At the short wavelengths, reflection was very low because of the absorption in the aging lens. At ~460 nm, the macular pigment reduced reflection. At longer wavelengths (>550 nm) reflection was seen to increase because of decreasing absorption by melanin and blood. At still longer wavelengths, water absorption reduced reflection.

Instead of a homogeneous layer of constant thickness, mimicking the range of pathways through the center or edge of small and large blood vessels. This thickness gradient varies from 0 to a certain maximum value, found with the model fit procedure. To use the model fit in advanced AMD (with noisy, or absent SCE), we made it more robust by fixing a number of non–age-dependent parameters. This involved ρ, a measure of how steep the optical SCE in the pupil plane, set at a mean value of 0.149 based on data of 102 healthy subjects.20 In addition, two parameters of the eye media that showed minimal losses was set at 0.58.20 The fundus reflectance model also provided information on the goodness of fit of each measurement by means of aχ2 value. The mean χ2 value ± SD of the healthy subjects was 13.2 ± 6.6. This was 24.9 ± 57.6 for the patients with AMD, indicating more noisy measurements in this group, mainly caused by spatially irregular lens densities together with AMD-related foveal disturbances. To limit the number of parameters generated by data that showed a very bad fit, measurements with a χ2 exceeding 53.1 (mean of normal subjects ± 3 SD) were discarded. This resulted in the loss of 1 of 230 healthy subject measurements (0.4%) and 55 of 388 AMD patient measurements (14.2%). In five eyes, all measurements were thus discarded. After this correction, the mean χ2 ± SD was 13.0 ± 6.0 for the healthy control subjects and 12.7 ± 5.6 for the patients with AMD.

The main parameter in this study was Rd, the reflection at the cone photoreceptor layer. To avoid confusion about the origin of this reflection (i.e., discs in outer segments of foveal cone photoreceptors, rather than the optic disc) the term Rdisc of the original model20 was changed to Rd. Likewise, the other two model parameters of interest were called MPOD and MOD, rather than dMP and dMEL.

Statistical Analysis

Because both eyes of several patients with AMD were used, we clustered them (Complex-Samples feature; SPSS; SPSS, Chicago, IL). For because both eyes of several patients with AMD were used, we clustered them (Complex-Samples feature; SPSS; SPSS, Chicago, IL). For those reasons we also used the complex-samples’ general linear model (GLM) analysis to compare Rd, MPOD, and MOD between the different AMD stages. Since Rd strongly varies with age,29 we used age as a covariate in analyzing the effect of AMD classification on this parameter. In a recent study,20 MOD showed no decrease with age. Because some other studies30–32 point to a slight decrease in melanin with age, we also used age as a covariate for this parameter. MPOD is not thought to change with age. Analysis of variance, together with a post hoc Scheffé test, was used to test for differences in age between no, early, and late AMD. All statistical analyses were performed with commercial software (SPSS for Windows, release 15.0) by SPSS.

RESULTS

Examples of single measurements of a healthy 64-year-old eye and a typical early AMD eye of similar age are presented in Figure 1A. Such measurements take about 1 second each. The optical SCE in the healthy eye showed a Gaussian-shaped light output across the pupil. The distribution in the early-AMD eye was essentially flat, except where the pupil edge cut off the light. The spectral reflection of these eyes is shown in Figure 1B. At short wavelengths, reflection was very low because of absorption in the aging lens and the macular pigment. At longer wavelengths (>550 nm), reflection was increased by decreasing absorption of melanin and blood and finally was decreased again by the absorption of water.

In Table 2, mean BCVA, Rd, MPOD, and MOD are compared between the different AMD stages. To investigate whether the FRA can detect foveal cone photoreceptor disturbances very early in the assumed process of AMD, we compared Rd in stage 0 AMD with Rd in stage 1 AMD, after having corrected for age and the (infrequent) use of two eyes of the same subject (not shown in Table 2). Stage 0 AMD contained 43 eyes of 42 subjects; mean eye age ± SD was 48.5 ± 16.4 years. Stage 1 AMD contained 14 eyes of 12 subjects with a mean age ± SD of 71.3 ± 8.55 years. After correcting for age and the use of both eyes in two subjects, Rd

