MACULAR FUNCTION IN EYES WITH EARLY AGE-RELATED MACULAR DEGENERATION WITH OR WITHOUT CONTRALATERAL LATE AGE-RELATED MACULAR DEGENERATION

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Purpose: To evaluate psychophysical and electrophysiologic responses in eyes with early age-related macular degeneration (AMD) without a decrease in visual acuity and with or without late AMD in the fellow eye.

Methods: Fifteen patients (mean age: 67.9 ± 7.20 years) with early AMD in both eyes (AMD1 group, 15 eyes) and 15 patients (mean age: 71.40 ± 7.06 years) with early AMD in one eye and late AMD in the fellow eye (AMD2 group, 15 eyes) were enrolled. They were compared to 15 age-similar normal control subjects. LogMAR visual acuity (VA), macular sensitivity by MP-1 microperimetry, and multifocal electroretinograms (mfERG) were assessed in control, AMD1, and AMD2 eyes. mfERG response amplitude density (RAD, nV/deg^2) of the N1-P1 component of first order binary kernels was measured.

Results: When compared to controls, AMD1 and AMD2 eyes showed a significant (analysis of variance, $P < 0.01$) decrease in MP-1 microperimetry assessed in the 0–2.5 and 2.5–5 degrees of the macula, significantly correlated (Pearson test, $P < 0.01$) to the corresponding significant decrease ($P < 0.01$) in mfERG N1-P1 RADs assessed in the 0–2.5 and 2.5–5 degrees. In AMD1 and AMD2 eyes, VA and mfERG N1-P1 RADs assessed in the 5–20 degrees were similar ($P > 0.01$) to controls. VA, MP-1, and mfERG values were not significantly different in AMD1 and AMD2 eyes.

Conclusion: In eyes with early AMD there is a dysfunction of preganglionic elements in the central 0–5 retinal degrees detectable by mfERG or MP-1 microperimetry. This impairment is not further influenced by the presence of late AMD in the fellow eye.

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Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in industrialized countries among people aged 65 years or older.[1–5]

Patients with early AMD (E-AMD), characterized by ophthalmoscopic signs such as macular drusen (≥63 μm) and changes in retinal pigment epithelial pigmentation, may show a normal visual acuity but sometimes complain of a worsened quality of vision.[6] Late AMD (L-AMD) is characterized by choroidal neovascularization or geographic atrophy and is associated with severe visual loss.[7,8]

Previous studies report that in eyes with E-AMD, the presence of L-AMD in the fellow eye represents a

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risk factor for the development of a choroidal neovascularization or geographic atrophy involving the center of the macula. On the other hand, this risk factor is reduced by the presence of E-AMD in the fellow eye.[3,9–13]

As a result, it would be interesting to investigate whether, in the presence of different conditions in the fellow eye (i.e., eyes with E-AMD or eyes with L-AMD), eyes with E-AMD may show dissimilar conditions of functional impairment of the macula.

In E-AMD with normal visual acuity, it would be clinically helpful to use methods of functional macular investigation to detect a possible visual dysfunction. This could be obtained by other psychophysical tests or by electrophysiologic evaluation.

Macular microperimetry represents a psychophysical method that allows the assessment of central field sensitivity under ophthalmoscopic monitoring, overlaying the visual field data over the fundus photograph.[14] Macular microperimetry data can be averaged, obtaining retinal sensitivity values measured in the entire central retina or, as suggested in our previous study,[15] in the central 1–2 degrees of the macula and in an annular area from 2 to 7 central degrees. This particular approach allows detection of whether the reduction in retinal sensitivity is located in the central (1–2 degrees) or in the paracentral (2–7 degrees) macular region.[15]

The electrophysiologic evaluation of macular function may be performed by focal-electroretinogram (F-ERG) or multifocal electroretinogram (mfERG) recordings.

F-ERG allows the electrophysiologic evaluation of the function of preganglionic elements [15–19] in response to luminance stimuli presented in the central 4–9 degrees.[15,16,19]

MfERGs assess localized retinal responses originating from photoreceptors and bipolar cells.[20] The average of the bioelectrical responses obtained in relation to different degrees of eccentricity from the fovea allows the differential functional evaluation of retinal areas enclosed between 1 and 25 degrees (i.e., 1, 2–5, 6–10, 11–15, 16–20, and 21–25 degrees).[21] The possibility of separately measuring the bioelectrical responses obtained in localized macular areas (i.e., 1, 2–5, and 6–10 degrees) may represent an advantage of mfERG with respect to F-ERGs, in which the bioelectrical response is obtained from the entire central retina (i.e., 4–9 central degrees[15,16,19]).

Thus, microperimetry and mfERG may psychophysically or electrophysiologically explore the entire central retina but, through an appropriate analysis of the data, may also give selective information regarding the function of retinal areas located in the central (i.e., 1–2 degrees) or paracentral (i.e., 2–5 degrees) macular region.

A reduction in central field sensitivity[22–26] and a dysfunction of macular preganglionic elements detected by F-ERG[27–32] or by mfERG[21,33–37] have been reported in the early stages of AMD.

Therefore, the aim of our study was to evaluate, in eyes affected by E-AMD without reduction of visual acuity, the presence of changes in psychophysical (microperimetry) and electrophysiologic (mfERG) responses with respect to normal age-similar, healthy, control subjects and whether any possible psychophysical and/or electrophysiologic difference could be detected between patients with bilateral E-AMD and patients with E-AMD in one eye and L-AMD in the fellow eye.

Methods

Patients

Seventy-eight patients binocularly affected by E-AMD or by E-AMD in one eye and L-AMD in the fellow eye (31 men and 47 women; range: 54–80 years) were initially enrolled for ophthalmoscopic examination; 30 patients were later selected according to our exclusion criteria (see below).

