Carotenoids and Antioxidants in Age-Related Maculopathy Italian Study

Multifocal Electroretinogram Modifications after 1 Year

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Objective: To evaluate the influence of short-term carotenoid and antioxidant supplementation on retinal function in nonadvanced age-related macular degeneration (AMD).

Design: Randomized controlled trial.

Participants: Twenty-seven patients with nonadvanced AMD and visual acuity ≥0.2 logarithm of the minimum angle of resolution were enrolled and randomly divided into 2 age-similar groups: 15 patients had oral supplementation of vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg) (AZYR SIFI, Catania, Italy) daily for 12 months (treated AMD [T-AMD] group; mean age, 69.4±4.31 years; 15 eyes); 12 patients had no dietary supplementation during the same period (nontreated AMD [NT-AMD] group; mean age, 69.7±6.23 years; 12 eyes). At baseline, they were compared with 15 age-similar healthy controls.

Methods: Multifocal electroretinograms in response to 61 M-stimuli presented to the central 20° of the visual field were assessed in pretreatment (baseline) conditions and, in nonadvanced AMD patients, after 6 and 12 months.

Main Outcome Measures: Multifocal electroretinogram response amplitude densities (RAD, nanovolt/deg^2) of the N1–P1 component of first-order binary kernels measured from 5 retinal eccentricity areas between the fovea and midperiphery: 0° to 2.5° (R1), 2.5° to 5° (R2), 5° to 10° (R3), 10° to 15° (R4), and 15° to 20° (R5).

Results: At baseline, we observed highly significant reductions of N1–P1 RADs of R1 and R2 in T-AMD and NT-AMD patients when compared with healthy controls (1-way analysis of variance $P<0.01$). N1–P1 RADs of R3–R5 observed in T-AMD and NT-AMD were not significantly different ($P>0.05$) from controls. No significant differences ($P>0.05$) were observed in N1–P1 RADs of R1–R5 between T-AMD and NT-AMD at baseline. After 6 and 12 months of treatment, T-AMD eyes showed highly significant increases in N1-P1 RADs of R1 and R2 ($P<0.01$), whereas no significant ($P>0.05$) change was observed in N1–P1 RADs of R3–R5. No significant ($P>0.05$) changes were found in N1–P1 RADs of R1–R5 in NT-AMD eyes.

Conclusions: In nonadvanced AMD eyes, a selective dysfunction in the central retina (0°–5°) can be improved by the supplementation with carotenoids and antioxidants. No functional changes are present in the more peripheral (5°–20°) retinal areas. *Ophthalmology 2008;115:324–333 © 2008 by the American Academy of Ophthalmology.*

Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in industrialized countries among people aged ≥65 years.[1–5] Patients affected by nonadvanced AMD, characterized by ophthalmoscopic signs such as macular drusen (≥63 μm) with or without changes in retinal pigment epithelium (RPE) pigmentation may show normal visual acuity but sometimes complain of a worsened quality of vision.[6,7] Late AMD is characterized by choroidal neovascularization or geographic atrophy involving the center of the macula and is associated with severe visual loss.[1,8]

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Several studies evaluated risk or protective factors for AMD. In particular, AMD risk is increased by conditions such as female gender, blue iris, and smoking; these conditions seem to be associated with a decrease in retinal concentrations of antioxidants.[9,10] Several studies suggest that a certain degree of protection from AMD can be obtained by the intake of lutein and zeaxanthin, constituents of the macular pigment,[9,11–15] and by vitamin E supplementation.[16] Recently, the Age-Related Eye Disease Study (AREDS) provided evidence that the supplement of antioxidants plus zinc reduces the risk of developing advanced AMD in a higher risk group.[17]

Recently, Falsini et al[18] assessed the effects of 180 days of supplementation with lutein, vitamin E, and nicotinamide in early AMD by using focal electroretinogram (F-ERG) recordings. Focal electroretinogram represents an objective method of evaluating the function of preganglionic macular elements.[19–21] In this study, increased F-ERG responses were observed in early AMD patients who had undergone antioxidant supplementation.[18]

However, Falsini et al used a visual stimulus presented in the central 18°[18]; therefore, this study did not provide selective information regarding the potential effects of antioxidants on each different retinal area located within the central 18°.

Multifocal electroretinogram (mfERG) recordings are an electrophysiologic method of evaluating the function of localized retinal or macular areas.[22] Indeed, by averaging out the bioelectrical responses obtained in relation to different degrees of eccentricity from the fovea, mfERGs allow the functional evaluation of different retinal areas included between 1° and 25° (1°, 2°–5°, 6°–10°, 11°–15°, 16°–20°, and 21°–25°),[23] and this may represent a great advantage of mfERG over F-ERG. In particular, mfERGs selectively detect a dysfunction of preganglionic elements located in the central retinal 0° to 5° or 6° that also appear in the early stage of AMD.[24–28]

The present study is ancillary to a larger clinical trial aiming to evaluate the possible effects of carotenoids and antioxidants in patients suffering from nonadvanced AMD (Carotenoids in Age-Related Macular degeneration Italian Study), an ongoing multicenter, randomized, controlled clinical trial, designed with the objective of evaluating whether short-term supplementation with a fixed combination of selected antioxidants and carotenoids could influence psychophysical and psychometric parameters in AMD patients by measuring visual acuity, contrast sensitivity, and vision-related quality of life. We enrolled 147 patients for the main study; 102 were randomly assigned to receive a supplementation of carotenoids and antioxidants and 47 were followed as nontreated controls. End points were measured at 6, 12, and 24 months after starting the supplementation.

In this ancillary study, we evaluated the possible presence of abnormal electrophysiologic (mfERG) responses originating from localized retinal areas enclosed between 0 and 20 central retinal degrees in patients with nonadvanced AMD, and whether the supplementation with carotenoids and antioxidants may induce any effect on mfERG responses. Our aim was to assess whether the effect of supplementation with carotenoids and antioxidants was exclusively located in the macular region or a possible improvement of retinal function could also be present in the peripheral retinal areas.

Because it is already known that dietary supplementation with lutein in healthy individuals may result in a significant increase of macular pigment density, as suggested by studies evaluating electrophysiologic,[18] psychophysical,[29] and reflectometric[30] data, in our study design we decided to not supplement the selected combination of carotenoids and antioxidants in healthy controls.

