Clinical Trials

Influence of Saffron Supplementation on Retinal Flicker Sensitivity in Early Age-Related Macular Degeneration

Benedetto Falsini,[1,2] *Marco Piccardi,*[1,2] *Angelo Minnella,*[1] *Cristina Savastano,*[1] *Ettore Capoluongo,*[3] *Antonello Fadda,*[4] *Emilio Balestrazzi,*[1] *Rita Maccarone,*[5] *and Silvia Bisti*[5,6,7]

Purpose. To evaluate the functional effect of short-term supplementation of saffron, a spice containing the antioxidant carotenoids crocin and crocetin, in early age-related macular degeneration (AMD).

Methods. Twenty-five patients with AMD were randomly assigned to oral saffron 20 mg/d or placebo supplementation over a 3-month period and then reverted to placebo or saffron for a further 3 months. Focal electroretinograms (fERGs) and clinical findings were recorded at baseline and after 3 months of saffron or placebo supplementation. fERGs were recorded in response to a sinusoidally modulated (41 Hz), uniform field presented to the macular region (18°) at different modulations between 16.5% and 93.5%. Main outcome measures were fERG amplitude (in microvolts), phase (in degrees), and modulation thresholds.

Results. After saffron, patients' fERGs were increased in amplitude, compared with either baseline or values found after placebo supplementation (mean change after saffron, 0.25 log μV; mean change after placebo, -0.003 log μV; $P < 0.01$). fERG thresholds were decreased after saffron supplementation but not placebo, compared with baseline (mean change after saffron, -0.26 log units; mean change after placebo, 0.0003 log units).

Conclusions. The results indicate that short-term saffron supplementation improves retinal flicker sensitivity in early AMD. Although the results must be further replicated and the clinical significance is yet to be evaluated, they provide important clues that nutritional carotenoids may affect AMD in novel and unexpected ways, possibly beyond their antioxidant properties. (ClinicalTrials.gov number, NCT00951288.) (*Invest Ophthalmol Vis Sci.* 2010;51:6118–6124) DOI:10.1167/iovs.09-4995

Age-related macular degeneration (AMD) is a degenerative disease of the macula characterized in the early stage by large, soft drusen, hyper-/hypopigmentation of the retinal pigment epithelium (RPE), and a moderate loss of central vision (age-related maculopathy, according to the International Classification,[1]). In its late stages (i.e., the geographic atrophy of the RPE or the subretinal neovascular membranes), the disease is associated with more severe central visual impairment and can be considered a leading cause of severe, irreversible low vision in elderly persons in the developed world.[2] Changes of the RPE and photoreceptor cells are early events in AMD[3,4] and may significantly affect visual function. There is indeed evidence that subtle visual losses, involving a variety of functions mediated by subpopulations of photoreceptors and/or postreceptoral neurons, can be detected by psychophysical and electrophysiological methods.[5–10] Epidemiologic data[11,12] indicate that several factors may protect against or increase the individual risk of photoreceptor degeneration and dysfunction in AMD. Many risk factors appear to be oxidative,[13] whereas many protective factors are known to act as antioxidants.[14–20] Smoking, being female, and having blue irises,[12,15]—associated with lower retinal concentrations of antioxidants—may increase the risk of AMD. By contrast, various studies[18,19] have indicated that protection from AMD is conferred by the dietary carotenoids lutein and zeaxanthin, which are constituents of macular pigment and antioxidants, whose proposed role in the retina is to quench reactive oxygen species that result from exposure to light.[21,22] As already suggested,[23–25] it is possible that, by increasing the level of protection exerted by retinal antioxidants, the function of damaged, but still viable, photoreceptors could recover. Recent clinical studies[23–25] in which focal, psychophysical, or multifocal electroretinographic (ERG) techniques were used as assays of the outer retinal function (cone photoreceptors/bipolar cells) have shown that dietary antioxidant supplementation may influence macular cone-mediated function early in the disease process. Most important, the large-scale AREDS investigation[26] results indicate that antioxidant supplementation may prevent the development of the most advanced stage of AMD.

Recent experimental findings[27] indicate that saffron, derived from the pistils of *Crocus sativus*, may have a role as a retinal neuroprotectant against oxidative damage. Indeed, saffron has been shown to be protective of both morphology and function in a rat model of light-induced photoreceptor degeneration.[27] Saffron's major constituents, the compounds crocin and crocetin, which are derivatives of carotenoids, are powerful antioxidants, with antiapoptotic characteristics. These

From [1]Dipartimento di Scienze Oftalmologiche e Otorinolaringologiche e [3]Dipartimento di Biochimica e Biologia Molecolare, Università Cattolica del S. Cuore, Rome, Italy; [4]Technology and Health Department, Istituto Superiore di Sanità, Rome, Italy; [5]Dipartimento di Scienze e Tecnologie Biomediche, Università dell'Aquila, L'Aquila, Italy; [6]ARC (Australian Research Council) Centre of Excellence in Visual Science, Canberra, Australia; and [7]Istituto Nazionale Biostrutture e Biosistemi (INBB), Rome, Italy.