The clinical diagnosis of AMD was based on slit-lamp and indirect ophthalmoscopic examination using +90–78 D no-contact lens (Volk Optical, Mentor, OH) after pupillary dilatation using tropicamide 1%. In addition, a 30° color fundus photograph centered on the fovea was also taken. The stereoscopic photographs were independently analyzed and graded by two masked observers (M.T. and L.P.) in accordance with the Wisconsin Age-Related Maculopathy Grading System.[38] If the two observers disagreed, a third was asked to arbitrate (M.V.). Macular features included drusen number, size and confluence, and focal hyperpigmentation or hypopigmentation of retinal pigment epithelium (RPE).

Eyes with presence of soft drusen ($\geq$63 μm) with or without pigmentary abnormalities (hyperpigmentation or hypopigmentation) and more than 10 drusen within 1,500 μm of the fovea were identified as early AMD eyes (E-AMD).[26,39] Eyes with presence of geographic atrophy or exudative AMD were identified as late AMD eyes (L-AMD).[7,8]

Exclusion criteria, based on the data that several pathologies may influence the bioelectrical responses derived from the macular region,[16] were as follows: presence of moderate to dense lens opacities (13 patients excluded), implanted intraocular lens (8 patients excluded), presence of corneal opacities (2 patients ex-

cluded), previous history of refractive surgery (5 patients excluded); presence of glaucoma or ocular hypertension (4 patients excluded); previous history of intraocular inflammation such as anterior or posterior uveitis (1 patient excluded); previous history of retinal detachment or laser treatment for peripheral retinal diseases (2 patients excluded); presence of diabetes (4 patients excluded) or systemic hypertension in medical treatment (3 patients excluded), previous history of ocular trauma (1 patient excluded); drug therapies with toxic effects on the macula (e.g., chloroquine, oxazepam) (1 patient excluded); presence of neurologic diseases (1 patient excluded); presence of angiographic sings of exudative AMD in the studied eye (3 patients excluded).

E-AMD eyes were compared to 15 eyes from 15 age-similar normal control subjects (7 men and 8 women; mean age: 69 ± 8.10; range: 55–78 years). Control subjects were enrolled following the same exclusion criteria used for AMD patient enrollment and particular attention was posed to exclude ophthalmoscopic signs of macular alterations (e.g., macular drusen or pigment epithelium alterations).

Informed consent was obtained from all subjects or patients before testing. The research followed the tenets of the Declaration of Helsinki and the study was approved by the local ethics committee.

Visual acuity, macular sensitivity by MP-1 microperimetry, and mfERGs were assessed in all patients with AMD and controls, using the following methods.

Best-Corrected Visual Acuity

Best-corrected visual acuity (VA) was assessed using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) chart; VA was expressed in logMAR values obtained at a distance of 4 m with the best refractive correction.

MP-1 Microperimetry

Macular sensitivity was evaluated using the MP-1 Microperimeter (Nidek Inc., Italy) with the software version available in 2003 (Version: MP1 SW 1.4.0.1.SP2).

MP-1 Microperimeter allows visualization of the location of sensitivity threshold measurement on the fundus image. An infrared fundus camera (768 × 576 pixels resolution) captures the retinal image with 45° field of view. The operator can visualize the retina on a liquid crystal color monitor in real-time and in a dynamic way. MP-1 software allows infrared light intensity variation to have a better quality of the image. Moreover, the operator can correct the patient's refractive error within a range of ±15 diopters through a system of lenses in the fundus camera. During the examination, the patient is required to fix a light target set at 100 apostilb.

The fixation target may be varied in size (ranging from I to V Goldmann standards) and shape (cross or ring). A random series of light stimuli are presented on a background with luminescence set on 4 apostilb (1.27 candles/m^2). The intensity of the stimulus can be varied on a scale from 0 to 20 dB (0 dB represents the brightest luminance of 400 apostilb). An autotracking system allows detection and measurement of eye movements during the test, calculating the shifts relative to a preplanned retinal area every 40 msec, thus allowing the accurate determination of the location and stability of fixation.

In our study the following parameters were used: grid of 32 stimuli covering the central 5 degrees, time between stimuli equal to 1 second, stimulus size equivalent Goldmann I, white background, bright red cross of 1 degree in size as the fixation target. A 4-2-1 double staircase strategy was carried out and the first stimulus was presented at the level of 15 db. Light intensity of each presented stimulus was decreased by 2 db after a correct answer and increased by 1 db after a negative one and the last seen threshold value was taken as final threshold.

At the end of the examination, MP1 allows to overlap the infrared image with a digital color fundus image acquired by a color CCD camera (780 × 580 pixels, with Xenon flash). A color graduate scale ranging from red (corresponding to 0 dB) to dark green (corresponding to 20 dB) reports the retinal sensitivity over the fundus image.

We studied macular sensitivity, testing the mean value, expressed in dB, of four retinal points of the central 0–2.5° of the macula (central microperimetry, CM) and the mean value of 28 retinal points located in an annular area from 2.5 to 5 central degrees (paracentral microperimetry, PM).

In each patient or control subject, CM and PM microperimetry was performed three times on three different days. The last evaluation was considered in the statistical analysis (see below).

Multifocal Electroretinogram (mfERG)

VERIS Clinic 4.9 (EDI, S. Mateo, CA) was used for mfERG assessment.