<table>
<thead>
<tr>
<th>AMD Stage</th>
<th>LogMAR BCVA (95% CI)*</th>
<th>P</th>
<th>Rd % (95% CI)†</th>
<th>P</th>
<th>MPOD (95% CI)*</th>
<th>P</th>
<th>MOD (95% CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AMD</td>
<td>0.00 (−0.06–0.05)</td>
<td>Ref.</td>
<td>1.76 (1.53–1.99)</td>
<td>Ref.</td>
<td>0.42 (0.37–0.46)</td>
<td>Ref.</td>
<td>1.23 (1.17–1.30)</td>
<td>Ref.</td>
</tr>
<tr>
<td>Early AMD</td>
<td>0.14 (0.08–0.20)</td>
<td>&lt;0.001</td>
<td>0.92 (0.74–1.11)</td>
<td>&lt;0.001</td>
<td>0.53 (0.43–0.63)</td>
<td>0.05</td>
<td>1.09 (1.03–1.14)</td>
<td>0.001</td>
</tr>
<tr>
<td>Late AMD</td>
<td>0.71 (0.49–0.93)</td>
<td>&lt;0.001</td>
<td>0.86 (0.55–1.18)</td>
<td>&lt;0.001</td>
<td>0.19 (0.09–0.29)</td>
<td>&lt;0.001</td>
<td>1.01 (0.86–1.15)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Estimated means of the different parameters are presented together with their 95% CIs. Standard errors are adjusted by clustering eyes of individual subjects.
† Additional adjustment for age, because Rd29 and, to a lesser extent, MOD30–32 show a decrease with age. MPOD is not thought to change with age.33
(95% CI) was 1.84% (1.53–2.16) in stage 0 AMD and 1.60% (1.18–2.03) in stage 1 AMD. This difference was not significant (P = 0.41), nor were the differences in MPD (P = 0.62) and MOD (P = 0.45).

Our dataset contained only 11 eyes with late AMD, of which 5 eyes had the atrophic form and 6 eyes had the neovascular form. After correction for age and the use of both eyes of one subject, Rd (95% CI) was 0.38% (0.00–0.87) in atrophic AMD and 0.56% (0.22–0.90) in neovascular AMD (P = 0.54).

**DISCUSSION**

In our study, the amount of directionally reflected light from the foveal cone photoreceptors (Rd) was halved in early AMD compared with that in eyes without AMD. DeLint et al. have argued that the optical SCE may replace cone visual pigment kinetics as a sensitive measure for detecting cone photoreceptor disturbances. Thus, our results are in line with those in a study by Elsner et al., who demonstrated that subjects with tor disturbances. Thus, our results are in line with those in a study by Elsner et al., who demonstrated that subjects with tor disturbances.

Rd). Severe central retinal changes in advanced AMD generate noisy reflections in the spatial dimension that may show up as a limited directional component (~optical SCE). Most, but apparently not all, of these artificial fits were removed on the basis of their higher χ² values (see the Methods section), inhibiting a further decrease in Rd from a certain stage on in advancing AMD. Because the estimation of MPD and MOD depends on both directional and background reflectance, these parameters are far less susceptible to the pathologic changes in late AMD.

We found no significant difference in the MPD between the no-AMD and the early-AMD stages, but a significant decline was seen in late AMD. Whether MP plays a protective role in AMD is a subject for debate. Evidence in favor of such a hypothesis is counterbalanced by evidence against. The present study may suggest some protective effect of MP in the later stages of AMD. However, causal relationships cannot be established with a cross-sectional study. With a firm protective role, MPD was expected to be lower in early AMD compared with the no-AMD stage, which was not the case in this study. The hypothesis that the process of AMD itself had reduced the amount of MP in late AMD is also quite plausible. Macular pigment is concentrated in the central area of the retina along the axons of the cone photoreceptors. In addition, MP has been detected in the photoreceptor outer segment layer in the central fovea.

In late AMD, large areas of RPE atrophy and/or neovascularization are present in the central retinal area, leading to advanced parafoveal rod loss and eventually to foveal cone degeneration. In the end, all photoreceptors may disappear, causing a decrease of central MP in late AMD.

MOD, representing the sum of the optic density of RPE and choroidal melanin, was lower in early and late AMD than in the no-AMD stage. Our findings might point to a protective effect of melanin in AMD, possibly by reducing backscattering and exerting antioxidative action. Again, the AMD-related process of RPE degeneration itself may also have led to the smaller amount of melanin measured. Changes in choroidal melanin are less likely, because its central MOD is 2.4 times higher than the central RPE melanin in white persons. In line with our MOD findings, a higher prevalence of all forms of AMD was found in a white population compared with the more pigmented populations. In other studies, a higher prevalence of late AMD was found only in whites. Data derived from a longitudinal study recently performed in our laboratory do not suggest a protective effect of melanin on the incidence of early AMD.

In conclusion, foveal cones show signs of misalignment and/or deterioration of their outer segments in early AMD, with soft, indistinct, or reticular drusen with or without RPE changes, or soft, distinct drusen with RPE changes, but not in the no-AMD stage. Apparently hard drusen, soft distinct drusen, or RPE changes only are insufficient to change parameters measured with the FRA. We found no clear evidence of a protective role of macular pigment in AMD. The role of melanin in protecting against AMD may be underestimated and needs further research.

**References**