Materials and Methods

Patients

Ninety-three patients (41 men and 52 women; mean age, 66.2±7.23 years) affected by AMD were screened for enrollment in the study. 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 (MT, MV) in accordance with the AREDS classification.[17] Macular features included drusen number, size, and confluence and focal hyperpigmentation or hypopigmentation of the RPE.

Only eyes with AREDS category 3 features (nonadvanced AMD) were selected for this study. Inclusion criteria for the selected eyes were as follows: visual acuity ≥20/32 (0.2 logarithm of the minimum angle of resolution [logMAR]), 74 letters of Early Treatment Diabetic Retinopathy Study chart; extensive (as measured by drusen area) intermediate (≥63 μm, <125 μm) drusen; and at least one large (≥125 μm) drusen or geographic atrophy not involving the center of the macula.[17]

Exclusion criteria, based on the fact that several pathologies may influence the bioelectrical responses derived from the macular region,[20] were presence of moderate to dense lens opacities, implanted intraocular lens, presence of corneal opacities, previous history of refractive surgery, presence of glaucoma or ocular hypertension, previous history of intraocular inflammation such as anterior or posterior uveitis, previous history of retinal detachment or laser treatment for peripheral retinal diseases, presence of diabetes or systemic hypertension under medical treatment, previous history of ocular trauma, drug therapies with toxic effects on the macula (e.g., chloroquine, oxazepam), presence of neurologic diseases, presence of any sign of advanced AMD (choroidal neovascularization or central geographic atrophy) in the studied eye.

When both eyes fulfilled the inclusion criteria, the eye with the best visual acuity was selected; when both eyes had the same visual acuity, the right eye was chosen for analysis. As a result, 27 eyes with nonadvanced AMD from 27 patients (12 men and 15 women; mean age, 65.5±5.14 years) were enrolled in the study.

All enrolled AMD 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.

The 27 enrolled patients were randomly (see below) divided into 2 age-similar groups: 15 patients took oral daily supplementation of vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg; AZYR SIFI, Catania, Italy) for 12 months (treated AMD [T-AMD]; 6 men and 9 women; mean age 69.4±4.31 years; 15 eyes); 12 patients received no dietary supplementation during the

same period (not treated AMD [NT-AMD]; 6 men and 6 women; mean age, 69.7±6.23 years; 12 eyes).

The AMD eyes were compared to 15 eyes from 15 age-similar normal control subjects (6 men and 9 women; mean age, 69.6±5.10 years). Control subjects were enrolled after the same exclusion criteria used for AMD patients and particular attention was paid to exclude ophthalmoscopic signs of macular alterations (e.g., macular drusen or pigment epithelium abnormalities). 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.

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.

Multifocal Electroretinograms

VERIS Clinic 4.9 (EDI; San 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 (size, 30 cm wide and 30 cm high) with a frame rate of 75 Hz. The array of hexagons subtended 20° 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 $2^{13}-1$ elements and total recording time was approximately 4 minutes. Total recording time was divided into 8 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.

In all controls and AMD eyes, mfERGs were recorded in the presence of pupils that were maximally pharmacologically dilated with 1% tropicamide to a diameter of 7 to 8 mm. Pupil diameter was measured by an observer (GG) by means of a ruler and a magnifying lens and stored for each tested eye. The cornea was anesthetized with 1% dicaine. The Dawson Trick Litzkow bipolar contact electrode was used to record mfERGs. 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 <3 KOhms.

The signal was amplified (gain 100 000) and filtered (band pass 1–100 Hz) by BM 6000 (Biomedica Mangoni, Pisa, Italy). After automatic rejection of artifacts (by VERIS Clinic 4.9 software), the first-order kernel response (K1) was examined. We analyzed the average response amplitude densities (RAD) between the first negative peak (N1) and the first positive peak (P1) obtained in 5 concentric annular retinal regions (rings) centered on the fovea. Therefore, we analyzed the N1–P1 RADs derived from 0° to 2.5° (ring 1 [R1]), from 2.5° to 5° (ring 2 [R2]), from 5° to 10° (ring 3 [R3]), from 10° to 15° (ring 4 [R4]), and from 15° to 20° (ring 5 [R5]).

We performed MfERGs 3 times on 3 different days in each AMD patient or control subject. The recording with the highest R1-R5 N1-P1 RADs was considered in the statistical analysis (see below).

To evaluate the presence of normal or abnormal mfERG responses, independent of the clinical conditions of the tested subjects, all electrophysiologic examinations were performed at baseline conditions in the presence of 2 operators (VP and GG), who did not know if the tested subject belonged to the category of control subjects or AMD patients (treated or untreated), as classified by 2 other operators (MT and MV). The random separation in treated and untreated patients was performed in accordance with an electronically generated randomization table, by one operator (MT) who was the only one to know the key. Indeed, during all mfERG recordings performed in AMD patients at 6 and 12 months of follow-up, VP and GG did not know whether the tested patient belonged to the treated or untreated group. The key was opened only at the end of the follow-up period.

Follow-up

Multifocal-ERG recordings were assessed after 6 and 12 months in T-AMD and NT-AMD eyes. During all follow-up examinations, mfERG recordings were performed in a condition of pupil dilatation equal to that measured in baseline conditions (see above).

Statistics

Sample size estimates were obtained from pilot evaluations performed in 10 nonadvanced AMD patients and 10 control subjects, other than those included in the current study (unpublished results). Interindividual variability, expressed as data standard deviation (SD) was estimated for mfERG measurements. It was found that data SDs were significantly higher for patients when compared with controls (35% vs 15%). It was also established that, assuming the above between-subjects SD in the current study, sample sizes of control subjects and patients belonging to AMD groups provided a power of 90%, at $\alpha = 0.05$, for detecting a between-group difference of ≥55% in mfERG amplitude. 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 treated or untreated AMD eyes observed in baseline conditions versus those observed at 6 and 12 months.

Test–retest data of mfERG results were expressed as the mean difference between 2 recordings obtained in separate sessions ± SD of this difference. The 95% confidence limits of test–retest variability in normal subjects and patients were established assuming a normal distribution. In AMD patients, test–retest data were calculated considering the entire cohort of enrolled patients (27 studied AMD eyes).

The differences of mfERG responses between groups (control eyes, T-AMD eyes, and NT-AMD eyes) were evaluated by one-way analysis of variance. Changes in mfERG responses observed in T-AMD and NT-AMD eyes after 6 and 12 months were compared with baseline (pretreatment) values by one-way analysis of variance. In all analyses, $P<0.05$ was considered statistically significant. When the $P<0.01$, it was considered highly statistically significant.