The multifocal stimulus, consisting of 61 scaled hexagons, was displayed on a high-resolution, black-and-white monitor (dimensions were 30 cm horizontally and 30 cm vertically) with a frame rate of 75 Hz. The array of hexagons subtended 20 degrees of visual field. Each hexagon was independently alternated between black (1 cd/m^2) and white (200 cd/m^2) according to a binary m-sequence. This resulted in a contrast

of 99%. The luminance of the monitor screen and the central fixation cross (used as target) was 100 cd/m^2. The m-sequence had 2^{13}–1 element and total recording time was approximately 4 minutes. Total recording time was divided into eight segments. Between segments, the subject was allowed to rest for a few seconds. Focusing lenses were used when necessary. At every mfERG examination, each patient positively reported that he or she could clearly perceive the cross fixation target. The eye's position was monitored by a video system in the screen of the computer.

Pupils were pharmacologically dilated to 7–8 mm with 1% tropicamide and the cornea was anesthetized with 1% dicaine. The DTL bipolar contact electrode was used to record mfERG. A small Ag/AgCl skin earth electrode was placed at the center of the forehead. The contralateral eye was occluded to help suppress blinking. Interelectrode resistance was less than 3 KOhms.

The signal was amplified (gain 100,000) and filtered (band pass 1–100 Hz) by BM 6,000 (Biomedica Mangoni, Pisa, Italy). The first order response component, K1, was examined. We analyzed the averaged response amplitude densities (RAD), obtained after rejection of artifacts using the VERIS Clinic 4.9 software, between the first negative peak, N1, and the first positive peak, P1, obtained in five concentric annular retinal regions (rings) centered on the fovea. Therefore we analyzed the N1-P1 RADs derived from 0 to 2.5 degrees (ring 1, R1), from 2.5 to 5 degrees (ring 2, R2), from 5 to 10 degrees (ring 3, R3), from 10 to 15 degrees (ring 4, R4), and from 15 to 20 degrees (ring 5, R5).

In each patient or control subject, mfERG was performed three times on three different days. The evaluation with the highest R1-R5 N1-P1 RADs was considered in the statistical analysis (see below).

Statistics

Sample size estimates were obtained from pilot evaluations performed on 10 ARM and 10 control subjects not included in the current study (unpublished results). Interindividual variability, expressed as data SD, was estimated for electrophysiologic (mfERG) and psychophysical (MP1 microperimetry) measurements. It was found that data SDs were significantly larger for patients as compared to controls (35% versus 15%) for both measurements. It was also established that, assuming the above between-subjects SD in the current study, sample sizes of control subjects and patients belonging to AMD1 and AMD2 groups provided a power of 90%, at an alpha = 0.05, for detecting a between group difference of 55% or greater in mfERG amplitude or MP1 microperimetric sensitivity. These differences were preliminarily observed by comparing patient and control data (see above). They were also expected to be clinically meaningful when comparing results of AMD1 versus those of ARM2 patients.

Lower 95% confidence limits were obtained from age-similar control subjects by calculating mean values minus 2 standard deviations for mfERG R1-R5 RADs and central and paracentral microperimetry.

Differences of functional parameters (VA, MP1, central and paracentral microperimetry, mfERG R1-R5 N1-P1 RADs) between groups (control eyes, AMD1, and AMD2 eyes) have been evaluated by one-way analysis of variance (ANOVA) with post hoc analysis by independent *t*-test plus Bonferroni correction.

Pearson correlation was used to correlate MP1 CM with mfERG R1 RADs and MP1 PM with mfERG R2 RADs in AMD1 and AMD2 eyes. mfERG R1 and R2 RAD values underwent logarithmic transformation to better approximate a normal distribution.

In all analyses, a *P* value less than 0.01 was considered statistically significant.

Results

Following our exclusion criteria, 30 patients with E-AMD were selected.

Fifteen patients (6 men and 9 women; mean age: 67.9 ± 7.20; range: 61–78 years) had E-AMD in both eyes (AMD1 group, 15 eyes) while 15 patients (7 men and 8 women; mean age: 71.4 ± 7.06; range: 54–80 years) had E-AMD in one eye and L-AMD in the fellow eye (AMD2 group, 15 eyes).

In AMD1 patients, only one eye of each patient was randomly selected, and in AMD2 patients, the eye with E-AMD was selected. All AMD1 and AMD2 eyes had a mean refractive error (when present) between –1.00 and +1.00 spherical equivalent and best-corrected visual acuity of 0 or 0.1 logMAR in the studied eye. All control subjects had a mean refractive error (when present) between –1.00 and +1.00 spherical equivalent and a best-corrected visual acuity of 0 or 0.1 logMAR in the studied eye. All age-similar control subjects had a mean refractive error (when present) between –1.00 and + 1.00 spherical equivalent and a best-corrected visual acuity of 0 or 0.1 logMAR in the studied eye.

The clinical characteristics of AMD1 and AMD2 eyes are reported in Table 1.

Figure 1, A and B, shows examples of a 45-degree color fundus photograph, MP-1 microperimetry, and mfERG recordings performed in one control eye and in different AMD1 and AMD2 eyes.

Mean data and relative statistical analysis of psychophysical and electrophysiologic parameters are shown in Tables 2 and 3.

Nonsignificant differences in mean age and in logMAR visual acuity among groups (Control, AMD1, and AMD2 eyes) were found.

In 5 AMD1 eyes and in 7 AMD2 eyes we observed CM values within our normal limits (mean values of control population minus 2 SDs) while 10 AMD1 eyes and 8 AMD2 eyes showed abnormal CM. In 7 AMD1 eyes and in 4 AMD2 eyes PM was within our normal limits (mean values of control population minus 2 SDs) while abnormal PM was observed in 8 AMD1 eyes and in 11 AMD2 eyes. On average, in both AMD1 and AMD2 eyes groups, MP1 central and paracentral microperimetry were significantly ($P < 0.01$) reduced when compared to the Control group, while no differences were observed between AMD1 and AMD2 groups.

