RETINAL DISORDERS

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Saffron therapy for the treatment of mild/moderate age-related macular degeneration: a randomised clinical trial

Geoffrey K. Broadhead [1,2,3] · John R. Grigg [1] · Peter McCluskey [1] · Thomas Hong [2,3] · Timothy E. Schlub [4] · Andrew A. Chang [1,2,3]

Abstract

Purpose To assess the efficacy and safety of oral saffron, a natural antioxidant, in treating mild/moderate age-related macular degeneration (AMD).

Methods Randomised, double-blinded, placebo-controlled crossover trial of 100 adults (> 50 years) with mild/moderate AMD and vision > 20/70 Snellen equivalent in at least one eye. Exclusion criteria included confounding visual lesions, or significant gastrointestinal disease impairing absorption. Participants were given oral saffron supplementation (20 mg/day) for 3 months or placebo for 3 months, followed by crossover for 3 months. Participants already consuming Age-Related Eye Diseases Study (AREDS) supplements or equivalent maintained these. Primary outcomes included changes in best-corrected visual acuity (BCVA) and changes in multifocal electroretinogram (mfERG) response density and latency. Secondary outcomes included safety outcomes and changes in mfERG and BCVA amongst participants on AREDS supplements.

Results Mean BCVA improved 0.69 letters ($p = 0.001$) and mean-pooled mfERG latency reduced 0.17 ms ($p = 0.04$) on saffron compared to placebo. Amongst participants on AREDS supplements, mean BCVA improved 0.73 letters $p = 0.006$) and mean-pooled mfERG response density improved 2.8% ($p = 0.038$). There was no significant difference in adverse event occurrence ($p > 0.10$).

Conclusion Saffron supplementation modestly improved visual function in participants with AMD, including those using AREDS supplements. Given the chronic nature of AMD, longer-term supplementation may produce greater benefits.

Keywords Clinical trials · Age-related macular degeneration · Saffron · Electroretinogram · Nutritional supplementation

Introduction

Age-related macular degeneration (AMD) remains the most common cause of irreversible visual loss in the developed world, and its impact is predicted to rise as populations age [1]. Despite recent advances in the treatment of advanced disease, particularly neovascular AMD (nAMD) [2, 3], therapeutic options for the most common earlier forms of AMD remain limited. More than 10 years after the publication of the initial landmark, Age-Related Eye Diseases Study (AREDS), dietary modification and nutritional augmentation with AREDS-based supplements still remain the basis of AMD management [4, 5]. Various additions to these therapies have been trialled, but only the addition of lutein/zeaxanthin, two carotenoids that accumulate in retinal tissue, have been well validated [6].

Saffron (*Crocus sativus*) is a well-known Middle-Eastern spice that has been shown to possess significant antioxidant potential, and has been trialled as a therapy for a range of chronic degenerative diseases [7, 8]. Much of its antioxidant potential stems from the presence of a range of molecules within saffron that have significant chemical similarities to carotenoids [9]. As some carotenoids are known to be effective in treating AMD, it is plausible that other carotenoid molecules may also be effective therapies for AMD without the potential systemic side-effects of carotenoids such as beta-

✉ Andrew A. Chang
  achang@sydneyretina.com.au

[1] Save Sight Institute, The University of Sydney, 8 Macquarie Street, Sydney, NSW 2000, Australia

[2] Sydney Institute of Vision Science, Sydney, Australia

[3] Sydney Retina Clinic & Day Surgery, Sydney, Australia

[4] Sydney School of Public Health, The University of Sydney, Sydney, Australia

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carotene. Saffron and its constituents also have no known significant side-effects at commonly consumed dosages, making it an attractive potential therapeutic for chronic conditions where long-term use may be required [10]. Preliminary investigations have suggested that saffron may be a useful treatment for AMD, however these studies may not have been adequately powered and standardised to assess efficacy [11, 12]. In an effort to better identify possible therapies for this increasingly burdensome condition, we investigated the role of saffron supplementation as a therapy for AMD.

Subjects & methods

A prospective, randomised, placebo-controlled, double blind crossover trial (Registered on Australian and New Zealand Clinical Trials Registry, June 2012, ACTRN 12612000729820) was conducted on 100 participants attending a single tertiary retinal clinic between January 2013 and March 2015. Power calculations indicated that such a sample provided 90% power to detect a difference of 15 nv·deg.$^{-2}$ in N1P1 response densities on multifocal electroretinogram (mfERG) to a significance level of 0.05, and allowed for a drop-out rate of 10%. All participants provided written consent, and Institutional Ethical Review was obtained via the Sydney South-West Area Health Human Research Ethics Committee prior to study commencement. This research adhered to the tenets of the Declaration of Helsinki.