Results

Individual values of mfERGs, obtained in T-AMD and NT-AMD eyes in response to visual stimuli presented in the 0 to 2.5 (R1) and 2.5 to 5 (R2) central degrees are reported in Table 1 (available at http://aaojournal.org). Figure 1 (available at http://aaojournal.org) shows examples of mfERG first-order response component (K1) recorded in one control eye and in different NT-AMD and T-AMD eyes at baseline conditions and after 12 months. Figure 2 shows examples of a 3-dimensional mfERG plot recorded in one control eye and in different NT-AMD and T-AMD eyes at baseline conditions and after 12 months.

No adverse events were reported from any of the T-AMD patients enrolled in the study during the entire period of treatment.

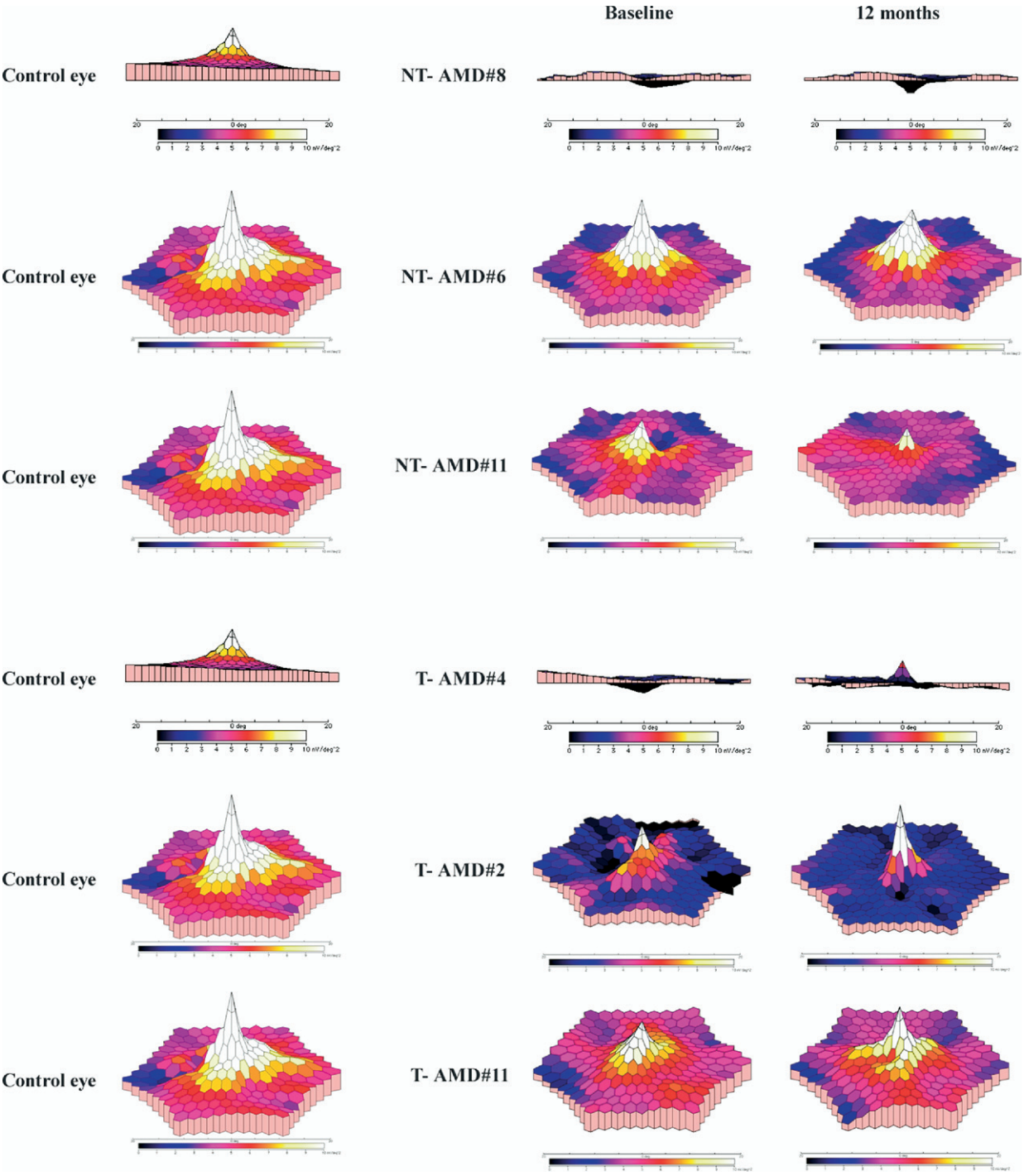

Figure 2. Examples of multifocal electroretinogram (mfERG) 3-dimensional plots, presented in different orientations, recorded in one control eye and in eyes affected by nonadvanced age-related macular degeneration (AMD) in baseline conditions and after 12 months without any treatment (NT-AMD#6, NT-AMD#8, NT-AMD#11) or supplemented with carotenoids and antioxidants (T-AMD#2, T-AMD#4, T-AMD#11). The 3-dimensional plot shows that, at baseline conditions, there is a decrease in amplitude in NT-AMD and T-AMD eyes, localized in the central retina. At 12 months, NT-AMD eyes showed a decrease similar to baseline conditions, whereas in T-AMD eyes there is still a decrease localized in the central retina with respect to control eyes, but the amplitude is increased with respect to baseline.

Multifocal Electroretinogram Responses: 0 to 5 Central Degrees (R1 and R2)

At baseline conditions, both NT-AMD and T-AMD eyes showed highly significant ($P<0.01$) R1 and R2 RADs reductions when compared with healthy controls. After 6 months, an increase in R1 RADs was found in 6 NT-AMD eyes and an increase in R2 RADs was found in 7 NT-AMD eyes; reduced R1 RADs were detected in 6 NT-AMD eyes and a decrease in R2 RADs was observed in 5 NT-AMD eyes. Nevertheless, the values of these differences with

respect to baseline conditions were within the intraindividual variability values resulting from test–retest analysis.

At the same end point (6 months), 14 eyes of the T-AMD group presented an increase in R1 RADs with values exceeding the intraindividual variability, whereas in one eye there was a RAD increase within the intraindividual variability. An increase in R2 RADs with values exceeding intraindividual variability was found in 12 T-AMD eyes. An increase in R2 RADs was also observed in 3 T-AMD eyes, although values were within the intraindividual variability.