Multifocal ERG R1 RADs were within our normal limits (mean values of control population minus 2 SDs) in 4 AMD2 eyes but not in AMD1 eyes, while 15 AMD1 eyes and 11 AMD 2 eyes showed abnormal mfERG R1 RADs. Multifocal ERG R2 RADs were within our normal limits (mean values of control population minus 2 SDs) in 12 AMD1 eyes and in 14 AMD2 eyes, while abnormal mfERG R1 RADs were observed in 3 AMD1 eyes and in 1 AMD2 eye. All AMD1 and AMD2 eyes showed mfERG R3–R5 RADs within our normal limits (mean values of control population minus 2 SDs). On average, mfERG R1 and R2 RADs recorded in both AMD1 and AMD2 eyes groups were significantly ($P <$

Table 1. Clinical Characteristics of AMD1 and AMD2 Patients

Patients	Age, yr	VA (logMAR)	CM (dB)	PM (dB)	mfERG R1 RADs, νV/degree^2	mfERG R2 RADs, νV/degree^2
AMD1						
AMD1#1	64	0.1	0.9	2.1	23.3	23.9
AMD1#2	65	0.1	1.5	2.6	40.3	27.8
AMD1#3	61	0.1	2	1.8	26.7	38.7
AMD1#4	78	0	3	3.3	20.4	32.1
AMD1#5	76	0	3.8	4.8	33.3	19.0
AMD1#6	65	0	4.3	8.5	68.0	45.8
AMD1#7	63	0	4.5	8	45.6	35.3
AMD1#8	61	0.1	1.6	0.9	24.6	21.1
AMD1#9	64	0	6	11.5	71.4	48.5
AMD1#10	78	0.1	6.8	6.6	77.3	26.8
AMD1#11	78	0	8.8	10.1	52.7	48.2
AMD1#12	62	0	9.6	13.1	60.7	50.1
AMD1#13	64	0.1	10	8.3	86.5	30.8
AMD1#14	62	0	11	4.5	57.1	28.9
AMD1#15	78	0	13.6	10.4	87.6	27.0
95% LNCL	—	—	7.18	7.2	87.78	23.96
AMD2						
AMD2#1	77	0.1	1.8	1.8	15.9	14.7
AMD2#2	79	0.1	3.8	2.6	46.4	26.3
AMD2#3	54	0.1	7.8	6.7	76.0	30.5
AMD2#4	70	0.1	5.7	6.9	37.0	32.2
AMD2#5	69	0.1	1.5	5.2	40.5	34.7
AMD2#6	79	0.1	2	4.5	63.3	35.1
AMD2#7	75	0.1	10	6.1	96.3	35.7
AMD2#8	74	0	5.8	5.0	72.4	36.5
AMD2#9	65	0	3.5	5.1	67.3	40.7
AMD2#10	67	0	14.0	4.9	73.3	42.4
AMD2#11	75	0	7.3	5.2	95.9	43.0
AMD2#12	63	0	10.6	14.3	89.4	44.0
AMD2#13	80	0	5.5	10.7	70.1	45.1
AMD2#14	71	0	14.6	10.2	104.4	45.7
AMD2#15	73	0	7.3	12.6	75.2	47.0
95% LNCL	—	—	7.18	7.2	87.78	23.96

AMD1 = eyes with early age-related macular degeneration (AMD) in both eyes; AMD2 = eyes with AMD in one eye (selected eye) and neovascular AMD in the fellow eye; VA = visual acuity; CM = MP-1 central microperimetry; PM = MP-1 paracentral microperimetry; mfERG = multifocal electroretinogram; RADs = N1-P1 response amplitude density; 95% LNCL = 95% lower normal confidence limits obtained from control subjects by calculating mean values minus 2 standard deviations for mfERG R1 and R2 RADs and central and paracentral microperimetry.