Inclusion/exclusion criteria

All participants underwent baseline dilated ophthalmic examination and general medical review to confirm the presence of AMD and to assess eligibility under the exclusion/inclusion criteria listed below. Inclusion criteria were as follows: (a) age greater than 50 years, (b) moderate severity AMD (defined as AREDS grade 2 or 3, Fig. 1) affecting at least one eye [13], c) best-corrected visual acuity (BCVA) better than 55 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters (approximately 20/70 Snellen equivalent) in the eye(s) meeting criteria a and b) and (d) ability to provide written consent. Exclusion criteria were: (a) presence of any confounding visual lesion in the study eye(s), including neovascular AMD, proliferative diabetic retinopathy, macular hole or epiretinal membrane, prior macular off retinal detachment, uncontrolled glaucoma, significant corneal or lenticular opacities or active uveitis; (b) prior macular laser therapy for AMD or other retinal disorders; c) prior or current intravitreal pharmacotherapy and (d) gastric or hepatic disorders altering either absorption or metabolism of orally administered saffron. In participants in whom both eyes met eligibility criteria, both eyes were included in the analysis, with 167 eyes meeting inclusion/exclusion criteria.

AMD

The diagnosis of AMD was confirmed by both dilated retinal examination by a retinal specialist (AC) and dilated retinal fundus photography (Zeiss Visucam NM/FA, Zeiss Industries, Dublin). Macular centred fundus photos (45°) were graded according to the AREDS trial criteria [13]. All participants also underwent baseline spectral domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF). Where necessary, additional investigations, including fundus fluorescein angiography and indocyanine green angiography, were undertaken to evaluate potential exclusion criteria such as nAMD.

Randomisation & treatment

Following enrolment, all participants were consecutively randomised by a pre-determined computerised random-number generator sequence to receive either 20 mg saffron (treatment; S) or placebo (P) for 3 months (90 days) administered as an unlabelled oral capsule consumed once daily in the morning with meals. Both types of capsule were identical in colour and size, and provided in numbered containers. This dosage of saffron was chosen based on results from previously published pilot studies of saffron in AMD [11]. After the initial 3-month period, all participants crossed-over into the other arm and received treatment with the other intervention (saffron or placebo as appropriate) for a further 3 months. Compliance with treatment was assessed by interview at monthly visits.

Participants, study personnel and statistical analysts were masked to treatment allocation throughout the study period. Capsules were prepared by an independent contractor (Vitex Pharmaceuticals, Eastern Creek, NSW, Australia) who de-identified the containers in which capsules were provided prior to delivery to study personnel. Saffron was sourced from a single supplier who produces saffron at a single Australian location in a standardised method to reduce variability in supply. A single, unrelated researcher not in direct participant contact had access to the treatment code. Analysis was conducted by an independent statistician with no direct participant contact who was provided coded, de-identified information by study personnel, and then independently provided the study code by the sole researcher with this code.

Participants were requested to maintain their usual diet throughout the trial, and to continue on any supplements (including AREDS-based therapies) they had been consuming prior to study commencement. For sub-group analysis purposes, supplements were further subdivided into AREDS-based therapies (containing zinc, copper, Vitamin C, Vitamin E, but no beta-carotene or lutein-zeaxanthin), and separately also into a lutein-zeaxanthin supplement group.

Study protocol

All participants underwent monthly assessment for a period of 6 months. At each visit, complete ophthalmic examination was undertaken, including: (a) intraocular pressure (IOP) monitoring via Goldmann applanation tonometry, (b) adverse event monitoring, (c) standardised BCVA assessment in ETDRS letters and (d) colour fundus photography.

Additionally, at the baseline, 3- and 6-month visits, participants also underwent flicker perimetry (FP), microperimetry (MP), SD-OCT, FAF, lens grading using AREDS lens assessment criteria [14] and multifocal electroretinogram (mfERG) assessment. Electroretinography and perimetric examinations were performed prior to any investigations that may have affected photoreceptor response, such as fundus examination, colour photography, OCT, FAF or fundus photography.

Autofluorescence and optical coherence tomography

SD-OCT was conducted using a 19-line, 1024 A-scans per line scan via a Heidelberg Spectalis system (Heidelberg Industries, Heidelberg, Germany), and inbuilt Heidelberg licenced software with eye tracking and image recognition (Tru-Track and AutoRescan respectively) was employed to ensure continuity of the scan location. All scans were reviewed, recentred and resegmented as necessary by two independent graders, with any disputes adjudicated by a third, independent grader. Central macular thickness (CMT) was measured via SD-OCT within the central 1-mm ETDRS circle, and was defined as the distance between the Internal Limiting Membrane and Bruch's Membrane.