The individual changes observed in NT-AMD and T-AMD eyes at 6 and 12 months of follow-up with respect to baseline conditions are shown in Figure 3A. On average, with respect to baseline conditions, NT-AMD eyes showed nonsignificant ($P>0.05$) changes in both R1 and R2 RADs, whereas a highly significant ($P<0.01$) increase in R1 and R2 RADs was found in T-AMD eyes.

After 12 months, NT-AMD eyes showed R1 and R2 RAD values similar ($P>0.05$) to those observed at baseline conditions. A highly significant ($P<0.01$) increase in R1 and R2 RADs was still observed in T-AMD eyes. Nevertheless, R1 and R2 RAD values were not further increased with respect to the values observed after 6 months (T-AMD, 12 months vs T-AMD, 6 months; $P>0.05$). Mean data and relative statistical analyses of mfERG responses are respectively shown in Figure 3B and Table 2.

Multifocal Electroretinogram Responses: 5° to 20° (R3, R4, and R5)

At baseline, NT-AMD, T-AMD, and control eyes showed similar values of R3, R4, and R5 RADs. Nonsignificant ($P>0.05$) differences were found when values of NT-AMD group were compared with those of T-AMD group and when both values of NT-AMD and T-AMD groups were compared with control group values.

After 6 months, an increase in R3 RADs was found in 8 NT-AMD eyes and in 6 T-AMD eyes, whereas reduced R3 RADs were detected in 4 NT-AMD eyes and in 9 T-AMD eyes. The R4 RADs were increased in 7 NT-AMD eyes and in 7 T-AMD eyes and reduced in 5 NT-AMD eyes and in 8 T-AMD eyes. An increase in R5 RADs was observed in 7 NT-AMD eyes and in 9 T-AMD eyes, and a decrease in R5 RADs was found in 5 NT-AMD eyes and in 6 T-AMD eyes. Nevertheless, the values of these differences observed in NT-AMD and T-AMD eyes were within the intraindividual variability values resulting from test–retest analysis.

The individual changes observed in NT and T-AMD eyes at 6 and 12 months of follow-up with respect to baseline conditions are shown in Figure 4A. On average, with respect to baseline conditions, both NT-AMD and T-AMD eyes showed nonsignificant ($P>0.05$) changes in R3, R4, and R5 RADs.

After 12 months, NT-AMD and T-AMD eyes showed R3, R4, and R5 RAD values similar ($P>0.05$) to those observed at baseline and at

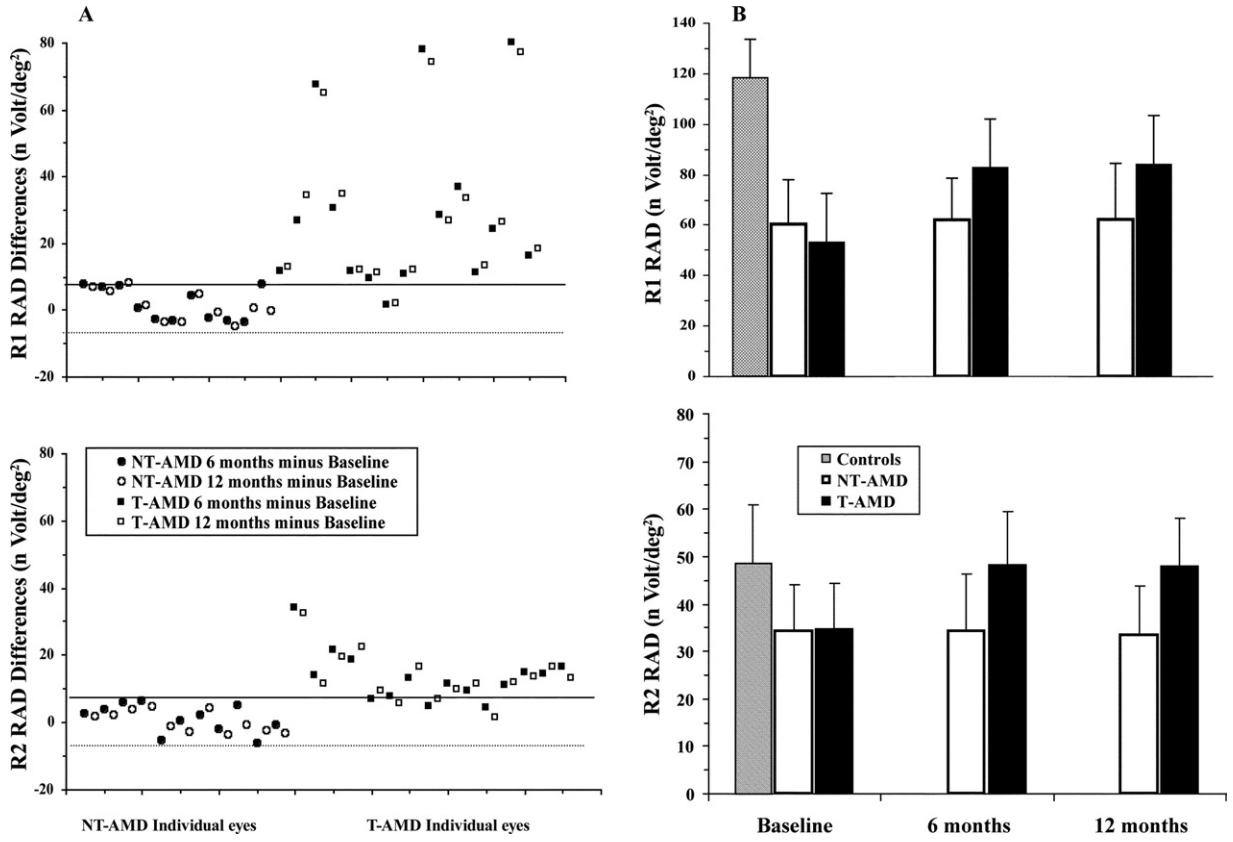

Figure 3. **A**, Individual changes of multifocal electroretinogram N1–P1 response amplitude densities (RADs) obtained in 2 retinal areas located at various degrees of eccentricity from the fovea: 0° to 2.5° (R1), and 2.5° to 5° (R2). The RAD values represent the difference between values observed after 6 and 12 months and baseline values in eyes affected by nonadvanced age-related macular degeneration (AMD) without any treatment (NT-AMD) and AMD eyes treated with a supplementation of carotenoids and antioxidants (T-AMD). Solid and dashed lines refer to the upper and lower 95% confidence limit of the intraindividual variability resulting from test–retest analysis, respectively. **B**, Graphic representation of mean values ± 1 standard deviation (vertical lines) of R1 and R2 RADs observed in NT-AMD and T-AMD eyes. The statistical analysis evaluating the differences between groups and within groups is reported in Table 2.