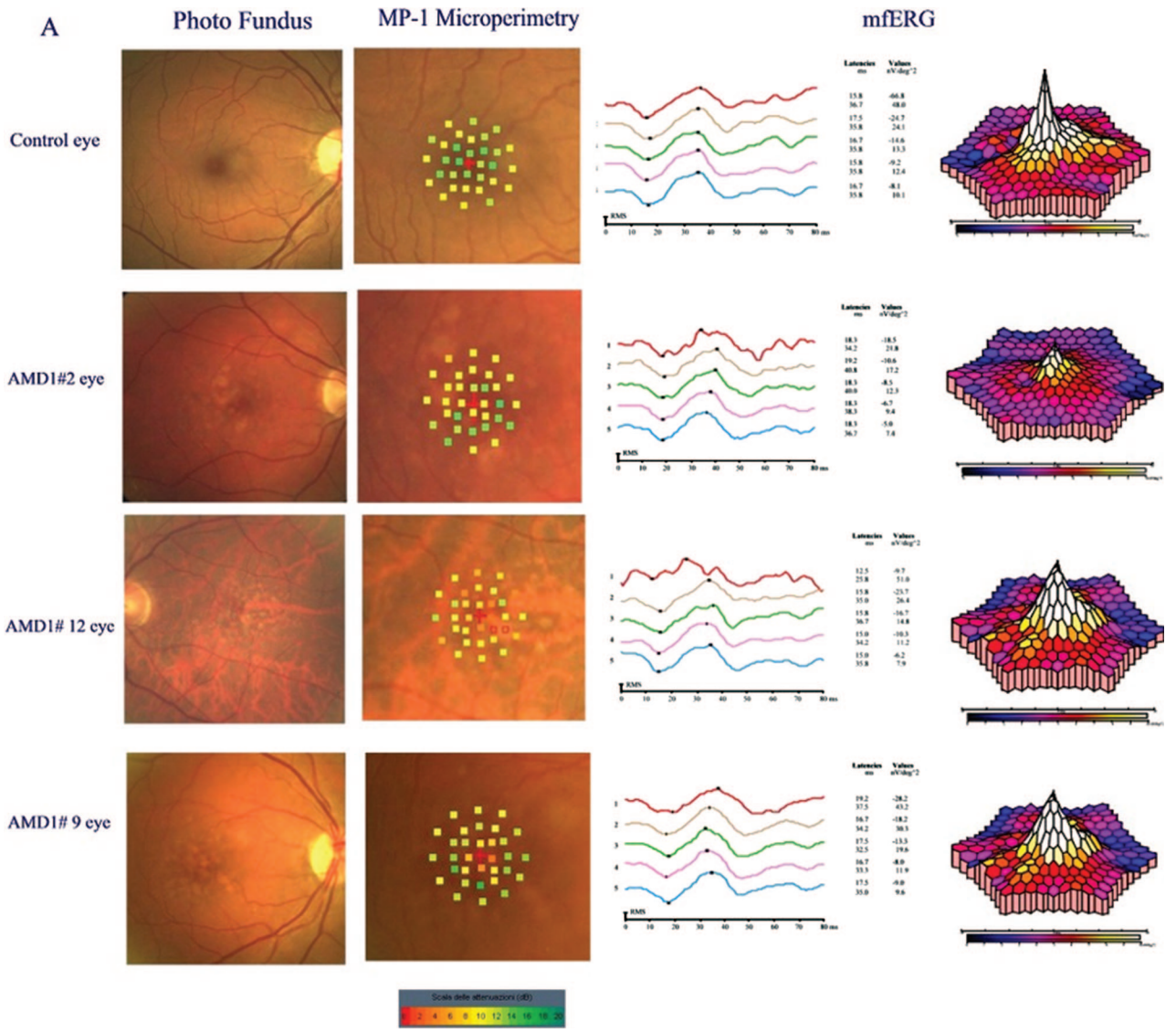

Fig. 1. Examples of a 45-degree color fundus photograph, MP-1 microperimetry, and multifocal electroretinogram (mfERG) recordings performed in one control eye and in eyes with bilateral early age-related macular degeneration (AMD1, **A**) or early AMD in the selected eye and late AMD in the fellow eye (AMD2, **B**). AMD1 and AMD2 eyes showed a decrease in both central and paracentral macular sensitivity with respect to control eyes. MfERG responses observed in AMD1 and AMD2 eyes were decreased in amplitude with respect to control eyes only when recorded in the 0–2.5 and 2.5–5 degrees. The three-dimensional plot shows that in AMD1 and AMD2 eyes there is a decrease in amplitude localized in the central retina.

0.01) reduced when compared to Control group ones. Nonsignificant differences were found in mfERG R3, R4, and R5 RADs between AMD1 and AMD2 with respect to Controls or between AMD1 and AMD2 groups.

In Figure 2, individual MP1 central and paracentral microperimetry values observed in AMD1 and AMD2 eyes are plotted as a function of the corresponding values of mfERG R1 and R2 RADs. In both AMD1 and AMD2 eyes, a significant correlation ($P < 0.01$) was observed between MP-1 CM and mfERG R1 RADs and between MP-1 PM and mfERG R2 RADs.

Discussion

The aim of our study was to assess macular function in patients with E-AMD by evaluating psychophysical (VA, MP-1 microperimetry) and electrophysiologic (mfERG) responses. In addition, we evaluated differences between eyes with binocular E-AMD (AMD1 eyes) and eyes with E-AMD in one eye and L-AMD in the contralateral eye (AMD2 eyes).

Our AMD1 and AMD2 eyes showed, when compared to control eyes, a decrease in retinal sensitivity tested by MP-1 microperimetry in both 0–2.5 and 2.5–5 degrees of the central retina, related to the