FAF was conducted using a Heidelberg Spectralis FAF acquisition module, and hypoautofluorescence area (a measure of retinal atrophy size) was measured in mm^2 using FAF images by two independent graders, with any disputes > 15% in the area being adjudicated by a third, independent grader.

Flicker perimetry

FP was conducted using a Medmont M7000 perimeter (Medmont Industries, Melbourne, Australia) in accordance with previously published methodology [15]. Briefly, the M700 is a bowl perimeter that presents Goldmann size III light-emitting diode stimuli with a maximum single point luminescence of 320 $cd·m^{-2}$ and a background illumination of 3.2 $cd·m^{-2}$. The automated flicker mode utilises a ZEST-fast Bayesian threshold logic, and tests participants using an array of 48-points at 1°, 3°, 6° and 10° from central fixation, with a duration of 800 ms^{-1} for flickering stimuli.

All participants initially received verbal instructions regarding test protocol and underwent a 2-min practice session in which non-flickering errors were highlighted and flickering responses received positive reinforcement as per previously published methodology. Following this, the test was conducted without operator involvement, and test reliability was ensured by blind-spot monitoring as well as non-flickering false-positives and flickering false-negatives randomly displayed throughout testing. A participant was retested if the rate of fixation loss, false negatives or false positives was more than 20%. Average sensitivities for the 1°, 3°, 6° and 10° rings were calculated at each time point, and the change in these values during the saffron and placebo phases were compared.

Microperimetry

MP was assessed undertaken with a Macular Assessment Integrity Analyser (MAIA, Centervue, Padova, Italy). The MAIA utilises a scanning light ophthalmoscope to perform fundus tracking, using the whole fundus as a reference. Participants were tested using an automated macular assessment protocol. Fixation was ensured via the use of a red circle target of 1° diameter, and stimuli were presented in a 4-2 strategy across an array of 37 points at 0°, 1°, 3° and 5° from central fixation. Throughout the test, Goldmann III stimuli are displayed across a dynamic range of 36 dB, with a background luminescence of 1.27 $cd·cm^2$.

All participants were dilated with 1% tropicamide/2.5% phenylephrine prior to testing, and all received a standardised set of instructions regarding test performance prior to test commencement. Tests were conducted in a standardised, non-illuminated room, prior to any tests that may have affected photoreceptor response (such as fundus photos). Participants with false positive responses of > 25% were retested, and if these responses persisted, they were excluded from analysis.

Results were grouped into concentric rings at 1°, 3° and 5° from central fixation (rings 1–3 respectively) and analysed as the average sensitivity of each of these rings, as well as the overall average macular sensitivity.