[2]Contributed to the work presented here and therefore should be regarded as equivalent authors.

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Corresponding author: Benedetto Falsini, Dipartimento di Scienze Oftalmologiche e Otorinolaringologiche, Universita' Cattolica del S. Cuore, Lgo F. Vito 1, 00168, Roma, Italy; md0571@mclink.it.

properties, together with preclinical evidence, provide a strong rationale for testing the effect of saffron supplementation in early AMD.

The purpose of the present double-blind, placebo-controlled, crossover study was to evaluate whether short-term saffron supplementation changes retinal function in patients with early AMD. The macular, cone-mediated ERG in response to high-frequency flicker (focal ERG, fERG), recorded according to a published protocol designed specifically to assess cone flicker sensitivity in early AMD,[28] was used as the main outcome variable. This was a no-sponsor, registered clinical trial.

Materials and Methods

Twenty-five consecutive patients (mean age, 65 ± 5 years; range, 54–84; 12 men and 13 women) with a diagnosis of bilateral early AMD, were recruited prospectively over an interval of 8 months from the outpatient service of the institution. Each patient underwent standard general and ophthalmic examinations. Clinical diagnosis of early AMD was established by direct and indirect ophthalmoscopy, as well as retinal biomicroscopy, when any of the following primary lesions in the macular area (i.e., the area within an eccentricity of approximately 2 disc diameters from the fovea) of one or both eyes was identified: soft distinct or indistinct drusen; areas of hyperpigmentation associated with drusen; or areas of hypopigmentation of the RPE associated with drusen, without any visibility of choroidal vessels,. All patients met the following inclusion criteria: best corrected visual acuity of 0.3 or better in the study eye, central fixation (assessed by direct ophthalmoscopy), normal color vision with Farnsworth D-15 testing, no signs of other retinal or optic nerve disease and clear optical media. Five patients had moderate systemic hypertension. No other systemic diseases were present. None of the patients was taking medications (e.g., chloroquine) that are known to affect macular function or to interfere with carotenoid absorption. AMD lesions of the study eyes were graded on stereoscopic fundus photographs, as previously described.[28] A macular grading scale, based on the international classification and grading system,[1] was used by a single grader who evaluated the photographs while masked to subject characteristics and fERG results. The presence of basic AMD lesions was noted within each of the nine subfields delimited by a scoring grid. Fluorescein angiography was also performed in all study eyes at the time of the diagnosis, to confirm the presence of early AMD lesions and exclude geographic atrophy or RPE detachment. According to the results of grading, intermediate AMD was diagnosed in all eyes,[29] with one or more drusen (≥63 μm) and/or focal hypo-/hyperpigmentation within the macular region. The average number of drusen was 9 (range, 4–22). Focal RPE abnormalities extending for at least 10% of one of the middle subfield areas in the macular region were present in 6 of 25 patients. The research adhered to the tenets of the Declaration of Helsinki. The study was approved by the Ethics Committee/Institutional Review Board of the Catholic University. Written, informed consent was obtained from each study participant after the purpose and procedures of the study were fully explained.

Treatment and Testing Schedule

The 25 patients with early AMD were divided into two groups: 11 were treated with oral supplementation of a daily dose (20 mg) of saffron for 90 days, and 14 underwent placebo treatment during the same period. At the end of a 90-day period, the groups were crossed over and the patients were assigned placebo or saffron supplementation. There was a period of rest of 15 days between the two arms. This washout period was considered sufficient on the basis of the data provided by preclinical studies[27] (see also the Discussion section). Patients were assigned to the two treatment groups by two ophthalmologists (AM, CS) who did not participate in electrophysiological and clinical data collection. Clinical and demographic data of the patients are summarized in Table 1. In all patients, a clinical examination, including visual acuity testing with a calibrated standard Snellen chart, fundus examination by direct and indirect ophthalmoscopy, and fERG testing, was performed at study entry (baseline) and after 90 days of treatment or placebo. Clinical and fERG examinations were conducted on the same day, with ophthalmoscopy always performed after fERG recordings. During the entire period of supplementation or placebo, no other systemic pharmacologic treatments were given. In all cases, compliance was judged to be satisfactory, since none of the treated subjects refrained, for any reason, from taking the daily dose of supplement or placebo during the treatment period. No adverse side effects were reported.