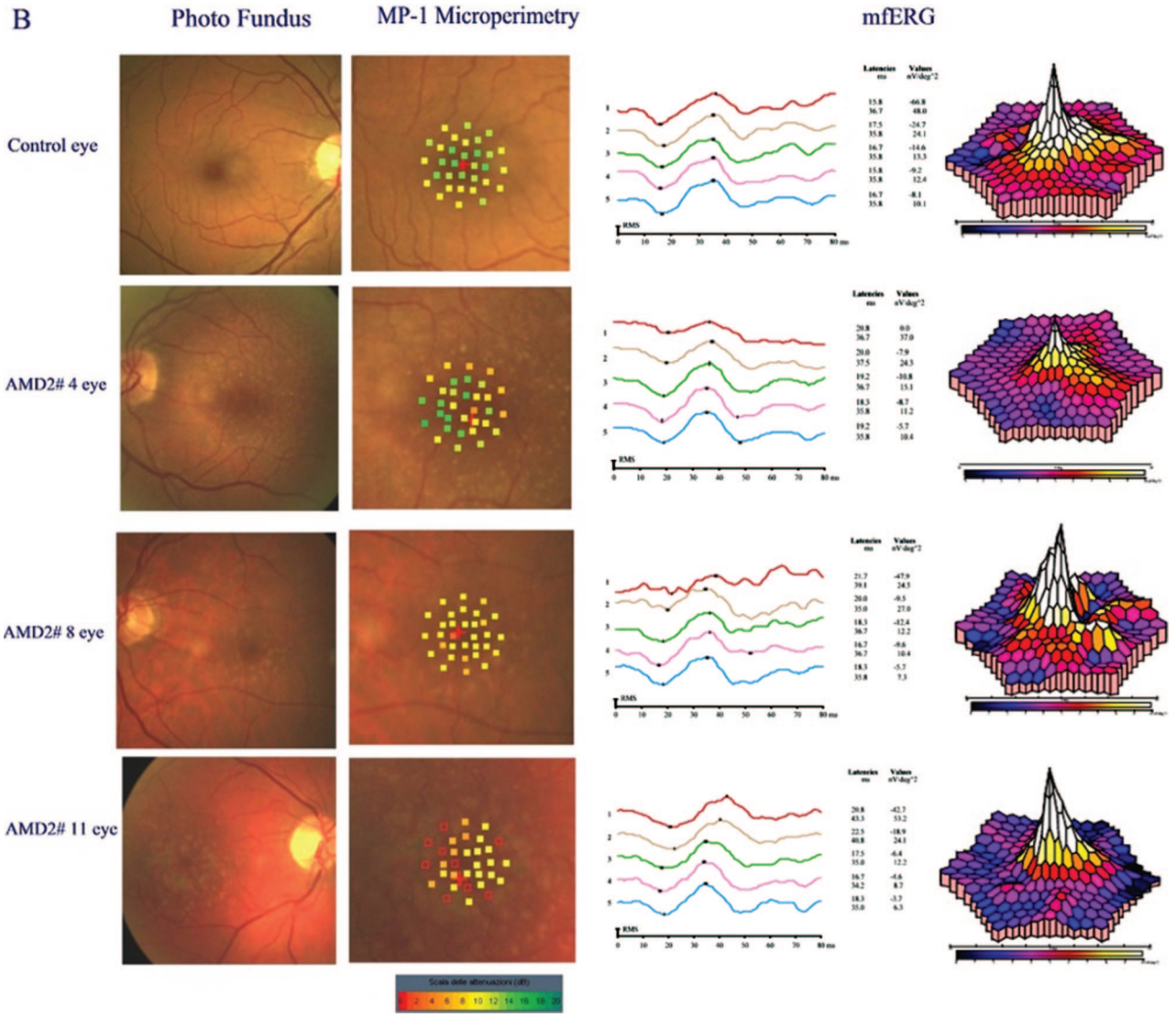

Fig. 1. *Continued*

corresponding decrease in mfERG N1-P1 RADs assessed in 0–2.5 (ring 1) and in 2.5–5 (ring 2) degrees. There were nonsignificant differences in all parameters evaluated between the AMD1 and AMD2 eyes.

The observed decrease in macular sensitivity (reduced CM and PM) in our AMD eyes is consistent with other findings in which different psychophysical methods (i.e., S cone-mediated sensitivity, Rayleigh color matching, scotopic threshold, central visual field sensitivity) revealed an early impairment in the central visual field.[22–26]

Our mfERG results obtained in both AMD1 and AMD2 eyes indicate that there is a decrease in the localized retinal bioelectrical responses obtained by stimulating the central retina (ring 1 and 2), while the responses obtained beyond the five central degrees (rings 3–5) are not statistically different when compared with controls. This suggests that in E-AMD the retinal dysfunction could be localized in the 0–5 central degrees with a functional sparing of the more external annular areas tested (5–20 degrees). Our results are in agreement with other studies obtained by carrying out a separation of rings of local mfERG responses. In fact, a decrease in N1 and P1 amplitude was only observed in the central rings, and no significant decrease in amplitude was observed in the more external rings.[33–36] However, these studies[33–36] used different criteria to separate the ring analysis (different degrees of eccentricity from the fovea); in addition, the criteria used to establish early AMD are not entirely specified and different types of visual stimuli (i.e., rod mfERG[36] or mfERG[33–35]) have been employed.

Nevertheless, notwithstanding the presence of normal mfERGs obtained in the more external annular

Table 2. Mean ± 1 Standard Deviation of LogMAR Visual Acuity and MP-1 Central (CM) and Paracentral (PM) Microperimetry Values Observed in Control Eyes and AMD1 and AMD2 Eyes and ANOVA Between Groups and Post Hoc (Bonferroni Test) Analysis

	C (n = 15)	AMD1 (n = 15)	AMD2 (n = 15)
Visual acuity (LogMAR)	0.026 ± 0.046	0.040 ± 0.051	0.047 ± 0.052
CM (dB)	11.8 ± 2.31	5.83 ± 3.97	6.75 ± 4.13
PM (dB)	11.7 ± 2.25	6.43 ± 3.88	6.79 ± 3.58

ANOVA

	Between Groups	AMD1 vs C	AMD2 vs C	AMD1 vs AMD2
Visual acuity	f(2,44): 0.63, $P = 0.534$	t = 0.013, $P > 0.01$	t = 0.020, $P > 0.01$	t = 0.006, $P > 0.01$
CM	f(2,44): 12.09, $P < 0.01$	t = 5.95, $P < 0.01$	t = 5.03, $P < 0.01$	t =0.92, $P > 0.01$
PM	f(2,44): 11.81, $P < 0.01$	t = 5.27, $P < 0.01$	t = 4.91, $P < 0.01$	t =0.35, $P > 0.01$

C = control eyes; AMD = age-related macular degeneration; ANOVA = analysis of variance.

areas tested (5–20 degrees, rings 3–5), the possible presence of isolated areas of retinal dysfunction cannot be entirely excluded. This is supported by data[20,34] showing that when a ring analysis is performed, the individual hexagons are not singularly evaluated, but there is an average of the bioelectrical responses obtained in the tested areas. Therefore, some individual abnormal responses could be masked by the adjacent normal responses with consequent normal averaged responses obtained in the entire tested area. This could represent a potential limitation of the ring analysis of mfERG.