Table 2. Statistical Evaluation (1-Way Analysis of Variance) between Groups and within Groups with Respect to Baseline Values

	Baseline vs. Controls	6 Months vs. Baseline	12 Months vs. Baseline	Baseline vs. NT-AMD
		NT-AMD eyes (n = 12)		
R1 RAD	$F_{1,26}$: 74.17; $P<0.001$	$F_{1,23}$: 0.040; $P = 0.850$	$F_{1,23}$: 0.090; $P = 0.752$	
R2 RAD	$F_{1,26}$: 13.80; $P<0.001$	$F_{1,23}$: 0.060; $P = 0.810$	$F_{1,23}$: 0.080; $P = 0.775$	
R3 RAD	$F_{1,26}$: 1.03; $P = 0.320$	$F_{1,23}$: 0.460; $P = 0.504$	$F_{1,23}$: 0.001; $P = 0.965$	
R4 RAD	$F_{1,26}$: 2.49; $P = 0.127$	$F_{1,23}$: 0.993; $P = 0.329$	$F_{1,23}$: 0.250; $P = 0.622$	
R5 RAD	$F_{1,26}$: 0.03; $P = 0.860$	$F_{1,23}$: 0.025; $P = 0.875$	$F_{1,23}$: 0.060; $P = 0.814$	
		T-AMD eyes (n = 15)		
R1 RAD	$F_{1,29}$: 104.7; $P<0.001$	$F_{1,29}$: 14.04; $P<0.001$	$F_{1,29}$: 15.7; $P<0.001$	$F_{1,26}$: 1.09; $P = 0.307$
R2 RAD	$F_{1,29}$: 11.72; $P = 0.002$	$F_{1,29}$: 12.16; $P = 0.002$	$F_{1,29}$: 14.1; $P<0.001$	$F_{1,26}$: 0.470; $P = 0.500$
R3 RAD	$F_{1,29}$: 0.22; $P = 0.643$	$F_{1,29}$: 0.006; $P = 0.937$	$F_{1,29}$: 0.13; $P = 0.717$	$F_{1,26}$: 1.550; $P = 0.225$
R4 RAD	$F_{1,29}$: 2.85; $P = 0.102$	$F_{1,29}$: 0.661; $P = 0.423$	$F_{1,29}$: 1.15; $P = 0.293$	$F_{1,26}$: 0.003; $P = 0.952$
R5 RAD	$F_{1,29}$: 1.10; $P = 0.303$	$F_{1,29}$: 1.01; $P = 0.322$	$F_{1,29}$: 2.79; $P = 0.106$	$F_{1,26}$: 0.568; $P = 0.457$

n = no. of eyes; NT-AMD = untreated eyes with nonadvanced age-related macular degeneration; R1–R5 = local multifocal electroretinogram; N1–P1 = response amplitude densities (RADs) averaged in 5 retinal areas located at various eccentricity from the fovea: 0° to 2.5° (R1), 2.5° to 5° (R2), 5° to 10° (R3), 10° to 15° (R4), and 15° to 20° (R5); T-AMD = eyes with nonadvanced age-related macular degeneration treated with antioxidants.

6 months. Mean data and relative statistical analyses of mfERG responses are shown in Figure 4B and Table 2, respectively.

Discussion

Multifocal Electroretinogram in Nonadvanced Age-Related Macular Degeneration: Baseline Conditions

At baseline, eyes with nonadvanced AMD showed a decrease in mfERG N1–P1 RADs assessed in 0° to 2.5° (R1) and in 2.5° to 5° (R2) degrees. These electrophysiologic abnormalities were independently observed in 2 age-matched groups (NT-AMD and T-AMD) of patients in whom a reduction of visual acuity had not yet been detected.

Our mfERG results obtained at baseline are consistent with results from 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.[24–27] However, these studies used different criteria to perform the ring analysis (different degrees of eccentricity from the fovea); in addition, the criteria used to classify AMD patients were not entirely specified and different types of visual stimuli (i.e., rod mfERG[27] or mfERG[24–26]) were used.

Our mfERG results could be ascribed to an impairment of macular preganglionic elements that may be functionally affected even in nonadvanced stages of AMD. This is supported by the results reported by Hood et al,[31] who showed that the first-order kernel response (our main electrophysiologic parameter evaluated) originates from photoreceptors and off 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.[31] At present, the mechanisms inducing the dysfunction of macular photoreceptors in the early stages of AMD are not entirely clear.

In early AMD, photoreceptor dysfunction could be the expression of impairment of RPE cells.[32–35] The relationship between photoreceptor function and RPE cell function is supported by the 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 an RPE dysfunction in patients with AMD.[36,37] Besides, abnormal RPE metabolism causes accumulation of indigestible materials between the RPE and Bruch's membrane (the 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.[32–35,37–40] All this may result in a loss of macular photoreceptors (in prevalence rods) that may also occur in the early stage of the disease.[41]

The hypothesis 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)[42,43] is supported by data showing that photoreceptor abnormalities are present in retinal areas overlying or immediately adjacent to drusen.[40]

Our observations reporting mfERG abnormalities in nonadvanced AMD eyes notwithstanding good visual acuity are consistent with other studies reporting impaired macular function, evaluated by different psychophysical methods.[44–47] This can be explained by the reported data that only 44% of the normal complement of foveal cones could maintain 20/20 visual acuity.[48] All this supports the hypothesis that in nonadvanced AMD the presence of an involvement of macular preganglionic elements may lead to a functional impairment detectable by mfERG assessment, even in the absence of visual acuity impairment. The variability of mfERG responses observed in our cohort of AMD eyes could be ascribed to possible variations in percentage of foveal cone damage; this damage should nevertheless be <44% of the normal complement, which represents a sufficient quota of normal cones to maintain preserved visual acuity.