Electrophysiological Methods

fERG testing was performed according to a previously published technique.[23,28] Briefly, ERGs were elicited by the LED-generated sinusoidal luminance modulation of a circular uniform field (diameter, 18°; mean luminance, 80 cd/m^2; dominant wavelength, 630 nm), presented at the frequency of 41 Hz on the rear of a Ganzfeld bowl, illuminated at the same mean luminance as the stimulus. This technique was developed according to the indications of published clinical studies, in which the fERG response to sinusoidal flicker stimulation was used to test retinal flicker sensitivity in comparison to psychophysical flicker sensitivity in normal and pathologic conditions.[30,31] In the general recording protocol, a series of fERG responses was collected at different modulation depths, quantified by the Michelson luminance contrast formula: $100\% \times (L_{max} - L_{min})/(L_{max} + L_{min})$, where L_{max} and L_{min} are maximum and minimum luminance, respectively, between 16.5% and 93.8% in 0.1- to 0.3-log-unit steps (16.5%, 33.1%, 44.8%, 63.6%, 77.2%, and 93.8%). In some patients, the signal-to-noise ratio (S/N) at the modulation of 93.5% was not large enough to allow recording of the whole response family. In those cases, response collection was limited to the highest or the two highest modulation depths. In Table 1, the number of responses that were significantly different from the noise level, collected at every visit, is reported for each patient.

fERGs were recorded monocularly by means of Ag-AgCl superficial cup electrodes taped over the skin of the lower eyelid. A similar electrode, placed over the eyelid of the contralateral, patched eye, was used as the reference (interocular ERG[32]). fERG signals were amplified, band-pass filtered between 1 and 250 Hz (−6 dB/octave), sampled with 12-bit resolution, (2-kHz sampling rate), and averaged. A total of 1600 events (in eight blocks of 200 events each) were averaged for each stimulus condition. The sweep duration was kept equal to the stimulus period. Single sweeps exceeding the threshold voltage (25 μV) were rejected, to minimize noise coming from blinking or eye movements. A discrete Fourier analysis was performed off-line to isolate the fERG fundamental harmonic and estimate its amplitude (in μV) and phase (in degrees). Component amplitude and phase were also calculated separately for partial blocks (200-event packets) of the total average, from which the standard error of amplitude and phase estimates were derived to test response reliability. Averaging and Fourier analysis were also performed on signals sampled asynchronously at 1.1 times the temporal frequency of the stimulus, to give an estimate of the background noise at the fundamental component. An additional noise estimate at the fundamental harmonic was obtained by recording responses to a blank, unmodulated field kept at the same mean luminance as the stimulus. In all records, the noise amplitudes recorded with both methods were ≤0.053 μV.

In all subjects, the fERG testing protocol was started after a 20-minute period of preadaptation to the stimulus mean illuminance. Pupils were pharmacologically (tropicamide 1%) dilated to 8 to 9 mm. Subjects fixated (from a distance of 30 cm) on the center of the stimulation field with the aid of a small (15 minutes of arc) fixation mark. An fERG response was first collected at the maximum modulation depth (93.5%) included in the protocol and was evaluated with respect to reliability and S/N ratio. In all patients, the responses at 93.5% modulation satisfied the following criteria: standard deviation estimates of <20% (variation coefficient) and 15° for