Multifocal electroretinograms

Multifocal electroretinograms are an objective test of retinal function that measures retinal conduction, and were acquired using a VERIS™ Science (Veris) device following International Society for Electroretinogaphy in Vision (ISCEV) guidelines [16], with refractive error assessed by either using the patient's own near glasses or by performing a subjective refraction for near and using this refraction in a trial frame. All participants' pupils were maximally dilated to at least 7-mm diameter using 0.5% tropicamide and/or 2.5% phenylephrine, with the cornea anaesthetised with 0.4% oxybupivicaine hydrochloride. The mfERG data was acquired using a gold foil electrode. Test stimuli consisted of 103 scaled hexagons presented in a pseudorandom fashion at a rate of
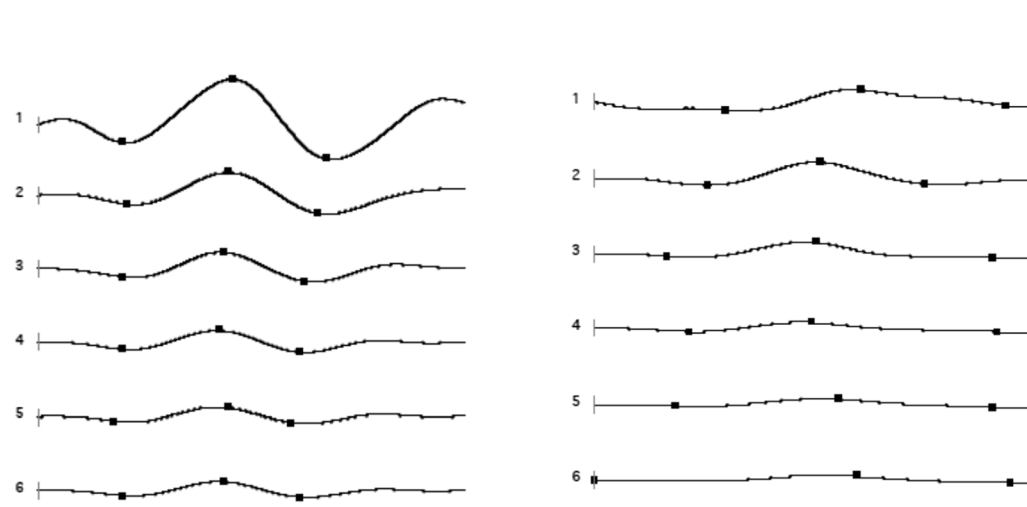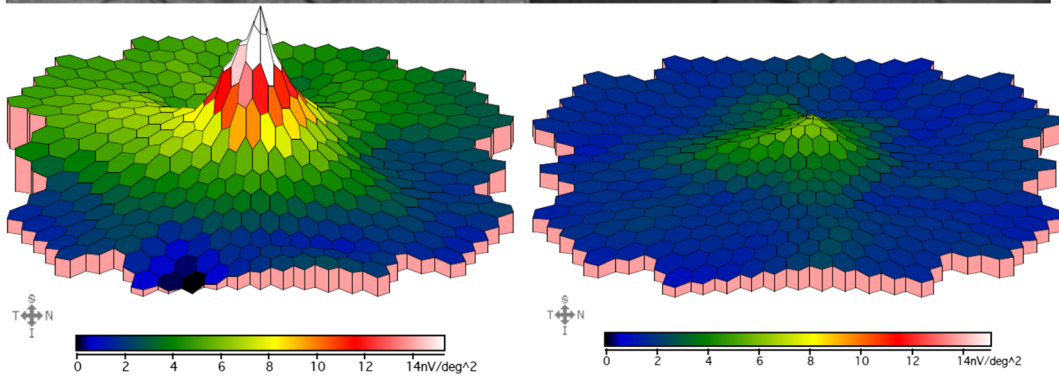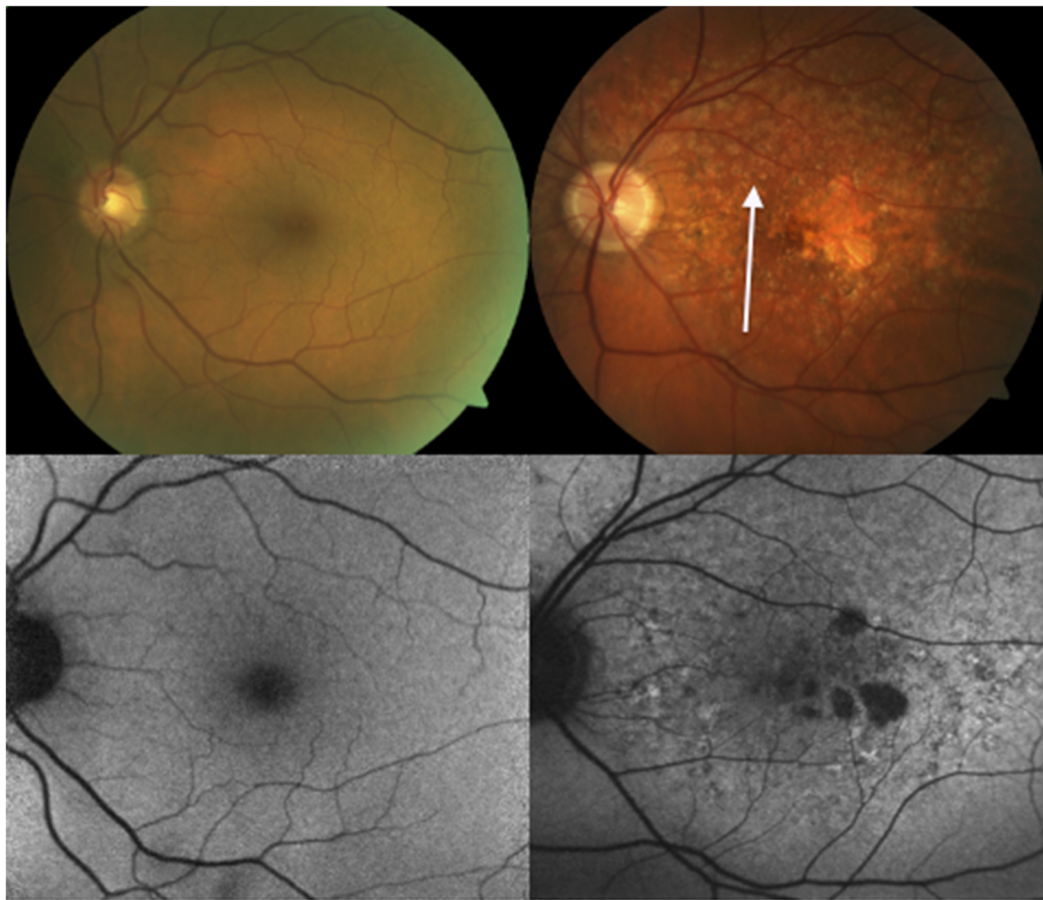
Fig. 1 Matched fundus photo and multifocal electroretingram output. Top left: normal participant, middle left: fundus autofluorescence (FAF) showing normal trace, bottom left: normal multifocal electroretinogram (mfERG) output with normal peak height; top right: fundus image of a patient with age-related macular degeneration (AMD), showing central atrophy and drusen (arrow); middle right: FAF from AMD patient showing areas of atrophy (dark patches); bottom right: mfERG output from AMD patient showing decreased peak height