Our mfERG results could be ascribed to an impairment of macular preganglionic elements that may be functionally affected in the early stages of AMD. This is supported by the data of Hood et al[40] showing that the first-order kernel response (our main electrophysiologic parameter evaluated) originates from photoreceptors and bipolar cells in an animal model. This is derived from mfERG changes obtained after suppression of inner retinal responses, blocking of signal transmission to ON-bipolar cells, or isolation of the contributions from the cone photoreceptors.[40]

The presence of a correlation between mfERG R1 and R2 responses with central and paracentral MP-1 microperimetry respectively may suggest that the reduction in macular sensitivity could be ascribed to the same retinal factors leading to abnormal mfERG responses such as a dysfunction in preganglionic macular elements.

At present, the mechanisms inducing the dysfunction of macular photoreceptors in the early stages of AMD are not entirely clear.

Table 3. Mean ± 1 Standard Deviation of mfERG Response Amplitude Densities (RAD) Values Observed in Control Eyes (C) and AMD1 and AMD2 Eyes

	C (n = 15)	AMD1 (n = 15)	AMD2 (n = 15)
R1 RAD (nanoV/degree^2)	118.4 ± 15.31	51.7 ± 23.25	68.2 ± 24.57
R2 RAD (nanoV/degree^2)	48.6 ± 12.32	33.6 ± 10.37	36.9 ± 8.69
R3 RAD (nanoV/degree^2)	22.8 ± 4.38	21.5 ± 6.03	20.9 ± 4.41
R4 RAD (nanoV/degree^2)	18.8 ± 6.56	15.6 ± 4.06	16.0 ± 5.10
R5 RAD (nanoV/degree^2)	14.3 ± 4.33	13.9 ± 4.65	13.7 ± 5.09

ANOVA

	Between Groups	AMD1 vs C	AMD2 vs C	AMD1 vs AMD2
R1 RAD	f(2,44): 39.34, $P < 0.01$	t = 66.66, $P < 0.01$	t = 50.14, $P < 0.01$	t =16.53, $P > 0.01$
R2 RAD	f(2,44): 8.38, $P < 0.01$	t = 15.03, $P < 0.01$	t =11.72, $P = 0.01$	t = 3.31, $P > 0.01$
R3 RAD	f(2,44): 0.54, $P = 0.585$	t = 1.27, $P > 0.01$	t = 1.86, $P > 0.01$	t = 0.59, $P > 0.01$
R4 RAD	f(2,44): 1.56, $P = 0.221$	t = 3.17, $P > 0.01$	t = 2.77, $P > 0.01$	t = 0.40, $P > 0.01$
R5 RAD	f(2,44): 0.06, $P = 0.940$	t = 0.35, $P > 0.01$	t = 0.60, $P > 0.01$	t = 0.25, $P > 0.01$

We analyzed the N1-P1 RADs derived from 0 to 2.5 degrees (ring 1, R1), from 2.5 to 5 degrees (ring 2, R2), from 5 to 10 degrees (ring 3, R3), from 10 to 15 degrees (ring 4, R4), and from 15 to 20 degrees (ring 5, R5) and ANOVA between groups and post hoc (Bonferroni test) analysis.

AMD = age-related macular degeneration; ANOVA = analysis of variance.