Table 1. Demographic and Clinical Findings in Patients with Early AMD

Patient	Age (y), Sex	Acuity	Treatment	Fundus*	Drusen *n*; Size (μm)†	RPE Abnormalities (% Area)‡	FERG Responses§
1	69, M	0.8	S, P	Soft drusen, middle subfield	9; ≥63 to <125	10	3 (B), 5 (S), 4 (P)
2	75, F	0.7	S, P	Soft drusen, middle subfield	6; ≥63 to <125	—	4 (B), 6 (S), 5 (P)
3	84, F	0.5	P, S	Soft confluent drusen, middle subfield	8; ≥125 to <175	25	1 (B), 1 (P), 3 (S)
4	75, F	0.8	S, P	Soft and hard drusen, central and middle subfield	10; ≥63 to <125	—	6 (B), 4 (S), 4 (P)
5	63, F	0.7	S, P	Soft drusen, middle subfield	6; ≥63 to <125	—	5 (B), 5 (S), 5 (P)
6	63, F	0.7	P, S	Soft drusen, central and middle subfield	10; ≥125 to <175	—	4 (B), 4 (P), 5 (S)
7	67, F	0.3	P, S	Soft drusen, central and middle subfield	12; ≥63 to <125	—	1 (B), 2 (P), 3 (S)
8	67, M	0.7	P, S	Soft confluent drusen, middle subfield	12; ≥125 to <175	—	2 (B), 2 (P), 3 (S)
9	83, M	0.9	S, P	Soft drusen, central and middle subfield	5; ≥125 to <175	—	5 (B), 4 (S), 4 (P)
10	67, F	0.8	P, S	Soft drusen, middle subfield	6; ≥63 to <125	—	4 (B), 6 (P), 6 (S)
11	80, F	0.4	P, S	Soft drusen, central and middle subfield	8; ≥63 to <125	10	4 (B), 5 (P), 3 (S)
12	73, F	0.8	S, P	Soft drusen, middle subfield	5; ≥63 to <125	—	6 (B), 6 (S), 6 (P)
13	83, M	0.5	P, S	Soft drusen; hyperpigm., middle subfield	8; ≥63 to <125	15	1 (B), 1 (P), 5 (S)
14	83, M	0.4	P, S	Soft confluent drusen, central and middle subfield	12; ≥175 to <250	—	2 (B), 3 (P), 5 (S)
15	68, M	0.8	P, S	Soft drusen, middle subfield	6; ≥175 to <250	—	6 (B), 6 (P), 6 (S)
16	70, F	0.8	S, P	Soft confluent drusen, hypopigm., middle subfield	22; ≥125 to <175	10	6 (B), 4 (S), 5 (P)
17	70, F	0.3	P, S	Soft drusen, hyperpigm., hypopigm., middle subfield	9; ≥175 to <250	40	6 (B), 3 (P), 5 (S)
18	55, F	0.9	S, P	Soft drusen, hyperpigm., middle subfield	4; ≥125 to <175	5	3 (B), 6 (S), 5 (P)
19	55, F	0.9	P, S	Soft drusen. and hyperpigm., middle subfield	4; ≥175 to <250	4	2 (B), 5 (P), 6 (S)
20	67, M	0.8	S, P	Soft drusen. and hyperpigm., middle subfield	18; ≥125 to <175	5	3 (B), 5 (S), 4 (P)
21	60, M	0.6	S, P	Soft drusen. and hyperpigm., central and middle subfield	16; ≥125 to <175	5	4 (B), 6 (S), 6 (P)
22	69, M	0.9	P, S	Soft confluent drusen., central and middle subfield	13; ≥125 to <175	—	5 (B), 5 (P), 6 (S)
23	68, M	1.0	P, S	Soft drusen, central and middle subfield	5; ≥63 to <125	—	4 (B), 5 (P), 6 (S)
24	63, F	1.0	S, P	Soft drusen, central and middle subfield	8; ≥63 to <125	—	4 (B), 6 (S), 5 (P)
25	58, M	0.8	P, S	Soft drusen, and hyperpigm., middle subfield	6; ≥63 to <125	5	5 (B), 5 (P), 6 (S)

S, saffron treatment; P, placebo; hyperpigm, hyperpigmentation.

* Macular appearance with reference to: drusen type, confluence and location; RPE abnormalities type and main location.[1]

† Drusen number and size according to the International Classification and Grading System.[1]

‡ Percentage of the main middle subfield area[1] affected with RPE abnormalities.

§ Number of FERG responses that were above noise level (i.e., S/N ratio ≥ 3) at the different modulation depths of the recording protocol; 6, S/N ratio ≥3 at all modulation depths; 5, S/N ratio <3 at the lowest modulation depth; 4, S/N ratio <3 at the two lowest modulation depths; (B), baseline; (S), after saffron supplementation; (P), after placebo supplementation.

the amplitude and phase, respectively, and an S/N ratio ≥4. In AMD patients having a response S/N ≥8, fERG signals were also acquired in sequence for six values of modulation depth between 16.5% and 93.5%, presented in an increasing order. For each stimulus modulation depth, fERG responses were accepted only if their S/N ratio was ≥2. As described elsewhere,[28] fERG log amplitudes were plotted for each patient as a function of log modulation depth. The resulting function slope was determined by linear regression. From the same regression line, fERG threshold was estimated from the value of log modulation depth yielding a criterion amplitude, corresponding to an S/N ratio of 3.[28]

Statistical Analysis

From each patient included in the study, one eye, typically the eye with the best visual acuity, was selected and designated as the study eye. The data from the study eyes were included in the statistical analysis. Main outcome variables were fERG amplitude, phase, fERG function slope, and threshold. A secondary outcome variable was visual acuity. fERG amplitude data underwent logarithmic transformation to better approximate normal distribution. fERG slope and threshold are reported as $\log_{10}$ values. In all statistical analyses, standard error and 95% confidence interval (CI) of the means were used for within-group comparisons.