75 Hz, using a luminance of 200 cd/m^2 for the white hexagons and 1.0 cd/m^2 for black hexagons. Fixation was ensured using a red fixation device, and recordings that contained blinks or other artefacts were not saved and were re-recorded. Signals obtained were band pass filtered from 10 to 100 Hz and amplified 100,000 times. Noise-contaminated segments were rejected and repeated.

The mfERG responses for the hexagons across the retina were separated into six concentric rings (rings 1 to 6) for data analysis. The first-order kernel responses were recorded according to the ISCEV guidelines [16]. The latencies and average response densities of the six concentric rings were measured (Fig. 1), with greater response density and lower latency indicative of better retinal function. The rings of 1–6 correspond to the foveola at 1°, 4°, 8°, 12°, 17°, and 22° radius of the stimulus, respectively according to the eccentricity, with the fixation target at the central 0.75°. The response amplitudes in each ring were measured between the first negative trough (N1) and the first positive peak (P1), yielding the N1P1 response densities (amplitudes per unit retinal area in nV/deg^2). The P1 peak latencies (ms) of the positive waveform were also measured.

An initial full-field ERG was also conducted under ISCEV conditions [17] to exclude the presence of potential confounding retinal degenerative diseases that may have mimicked AMD.

Statistical analysis

The primary outcome was mean change in BCVA. Secondary outcomes included mean change in mfERG N1P1 response density, and mean change in mfERG latency, change in individual ring mfERG N1P1 response density and latency, mean change in flicker perimetry ring response, mean change in microperimetry ring response, and safety of saffron as compared to placebo. Incidence of serious adverse events (SAEs) was recorded and analysed between the two groups to assess this final outcome.

Additional exploratory analyses were also conducted to explore the effect of saffron on those participants already consuming other supplementation therapy, and the effect of baseline atrophy on response observed. Participants on AREDS supplementation (current best-practice treatment) were analysed to assess the efficacy of saffron in this subgroup.

Of the 100 participants enrolled, 97 completed both placebo and saffron supplementation (Fig. 2). A single participant withdrew soon after study commencement due to family illness, one participant died of urinary sepsis during the saffron supplementation phase after completing the placebo phase, and another participant withdrew due to travel difficulties during the placebo supplementation phase after completing the saffron phase. All participants enrolled in the study were included in the safety analysis. Efficacy analysis was conducted on an intention to treat basis; however, participants who developed confounding visual pathologies (nAMD, significant cataract, etc.) during one arm of the trial had their visual results (mfERG, FP, MP and BCVA) excluded from analysis in these arms. There were thus 95 participants in the placebo arm and 96 participants in the saffron arm who were analysed to assess the efficacy of saffron.

Given the hierarchical nature of data (cross-over study in two eyes for some patients, and across six rings for mfERG results) a linear mixed effects model was used to account for within patient, eye and ring correlations using the *lme* function in R package *nlme*. To combine mfERG results over all rings, a linear mixed effects model was fitted on the mfERG logarithm with a quadratic term for reduction of log (mfERG) by ring. The choice for taking the logarithm of mfERG results, and for using a quadratic term for reduction by ring, was made to ensure assumptions on residual values were not violated. The fixed effects were treatment only for BCVA and treatment, ring and ring squared for log (mfERG) and for latency. Random effects were chosen to minimise Akaike information criterion (AIC) and Bayesian information criterion (BIC) values.

Analysis of mfERG latency was also conducted both including and excluding ring 1, as this ring had significantly more variability than rings 2–6. This variability was suggestive of potential noise in the signal, and analysis with and without this ring gave similar results. Both results are presented below and the conservative results of all rings included are presented in the abstract.

All analyses were conducted using the software *R: A Language and Environment for Statistical Computing* version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics of the cohort are displayed in Table 1. Fifty-one participants were male (52.6%), the mean age of participants was 73.9 years and 71 (73.2%) were already consuming AREDS supplements in both groups.