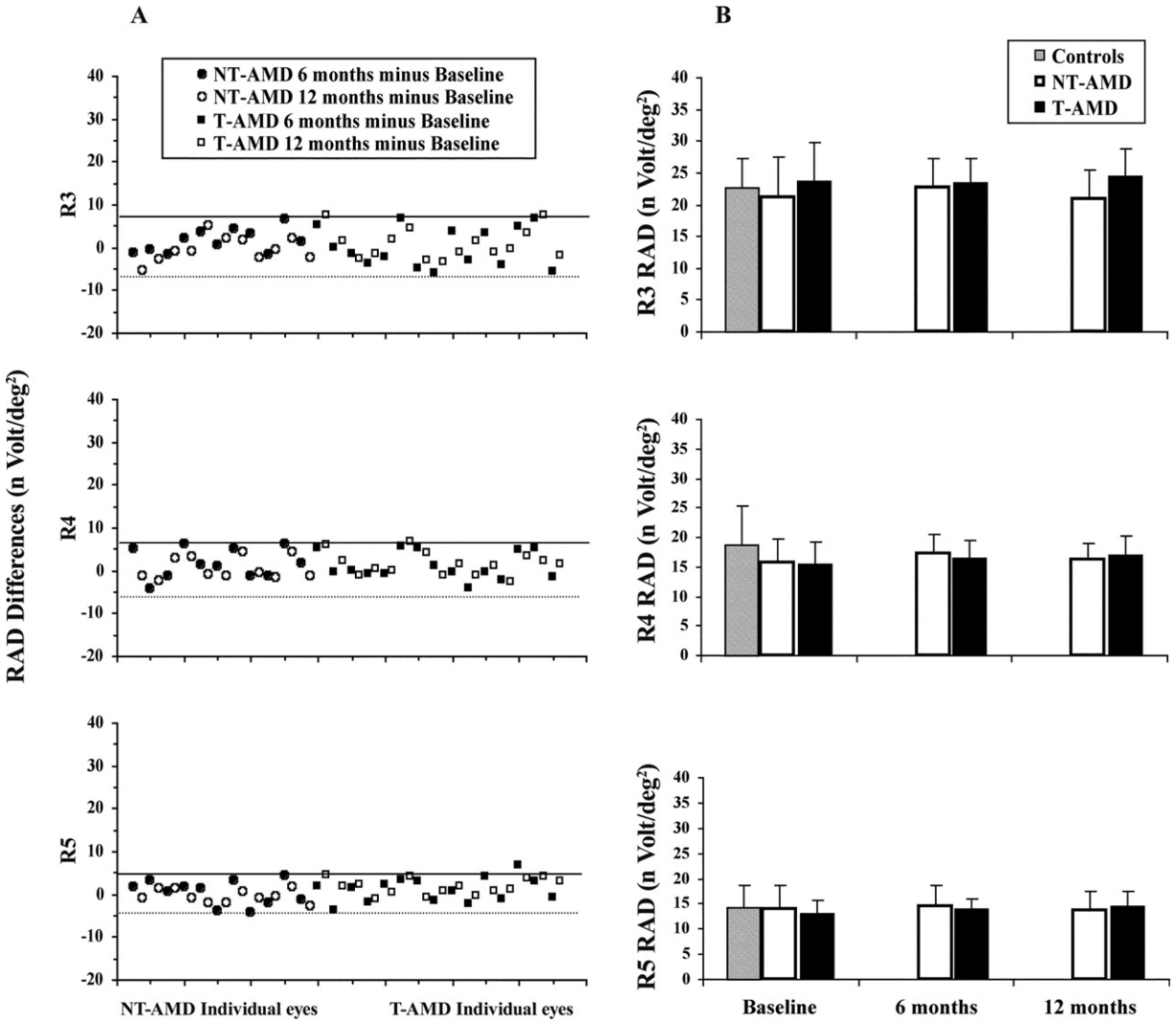

Figure 4. **A**, Individual changes of multifocal electroretinogram N1–P1 response amplitude densities (RADs) obtained in 3 retinal areas located at various degrees of eccentricity from the fovea: 5° to 10° (R3), 10° to 15° (R4), and 15° to 20° (R5). The RAD values refer to the difference between the values observed after 6 and 12 months and baseline values in eyes affected by nonadvanced age-related macular degeneration (AMD) without any treatment (NT-AMD) and nonadvanced AMD eyes treated with a supplementation of carotenoids and antioxidants (T-AMD). Solid and dashed lines refer to the upper and lower 95% confidence limit of the intraindividual variability resulting from test-retest analysis, respectively. **B**, Graphic representation of mean values ± 1 standard deviation (vertical lines) of R3, R4, and R5 RADs observed in NT-AMD and T-AMD eyes. The statistical analysis evaluating the differences between groups and within groups is reported in Table 2.

Multifocal Electroretinograms after 12 Months in Nonadvanced Age-Related Macular Degeneration with or without Antioxidant Supplementation

Untreated eyes with nonadvanced AMD (NT-AMD eyes) showed, after 6 and 12 months, unmodified mfERG responses with respect to baseline conditions. Our findings are in accordance with those of Feigl et al,[49] who did not find a progressive reduction in mfERG responses in patients with early AMD. A period >12 months (28–41 months) is reported to be necessary to detect a progression of mfERG impairment in the presence of a stable visual acuity.[25]

In eyes of treated patients (T-AMD eyes), the supplementation with the combination of vitamin C, vitamin E, zinc, copper, lutein, zeaxanthin, and astaxanthin induced an increase of mfERG responses derived from the central retina (0°–5°), whereas no changes in the bioelectrical responses were observed in the other retinal areas (5°–20°). The reduction of mfERG impairment was present after 6 months of supplementation and additional 6 months of treatment did not induce a further improvement of mfERGs. The improvement of mfERGs could be related to the effects of antioxidant supplementation contrasting these degenerative changes of RPE and photoreceptors occurring in AMD.[41]

In contrast with other studies evaluating the effects of antioxidants (e.g., Falsini et al,[18] AREDS[17]), our study also supplemented zeaxanthin (1 mg) and astaxanthin (4 mg) in addition to lutein, vitamin C, vitamin E, zinc, and copper,

whose effects in early AMD have been previously reported.[16–18] The nature of the study design does not allow us to ascribe the observed mfERG improvement, exclusively detected in the central 5°, to these supplemented compounds, but there are evidences described elsewhere that may provide possible reasonable interpretations.