Sample size estimates were based on those in previous investigations,[23,28] in which the between- and within-subjects variability (expressed as the standard deviation) of fERG parameters was determined in patients with early AMD. Assuming between- and within-subject SDs in fERG amplitude and phase of 0.1 log μV and 20°, respectively, the sample sizes of patients assigned to both saffron and placebo provided a power of 80%, at an $\alpha = 0.05$, for detecting test–retest differences in each group (i.e., 90 days minus baseline) of 0.1 (SD 0.1) log μV and 30° (SD 20) in amplitude and phase, respectively. Given the absolute mean amplitude and phase values of the patients' fERGs, these differences were considered to be clinically meaningful, since they corresponded approximately to a 25% to 30% change in either amplitude or phase. A study[23] in patients with early AMD showed that test–retest variabilities in fERG amplitude and thresholds are significantly smaller than this change. The patient sample size also provided a power of 90%, at an $\alpha = 0.05$ for detecting within-group differences in fERG amplitude and phase (i.e., 90 days minus baseline) of 0.15 (SD 0.1) log μV and 40° (SD 20), respectively.

Electrophysiological results were analyzed by multivariate statistics (multivariate analysis of variance for repeated measures, MANOVA). Dependent variables in the MANOVA design were fERG log amplitude and phase. Stimulus modulation depth and treatment (saffron versus placebo) were the independent variables. fERG log thresholds and slopes, estimated from the corresponding functions, were individually determined by considering only responses with an S/N ≥ 3. Repeated-measures ANOVA on log threshold and slope as dependent variables was used to compare the fERG results recorded at 90 days with the corresponding baseline values, after either saffron or placebo supplementation. Visual acuity changes across treatments were analyzed, either individually for every pa-

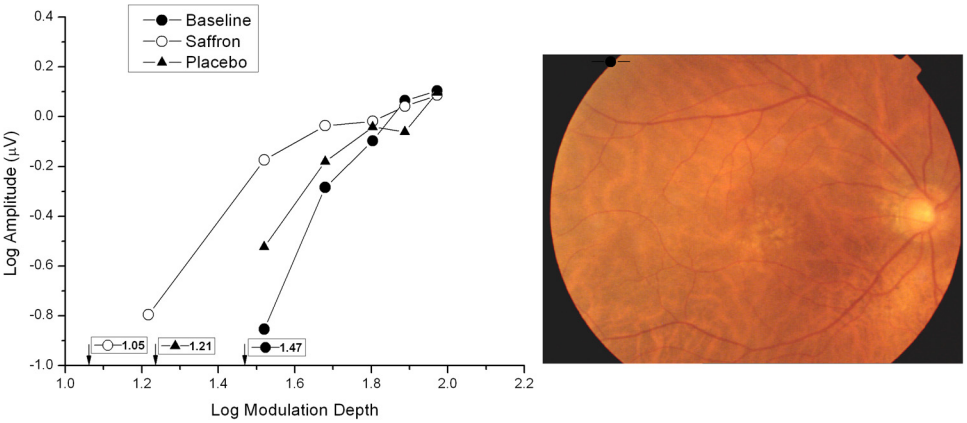

FIGURE 1. *Left*: Representative fERG functions, recorded from AMD patient 22 (Table 1) at baseline and after 90 days of saffron or placebo supplementation, are shown in plots of response amplitudes as a function of modulation depth. *Arrows*: fERG modulation thresholds determined for each fERG function. *Right*: a color fundus photograph from the study eye, showing confluent soft drusen in the foveal region.

tient or as group means by repeated-measures ANOVA, assuming normal distribution.

In all the analyses, results with a $P < 0.05$ were statistically significant.

RESULTS

In Figure 1, representative fERG functions, recorded from AMD patient 22 (Table 1) at baseline and after 90 days of saffron or placebo supplementation, are shown in plots of the response amplitudes as a function of modulation depth. Arrows in the plot indicate the fERG modulation thresholds determined for each fERG function. A color fundus photograph of the study eye shows confluent soft drusen in the foveal region. fERG amplitude increased from baseline after saffron but not placebo supplementation, mainly at intermediate stimulus modulations (33.1%–47.1%). Responses at the lowest modulation (16.5%) were recordable only after saffron supplementation. These changes resulted in a reduced fERG threshold, as predicted by the slope of linear regression best fitted to the data points, which decreased after saffron treatment, compared with baseline.