Fig. 2 Flow diagram of
participants within the trial
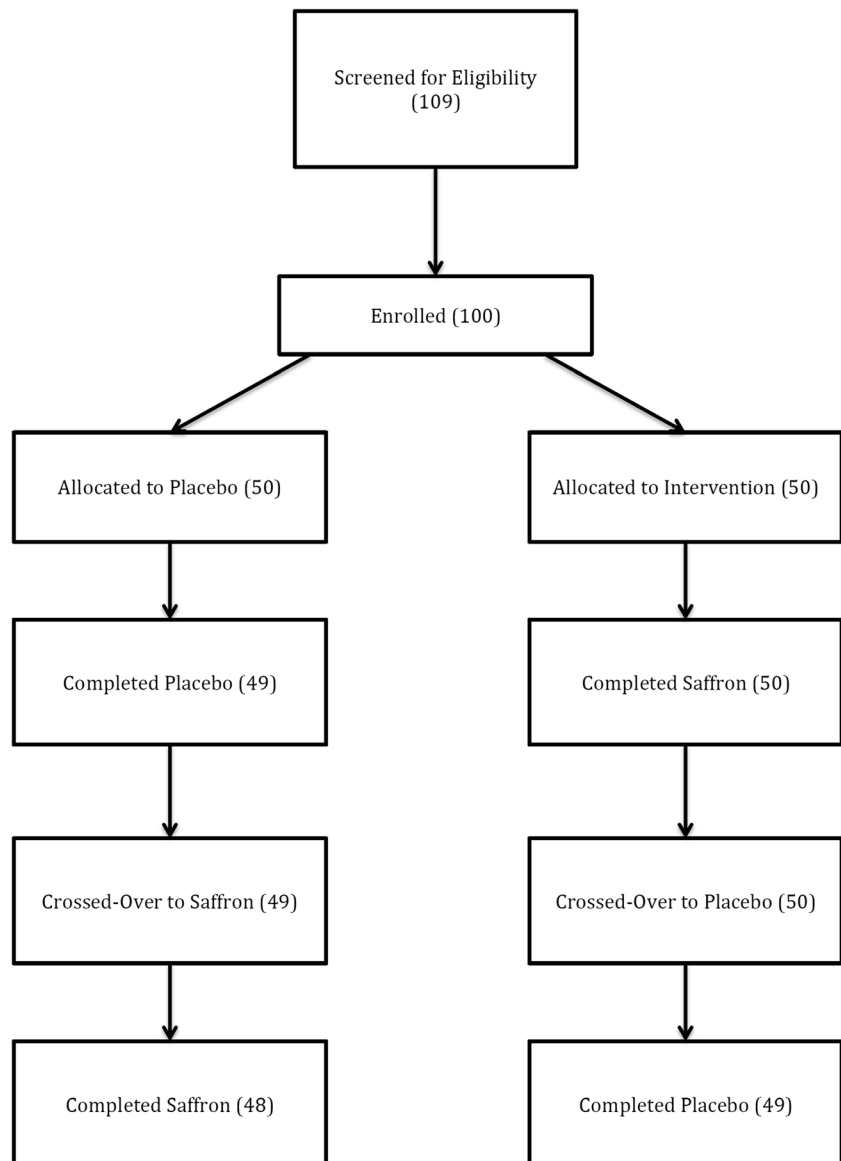
Vision

Mean BCVA improved by 0.69 ETDRS letters whilst on saf-
fron compared to placebo ($p = 0.001$). For those participants
already on best-practice supplementation (AREDS equiva-
lent) mean BCVA improved 0.73 letters on saffron compared
to placebo after 3 months ($p = 0.006$). There was no difference
in the change in BCVA between AREDS grades (i.e. no dif-
ference in response between AREDS grades 2 and 3,
$p > 0.05$).

Electroretinography

There was a trend toward improved mean pooled mfERG
response density (2.1% improvement) whilst on saffron
compared to placebo ($p = 0.08$). Individual ring response

density changes were greater in rings 1, 2, 3, 5 and 6 whilst
on saffron, and responses in ring 4 were greater on place-
bo; however, none of the individual ring response density
changes were significant ($p > 0.10$ for all, Table 2).

For those participants already on best-practice supplementa-
tion (AREDS equivalent) mean pooled mfERG response density
improved 2.8% on saffron compared to placebo ($p = 0.04$).

Mean pooled latency was 0.17 ms lower on saffron as
compared to placebo ($p = 0.04$). Individual ring latency was
lower for rings 3 and 6 on saffron ($p < 0.05$ for rings 3 and 6,
$p > 0.1$ for all other rings, Table 2).

For those participants on AREDS supplements, mean
pooled latency reduced 0.15 ms on saffron compared to pla-
cebo; however, this was not statistically significant ($p = 0.12$).