Lutein and zeaxanthin, which form the macular pigment and whose concentrations are directly proportional to the rod/cone ratio,[50] vary according to the eccentricity from the fovea: within 0.25 mm of the fovea, the ratio of lutein/zeaxanthin is approximately 1/2.4, whereas in the peripheral retina this ratio is 2/1.[51,52] The concentration and localization of lutein and zeaxanthin may be due to specific mechanisms of uptake, stabilization, and storage. Carotenoid uptake and stabilization is mediated by xanthophyll-binding proteins, which are saturable and bind lutein and zeaxanthin in a highly specific way.[53] Xanthophyll-binding proteins are thought to be located in macular cell membranes. After uptake, tubulin could act as a storage protein for lutein and zeaxanthin; tubulin is abundant in the axonal layer of the fovea and this localization is consistent with the high concentrations of lutein and zeaxanthin in Henle's fiber layer.[54]

The normal concentration of lutein and zeaxanthin seems to have a protective role against the development of AMD. Indeed, studies performed on AMD donor eyes reveal reduced retinal levels of macular pigments[55] and epidemiologic data highlighted that high dietary intake of lutein- and zeaxanthin-rich foods, as well as high plasma levels of the 2 carotenoids, are associated with a decreased risk of AMD.[14]

We believe that the supplementation of lutein and zeaxanthin induced an increase in mfERG N1–P1 R1 and R2 (0–5 central degrees) RAD, which reflects the functional improvement of preganglionic elements.[31] This finding, linked to the localization and concentration of macular pigments,[50–52] could be related to the different properties of lutein and zeaxanthin. In fact, these pigments prevent the light-induced damage, shielding the retina from the harmful effects of blue light,[56] and, by quenching reactive oxygen species, reduce the oxidative injury (one of the mechanisms involved in the pathophysiology of this disease[57]). This leads to a significant antioxidant effect, preventing or delaying photoreceptor dysfunction or death.[11,58]

Our results, and the results of other studies assessing the effects of antioxidant supplementation,[11,17,18,59] are supported by experimental evidence from monkeys fed a xanthophyll-depleted diet, in which the development of drusen was observed at the level of retinal pigmented epithelium,[60] and in quails supplemented with zeaxanthin, in which the number of light-induced apoptotic photoreceptors was inversely and significantly related to retinal zeaxanthin levels.[61] Nonadvanced AMD eyes showed mfERG responses, which were not different from those of control subjects when recorded from an annular peripheral ring included between 5 and 20 retinal degrees. This suggests the functional sparing of preganglionic elements located beyond the 5 central degrees.

An explanation for this is offered by the evidence that peripheral retinal areas contain a very small concentration of carotenoids (between 13 ng/mm^2 at the fovea and 0.05 ng/mm^2 at the periphery[51]) that is adequate to achieve the normal function of photoreceptors. It is likely that, in nonadvanced AMD, a decrease in lutein and zeaxanthin concentrations also occurs in the peripheral retina, but it could be hypothesized that photoreceptor function is maintained even in the presence of a further reduction of carotenoid concentration. On the contrary, the supplementation of lutein and zeaxanthin does not induce hypernormal photoreceptor function, as suggested by the mfERG responses recorded after 6 and 12 months in T-AMD eyes.

In accordance to other published studies using mfERG[23–28] or F-ERG[62–65] recordings, our findings suggest that mfERG may be a reliable method to detect early macular dysfunctions occurring in the central retina in nonadvanced AMD eyes. These MfERG abnormalities could represent risk factors in predicting the development of AMD from early to advanced stages and this could be of great relevance in clinical practice. However, to our knowledge there is only one published paper[23] in which mfERG abnormalities have been identified as important predictors of drusen progression; therefore, we believe that further prospective studies are necessary.

In conclusion, in our selected group of patients, the combined supplementation with vitamin C, vitamin E, zinc, copper, lutein, zeaxanthin, and astaxanthin induced a selective improvement of the function of the central retina (0°–5°), whereas no functional changes were observed in the peripheral (5°–20°) retinal areas. Because of the small number of patients enrolled, the present trial can be considered a pilot study and caution must be taken against drawing general conclusions. It is necessary to confirm our findings in a larger population and with long-term follow-up. For the same reason, even if we did not observe any side effects in treated patients, no final conclusions could be drawn regarding safety.

To clarify whether the improvement observed in T-AMD eyes was supplement dependent, it would be useful to perform mfERG recordings after a period of suspension of antioxidant supplementation. Nevertheless, considering the beneficial functional effects of antioxidant supplementation, the suspension of supplementation with consequent exposure of the AMD patient to a possible decrease in macular function could represent an ethical problem. All this is at present being debated within our local ethics committee.

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Table 1. Multifocal Electroretinogram (mfERG) Responses in Untreated and Treated Eyes with Nonadvanced Age-Related Macular Degeneration

Group	Age (yrs)	mfERG R1 RADs (Nanovolt/Degree^2)			mfERG R2 RADs (Nanovolt/Degree^2)		
		Baseline	*6 Months*	*12 Months*	*Baseline*	*6 Months*	*12 Months*
NT-AMD#1	64	40.5	48.3	47.5	34.7	37.3	36.5
NT-AMD#2	65	73.1	79.9	78.8	46	49.9	48.3
NT-AMD#3	66	57.1	64.3	65.2	28.9	34.7	32.8
NT-AMD#4	78	75.8	76.3	77.3	36.9	43.2	41.6
NT-AMD#5	76	77.3	74.4	73.8	26.8	21.3	25.7
NT-AMD#6	65	87.4	84.2	83.6	48.7	49.3	45.9
NT-AMD#7	63	33.3	37.7	38.3	19	21.2	23.2
NT-AMD#8	67	37.0	34.6	36.5	32.2	30.4	28.5
NT-AMD#9	64	63.3	59.9	58.6	42.4	47.4	41.6
NT-AMD#10	78	61.8	58.3	62.3	30.8	24.4	28.3
NT-AMD#11	78	56.8	64.4	56.4	18.3	17.4	15.2
NT-AMD#12	72	64.3	61.4	65.2	21.3	22.6	24.3
T-AMD#1	66	61.6	73.2	74.2	35.1	69.0	67.3
T-AMD#2	68	65.8	92.3	99.9	38.2	52.2	49.4
T-AMD#3	78	56.7	124.0	121.6	30.1	51.5	49.4
T-AMD#4	72	33.8	64.3	68.6	21.9	40.3	44.1
T-AMD#5	74	72.4	83.8	84.5	35.7	42.3	45.1
T-AMD#6	66	70.1	79.4	81.3	45.7	53.2	51.4
T-AMD#7	62	46.4	47.7	48.2	21.4	34.4	37.6
T-AMD#8	64	71.4	82.1	83.4	38.5	43.2	45.2
T-AMD#9	67	36.3	114.0	110.6	45.8	57.3	55.4
T-AMD#10	74	68.3	96.7	94.8	38.1	47.2	49.3
T-AMD#11	73	58.5	95.3	92.0	29.9	34.2	31.2
T-AMD#12	69	43.3	54.4	56.7	30.5	41.3	42.4
T-AMD#13	67	15.9	40.1	42.1	32.1	46.7	45.7
T-AMD#14	71	20.4	100.2	97.3	23.2	37.5	39.4
T-AMD#15	70	76.2	92.4	94.4	56.0	72.3	69.2