In Figure 2A, mean (± SEM) fERG log amplitudes recorded in patients at baseline and after saffron or placebo supplementation, are plotted as a function of log modulation depth. Data at the lowest modulation have been omitted from the plot, since there were too few data points at baseline to allow a suitable comparison of the group means. It can be seen that after saffron, but not after placebo, mean log amplitudes increased at 90 days compared with baseline values at all modulation depths. There was a tendency for the amplitude increase to be more pronounced at intermediate (44.8%, 63.6%, and 77.2%) compared with the lowest or highest modulation depth. fERG amplitude increased from baseline by 0.25 log units, taking the average change across the different modulations. MANOVA showed a significant ($P < 0.01$) effect of treatment (*F*-ratio, 12.6; *df*, 2, 23; $P < 0.001$) and modulation depth (*F*-ratio, 27.6; *df*, 5, 20; $P < 0.01$) and a significant interaction of treatment by modulation depth (*F*-ratio, 2.6; *df*, 10, 15; $P < 0.05$). The increase in mean amplitude after saffron treatment resulted in a decrease in both threshold and slope of the fERG versus modulation depth function. Slopes and thresholds are shown, as box plots in Figure 2B. In each box, the symbol is the mean, the box indicates the median and interquartile range, and the bars the 99 percentiles. Compared with baseline, modulation threshold of the fERG decreased by 0.26 log units (on average) after saffron supplementation, whereas it was virtually unchanged (0.003 log units on average) after placebo. fERG threshold changes across treatments were significant (*F*-ratio, 16; *df*, 2, 22; $P < 0.001$). fERG slope decreased from baseline after saffron (by 1.6 log units on average) and, although to a much lesser extent, after placebo (0.8 log units) supplementation. fERG slope changes across treatments were significant (*F*-ratio, 5.4; *df*, 2, 22; $P < 0.05$).

Figure 3 shows mean fERG phases (±SEM) recorded in all patients at baseline and after saffron or placebo supplementation, plotted as a function of modulation depth. Mean fERG phase did not show a consistent trend in its changes at 90 days compared with baseline values, either after saffron or placebo

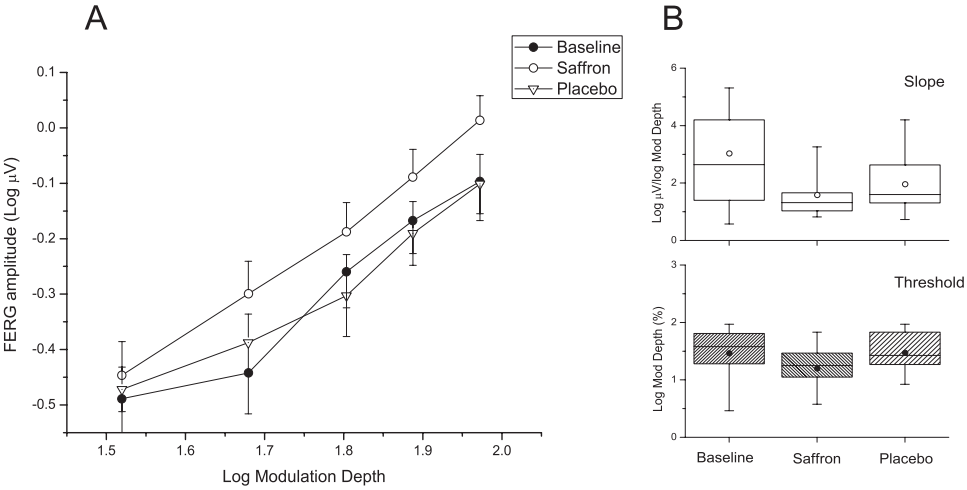

FIGURE 2. (**A**) Mean (±SEM) fERG log amplitudes recorded in patients at baseline and after saffron or placebo supplementation, are plotted as a function of log modulation depth. Data at the lowest modulation have been omitted from the plot, since there were too few data points at baseline to allow a suitable comparison of the group means. (**B**) fERG slopes and thresholds recorded at baseline and after saffron or placebo supplementation are shown as box plots. In each box, the symbol is the mean, the box indicates the median and interquartile range, and the bars the 99 percentiles.

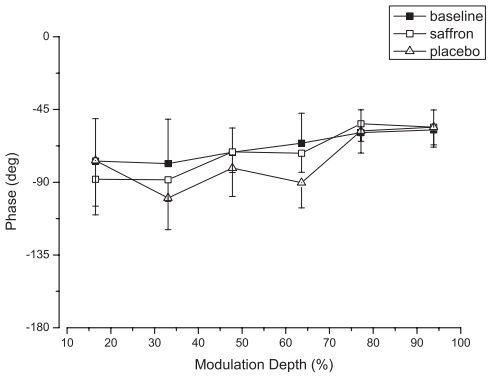

FIGURE 3. Mean fERG phases (±SEM), recorded from patients at baseline and after saffron or placebo supplementation, are plotted as a function of modulation depth.

supplementation. MANOVA did not show any significant effect of treatment or modulation depth.