Central ring latency (ring 1) was significantly more vari-
able than latency for rings 2–6. Excluding ring 1, mean pooled

Table 1 Baseline characteristics of trial participants

Characteristic	Number
Male (%)	51
Age (years)	73.9 ± 8.5 SD
Age range (years)	51.0–89.7
BCVA (ETDRS letters and Snellen equivalent)	81 (20/25+)z ± 7.5 SD
Smoking status (non-smoker, ex-smoker, current smoker)	52:47:1
Lens status (phakic: pseudophakic)	105 (63%):62 (37%)
AREDS grade (2/3)	45:122
AREDS supplement use (%)	71
Not using AREDS supplements (%)	29
Lutein supplement use (%)	52
Not using lutein supplements (%)	48

BCVA best-corrected visual acuity, *ETDRS* early treatment of diabetic retinopathy study, *AREDS* age-related eye disease study

latency was 0.25 ms lower on saffron compared to placebo ($p < 0.001$), and for those participants also on AREDS supplements, mean pooled latency decreased 0.16 ms on saffron compared to placebo ($p = 0.05$).

There was no difference in the change of the mfERG response density or latency to saffron depending on the grade of AMD (i.e. no difference in response between AREDS grades 2 and 3, $p > 0.05$ for all).

Perimetry

Microperimetry results were not different between saffron or placebo for any of rings 1–3 ($p > 0.10$ for all). Flicker perimetry results were also not different between saffron or placebo for any of rings 1–4 ($p > 0.10$ for all).

Impact of anatomical and demographic factors

After adjusting for baseline CMT and FAF area, mean mfERG response density improved 2.3% on saffron compared to placebo ($p = 0.05$), mean mfERG latency was reduced by 0.19 ms on saffron compared to placebo ($p = 0.03$), and mean BCVA improved 0.70 letters on saffron compared to placebo ($p = 0.001$).

No association was found between FAF area and latency ($p > 0.05$); however, increased FAF area was associated with decreased mean pooled mfERG response density ($p = 0.008$).

No interaction was found between age and the effect of treatment on BCVA ($p = 0.09$), response density ($p = 0.86$) or latency ($p = 0.58$).

Safety

A total of 19 SAEs occurred during the trial, with ten occurring during the saffron supplementation phase and nine during placebo supplementation (Table 3).

Table 2 mfERG Results seen with Saffron supplementation

mfERG response	Saffron	Placebo	*p* value
Ring 1 response density (nV/deg^2)	36.07 (18.91)	34.5 (18.10)	0.32
Ring 2 response density (nV/deg^2)	21.06 (8.40)	20.48 (7.66)	0.30
Ring 3 response density (nV/deg^2)	14.57 (5.38)	14.25 (5.20)	0.30
Ring 4 response density (nV/deg^2)	10.81 (4.22)	10.84 (4.34)	0.88
Ring 5 response density (nV/deg^2)	8.64 (4.13)	8.63 (4.51)	0.99
Ring 6 response density (nV/deg^2)	7.5 (4.13)	7.48 (4.20)	0.93
Pooled response density	1.021[a]	1.00	0.08
Pooled response density (AREDS supplements)	1.028[a]	1.00	0.04
Ring 1 latency (ms)	39.30 (4.04)	38.97 (3.95)	0.39
Ring 2 latency (ms)	38.40 (2.35)	38.38 (2.62)	0.92
Ring 3 latency (ms)	36.61 (2.28)	37.09 (2.45)	0.01
Ring 4 latency (ms)	35.86 (2.20)	36.09 (2.36)	0.20
Ring 5 latency (ms)	36.25 (2.22)	36.47 (2.10)	0.19
Ring 6 latency (ms)	36.43 (1.97)	36.76 (2.02)	0.03
Pooled latency	36.47	36.64	0.04
Pooled latency rings 2–6	36.32	36.57	<0.001
Pooled latency (AREDS supplements)	36.57	36.73	0.12

mfERG multifocal electroretinogram, *nV/deg^2* nanovolts/degree squared, *AREDS* age-related eye diseases study, *ms* milliseconds

[a] Proportional Increase in mfERG response compared to placebo

Table 3 Serious adverse events in mild/moderate age-related macular Degeneration

Adverse event	Number of events during placebo supplementation	Number of events during saffron supplementation
Death (urinary sepsis)	0	1
Fall	1	2
Acute torticollis	1	0
Self-induced substance Overdose	0	1
Neovascular AMD	1	2
SCC requiring excision	0	1
Cataract progression	1	1
Acute myocardial infarction	1	0
Idiopathic pancreatitis	0	1
Hip fracture	1	0
Bowel cancer	1	1
Bradycardia requiring Pacemaker insertion	1	0
Incarcerated hernia	1	0

AMD age-related macular degeneration, *SCC* squamous cell carcinoma

No significant difference was observed in the rate of SAE occurrence between the two treatment arms ($p > 0.10$, two-sample *t* test). Mean change in IOP was 1.02 mmHg in the saffron phase and 1.13 mmHg in the placebo phase ($p > 0.10$, paired t-test) for change in IOP between the two groups.