NT-AMD = untreated eyes with nonadvanced age-related macular degeneration; R1 = mfERGs recorded in 0 to 2.5 central degrees; R2 = mfERGs recorded in 2.5 to 5 central degrees; RADs = N1–P1 response amplitude densities; T-AMD = eyes with nonadvanced age-related macular degeneration treated with oral supplementation of vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg).

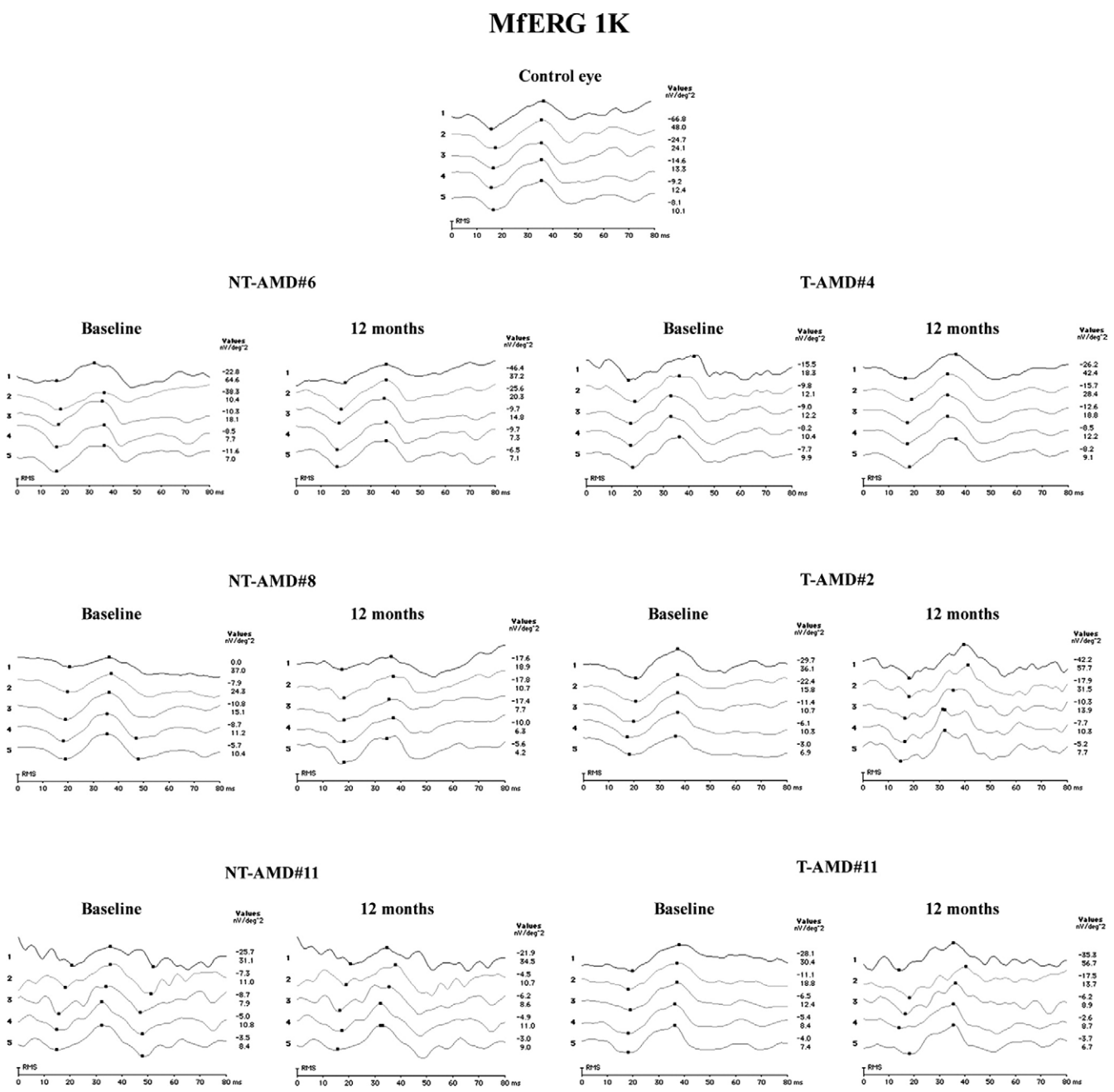

Figure 1. Examples of multifocal electroretinogram (mfERG) first-order response component (K1) recorded in one control eye and in eyes affected by nonadvanced age-related macular degeneration (AMD) in baseline conditions and after 12 months without any treatment (NT-AMD#6, NT-AMD#8, NT-AMD#11) or supplemented with carotenoids and antioxidants (T-AMD#2, T-AMD#4, T-AMD#11). The MfERGs were recorded in response to 61 M-stimuli presented to the central 20°. The local responses were averaged in 5 retinal areas located at various degrees of eccentricity from the fovea: 0° to 2.5° (R1), 2.5° to 5° (R2), 5° to 10° (R3), 10° to 15° (R4), and 15° to 20° (R5). The MfERG responses observed in NT-AMD and T-AMD eyes in baseline conditions were decreased in amplitude with respect to control eyes only when recorded in 0° to 2.5° and 2.5° to 5°. At 12 months, in T-AMD eyes, it was possible to observe an increase of mfERG responses recorded in 0° to 2.5° and 2.5° to 5°, whereas the other mfERG responses were substantially unmodified. In NT-AMD eyes, the five mfERG responses were similar to baseline.