Individual fERG log amplitudes recorded at baseline and after saffron or placebo supplementation are reported in Figure 4. Each symbol indicates an individual patient. It can be seen that, after saffron, fERG amplitudes increased especially at intermediate modulations, as indicated by the increased density of data points. The same effect cannot be seen after placebo. The result of saffron supplementation mainly consisted of an increase in fERG amplitude at intermediate modulations, resulting in most patients in a decrease in the slope and threshold from the baseline value. In 14 of 25 patients, fERG threshold decreased after saffron but not placebo supplementation by 0.3 log units or more compared with baseline, indicating a substantial increase in response sensitivity to the stimulus modulation depth.

The results of clinical examinations performed after 90 days of saffron or placebo supplementation were compared with baseline data, to evaluate whether changes in visual acuity and/or clinical picture would be detectable after treatment. Mean Snellen acuity was 0.70 (SD 0.22) at baseline, 0.80 (SD 0.20) after saffron supplementation, and 0.72 (SD 0.24) after placebo. The mean acuity changes across treatments were significant (*F*-ratio, 6.8; *df*, 2, 22; $P < 0.01$). After saffron supplementation, visual acuity increased in 20 patients (by one line) and remained unchanged in 5. After placebo, visual acuity was unchanged compared with baseline values in all patients. Ophthalmoscopic appearance was unchanged in all patients after both saffron and placebo supplementation.

DISCUSSION

In the present pilot study, daily supplementation of 20 mg/d saffron for 90 days was associated with statistically significant changes in the macular fERG parameters (amplitude and modulation threshold) in patients with early AMD. No such changes were observed in the same patients after placebo supplementation, supporting a specific effect of the supplement on improvement of retinal function. The present study had a randomized, placebo-controlled, crossover design. The crossing over of our AMD patients was justified by the preclinical results[27] (Maccarone et al., unpublished data, 2008) indicating that the protective effect of systemic saffron on the rat retinas vanishes rapidly right after the end of supplementation. Therefore, a relatively short resting period of 15 days between the two arms of the study was estimated to be a sufficient washout period. On the other hand, if saffron had exerted a carryover effect, then the statistical significance of the results, estimated by evaluating the effect of the drug in comparison with that of the placebo, should have been completely obscured or greatly reduced by the crossover design. The postsupplementation changes in the mean fERG threshold observed in our AMD patients reflected an increase in mean response amplitude that was more pronounced at intermediate modulation depths, as indicated by the data in Figures 2 and 4. The responses at the lowest modulation depth (16.5% and 33.1%) became recordable in some patients only after the supplementation (see Fig. 4). As a consequence, not only the mean threshold but also the mean slope of the fERG versus modulation depth function tended to decrease after supplementation. By contrast, temporal response properties were unaffected, as shown by the phase data reported in Figure 3, indicating that no saffron-induced effect was detectable on the time constant and/or temporal responsiveness of the fERG retinal generators.

The technique used in this study has been successfully used by other groups in several clinical studies, to test both physiologic and pathologic conditions.[30,31] The fERG threshold technique has been shown to be a valid clinical method of estimating retinal flicker sensitivity in normal retinal physiology as well as in patients with a degenerative disease of the outer retina,[30,31] thus supporting the use of such an approach to advancing research in the AMD field. Indeed, it is a sensitive tool for analyzing cone photoreceptor function and may be