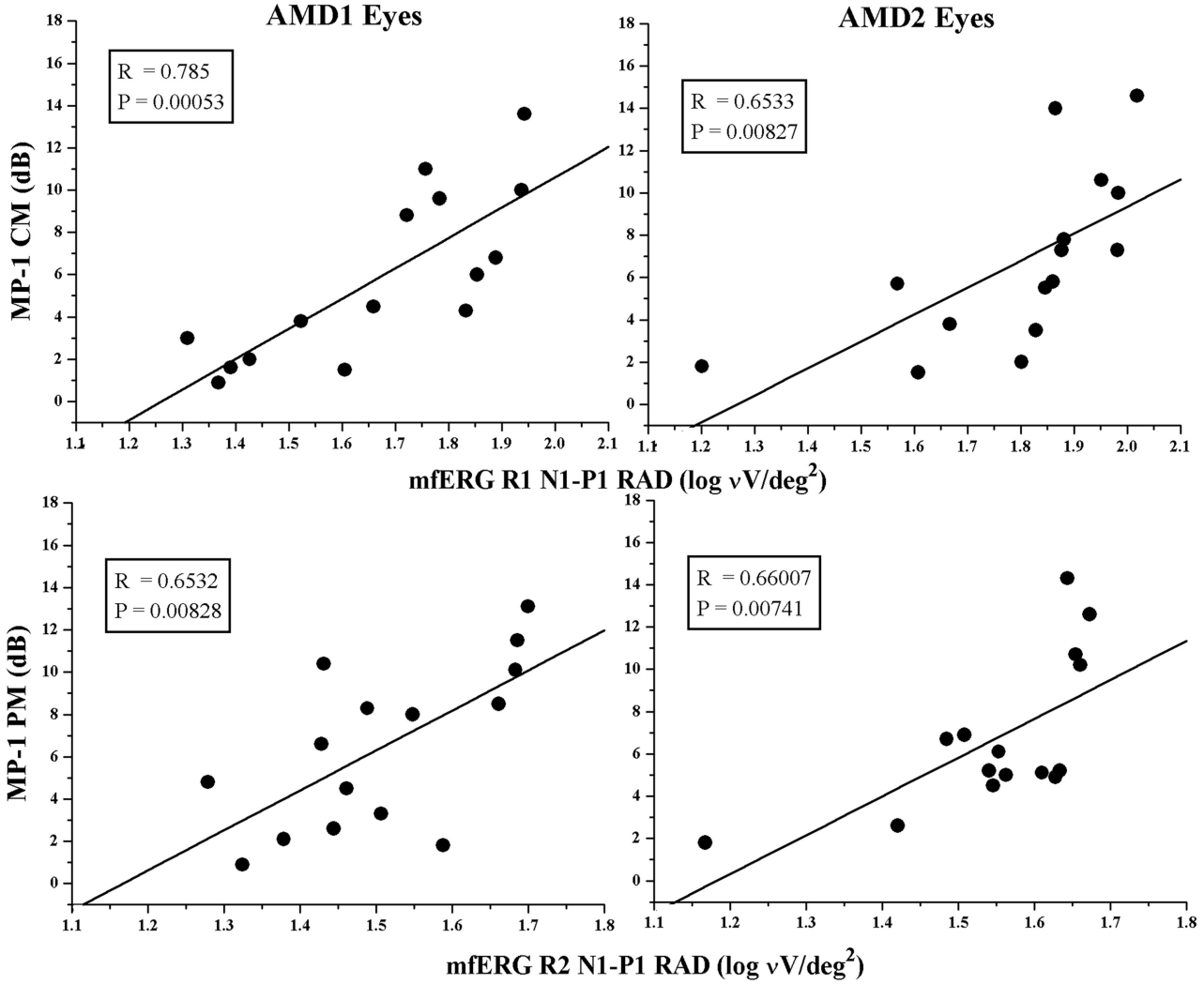

Fig. 2. Individual values of multifocal electroretinogram (mfERG) N1-P1 response amplitude density (RAD) recorded from 0 to 2.5 central degrees (R1) and from 2.5–5 paracentral degrees (R2) plotted against central (0–2.5 degrees, CM) and paracentral (2.5–5 degrees) MP-1 microperimetry in patients with bilateral early age-related macular degeneration (AMD1) or early AMD in one selected eye and late AMD in the fellow eye (AMD2). Pearson's test was used for regression analysis.

In early AMD, photoreceptor dysfunction could be the expression of impairment of RPE cells.[41–44] The relationship between photoreceptor function and RPE cell function is supported by evidence of a correspondence between the decrease in retinal sensitivity (above all scotopic sensitivity) and the increase in fundus autofluorescence (e.g., accumulation of lipofuscin within RPE cells), which can be considered the expression of a RPE dysfunction in patients with AMD.[39,45] Besides, abnormal RPE metabolism causes accumulation of indigestible materials between the RPE and Bruch's membrane (i.e., soft drusen) that could induce a mechanical displacement of the outer segments and/or a defect of the pathway of nutrient exchange between photoreceptors and choriocapillaris.[41–44,46–48] All this may result in a loss of macular photoreceptors (in prevalence rods) that may also occur in the early stage of the disease.[49]

That the dysfunction, or loss, of macular photoreceptors is related to the formation of drusen (for which inflammatory or immunologic factors may also be considered)[50,51] is supported by data showing that photoreceptor abnormalities are present in retinal areas overlying or immediately adjacent to drusen.[48]

Since it was observed that a dysfunction of macular cones (detectable by foveal-ERG) is dependent from a reduced choroidal perfusion in AMD eyes with L-AMD in the fellow eye,[52] we cannot exclude that at least in AMD2 eyes vascular abnormalities may induce the observed photoreceptor dysfunction.

In our tested E-AMD eyes, MP1 microperimetry and mfERG abnormalities were detected in the ab-

sence of a decrease in visual acuity, in agreement with other similar studies reporting impaired macular function evaluated by different psychophysical methods[26,53–55] in the presence of normal visual acuity. This can be explained by the reported data that only 44% of the normal complement of foveal cones could maintain 20/20 visual acuity.[56] All this leads us to believe that in E-AMD the presence of an involvement of macular preganglionic elements may give a functional impairment detectable by mfERG and microperimetry assessment but not by logMAR visual acuity evaluation.

Our work also aimed to detect the possible presence of differences between E-AMD eyes with contralateral L-AMD with respect to bilateral E-AMD eyes. The rationale for this quest is represented by the observation that E-AMD eyes with contralateral L-AMD may have a higher risk of developing a L-AMD.[9–13]

In our study, eyes with bilateral E-AMD (AMD1 eyes) and E-AMD eyes with L-AMD in the fellow eye (AMD2 eyes) showed no statistically significant differences in psychophysical or electrophysiologic responses. This is in agreement with some other studies evaluating macular function in patients with AMD similar to those enrolled in our study.[26,36]

The lack of difference between AMD1 and AMD2 eyes could reduce the significance of the clinical purpose of our study. Indeed, our results do not allow to state that MP1 microperimetry and mfERG may provide a clear-cut separation of patients at increased risk of visual loss due to L-AMD versus low-risk patients.

This implies that our methods are not able to identify potential prognostic electrophysiologic or psychophysical abnormalities leading to the evolution of E-AMD in L-AMD. Nevertheless, it cannot be entirely excluded that other psychophysical or electrophysiologic methods may be more useful in detecting differences related to the presence of L-AMD in the fellow eye. In this case, a follow-up study is required to confirm the predictive value of possible abnormalities observed.

To explain our findings, the limits of the current statistical power should be considered although a larger sample size for both groups could have disclosed small differences in macular function.

Therefore, at present, our findings suggest that in eyes with E-AMD, the degree of macular functional impairment, at least as measured with the present methods, is independent from the condition of the fellow eye.[26,57]

In conclusion, our results suggest that in E-AMD eyes with absence of reduced visual acuity, there is a dysfunction of preganglionic elements in the central 0–5 retinal degrees detectable by mfERG or MP-1 microperimetry. This impairment is not further influenced by the presence of late AMD in the fellow eye.

Key words: AMD, mfERG, microperimetry.

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