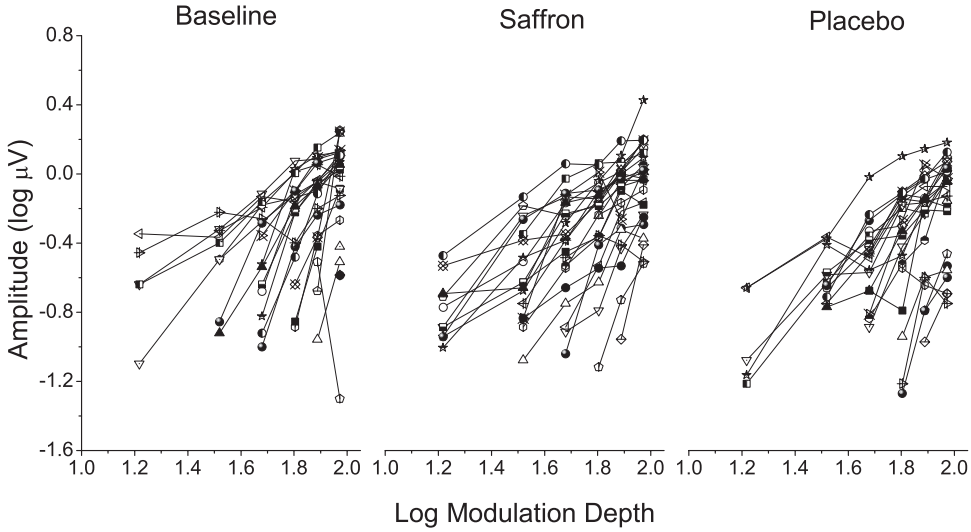

FIGURE 4. Individual fERG log amplitudes at the various modulation depths, recorded from all patients at baseline and after saffron or placebo supplementation. Each symbol indicates an individual patient.

useful in clinical trials,[23] such as this study, which was intended to test potential neuroprotective agents that delay and/or rescue photoreceptor damage in the early stages of AMD.

The current results appear to somewhat counter the traditional beliefs of the scientific community—that is, that oxidative stress represents chronic and cumulative damage, antioxidants (if successful) protect against further oxidative injury only, and such a benefit becomes apparent only after prolonged use of the antioxidant(s).[19,20] The present findings, obtained in a double-blind, placebo-controlled study, unambiguously show an improvement of retinal flicker sensitivity after short-term saffron supplementation in early AMD eyes. This is not the first study, however, to show a short-term functional effect of oral antioxidants. Trials conducted by our group[23] and others[24,25] have shown significant improvements in macular function (by using either electrophysiological or psychophysical techniques) after limited periods (6–12 months) of antioxidant supplementation. We believe that the ERG changes observed in the present study by no means indicate that short-term saffron supplementation may reverse the photoreceptor damage induced by chronic oxidative injury. Rather, a more plausible and parsimonious interpretation of our findings is that saffron exerts a measurable and temporary beneficial effect on the dysfunctional fERG generators, photoreceptors, and/or bipolar cells[33] through a mechanism that still must be fully elucidated, but involves the neuroprotective properties already documented in a preclinical study.[27]

In recent work, Maccarone et al.[27] provided evidence of the neuroprotective potential of saffron treatment in albino rats, in which an artificial induction of apoptosis was obtained in photoreceptors by exposure to high-intensity light. Cell death was thought to have resulted from oxidative stress induced by prolonged increase in oxygen tension and photo-oxidation. Saffron extracted from the stigmata of *Crocus sativus* contains high concentrations of two major compounds, crocin and crocetin,[34] whose multiple C=C bonds confer the stigmata color, fragrance, taste, and antioxidant potential.[35–37] Crocin and crocetin, the carotenoid derivatives, may act through a protective mechanism similar to that seen with carotenoid supplementation.[20,21,23] It has been recently reported[34] that crocins are able to activate metabolic pathways to protect cells from apoptosis and to reduce light-induced death in isolated photoreceptors,[35,36] whereas crocetin[34] increases oxygen diffusivity through liquids, such as plasma. Kanakis et al.[37] showed that metabolites of antioxidant flavonoids bind directly to DNA and induce its partial conformation to β-DNA, thereby protecting the cell from damage. These components have the potential of acting in humans as protective agents against oxidative damage for the aging and AMD retinas, whose disease pathophysiology has been linked (see, for example, Ref. 38) to light-induced oxidative damage to the outer retina.

This study did not provide clinical evidence of saffron neuroprotection in AMD. The observed marginal improvement of visual acuity in our AMD patients, although statistically significant when the group means are compared, is well within the range of test–retest variability in early AMD eyes,[23,24] and it is not strong enough to support a protective effect of saffron. A correlation between fERG sensitivity improvement and visual function could have been expected, given the reported correlation between macular ERG parameters and visual acuity in patients with macular degeneration. (see, for example, Ref. 39). However, it should be emphasized that the present fERG technique, clinically used and validated in the past by other investigators,[30,31] taps the activity of a central retinal area much larger than that subserving visual acuity. It is therefore possible that the effects of treatment involved predominantly the parafoveal regions, which are known to be more vulnerable early in the disease process.[40] Alternatively, psychophysical tests aimed at evaluating the function of central regions in the posterior pole may have correlated better with fERG[30,31] for detecting treatment-induced changes in visual function.

The present data indicate that saffron supplementation may induce a short-term, significant improvement in retinal function in early AMD. To our knowledge, this effect has not been previously documented. Although such results must be further replicated and the clinical significance is yet to be evaluated, they provide important clues that nutritional carotenoids may impact AMD in novel and unexpected ways, possibly beyond their antioxidant properties.

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