Discussion

Oral saffron supplementation modestly assists in preserving retinal function in participants with AMD, and this benefit appears to extend to participants already consuming current best-practice therapy (beta-carotene free AREDS supplementation). Although the recent AREDS2 trial is a notable exception [6], few other studies investigating supplementation therapies in AMD have maintained participants on concurrent supplement therapies [18, 19]. This limits the generalizability of the results obtained, as patients in clinical practice are often already consuming supplements for moderate AMD [20]. Restricting concurrent supplement use also limits understanding of both potential therapeutic mechanisms of action and AMD pathophysiology, as it can be unclear whether different therapies are working on separate pathways or whether they are all targeting a single pathophysiological mechanism.

The most interesting finding of this trial was that there was a small but additive response in those participants consuming other antioxidant supplements, including beta-carotene free AREDS supplements. Although the mechanism by which saffron affects retinal tissue is still under investigation, reduction of oxidative stress is the most heavily investigated pathway

[21]. Multiple constituents of saffron have been shown to reduce retinal oxidative stress in animal studies [22, 23], suggesting that there may be specific saffron constituents that are responsible for the results observed in our study. Whilst these are initial studies, they suggest that these constituents may be promising targets for future trials, and their benefits may be additive with existing therapies, potentially resulting in further reductions in AMD progression and visual loss.

Although electroretinography is not routinely used in the everyday clinical assessment of AMD, mfERG has successfully been utilised in AMD trials, including other trials of dietary supplementation [24, 25]. The major advantage of mfERG is that it is an objective functional assessment, meaning that it is less influenced by patient perceptions or other confounding factors than other, subjective functional tests. This allows for a more reliable measure of visual outcomes and may be highly specific in predicting visual loss over time [26, 27]. Responses represent ON and OFF-bipolar cell contributions plus smaller contributions from inner retina and photoreceptors, and thus the mfERG indicates function of the photoreceptors indirectly [28]. Previous studies of the mfERG in mild/moderate AMD have shown that compared to age-matched normal subjects, AMD sufferers have decreased amplitude/response density and increased latency [27, 29]. Changes in amplitude are thought to relate to loss of photoreceptor function, whilst alterations in latency have been suggested to reflect cellular dysfunction [30, 31]. As saffron supplementation improved both parameters, saffron may have a role in reducing cellular loss as well as stabilising photoreceptor function, although the short time frame of the study limits interpretation.

Microperimetry, which is a subjective measurement of retinal sensitivity at mesopic adaptation levels, may be mediated by both rod and cone photoreceptors. It was not found to show any difference between the two groups, suggesting that microperimetry in this study was not as sensitive as previous reports have suggested when comparing the techniques to matched normals [32]. Interestingly, flicker perimetry has previously been suggested to be less sensitive than mfERG at detecting altered retinal function in early AMD, and our results support this earlier finding [15].

There was no evidence that regular saffron supplementation is associated with increased safety risks to patients. The rate of cataract progression across the study was similar to that seen in major studies of cataract [33], as was the rate of nAMD development [5] although the study period is relatively short. Toxicological studies of saffron have suggested that saffron is not associated with serious side-effects at dosages below 5 g/daily, and the 20 mg/daily dose used in this study is well within such a therapeutic window [10]. It should be emphasised, however, that this study was not specifically designed to assess the safety of saffron supplementation, and as such, was not powered to detect potentially rare adverse

events. Further research is thus needed to fully define the risk profile of saffron as a therapeutic substance.

Some limitations do apply to this study. The visual gain detected was statistically but not clinically significant, and this may relate to the relatively short follow-up period of our study, particularly given the slow progressive nature of AMD. It may therefore be that with more prolonged supplementation, a greater neuroprotective response would be observed, and longer duration trials may thus provide greater insight into this. Additionally, efficacy has also thus far only been tested at a single dosage, and further research is needed to fully define the dose-response relationship of saffron in AMD sufferers. Further, as there is no reliable biomarker available for testing to assess response or compliance with dosing, it was not possible to biologically monitor compliance with therapy, meaning compliance could only be assessed subjectively. Additionally, the lack of reliable biomarkers and the complex natural components in saffron mean than accurate measurement of the pharmacokinetics of saffron could not be assessed. As a result of this, the association between pharmacokinetics and pharmacodynamics for oral saffron remains unknown.

Saffron supplementation was both efficacious and safe in preserving retinal function in participants with AMD, although clinically the degree of response obtained with this short-term study was small. These effects were complementary with current best practice therapies, suggesting that further protection than is currently obtained may be gained for patients by using these substances in tandem. Given the rising number of individuals with AMD, and its propensity to lead to later irreversible visual loss, further, longer-duration studies are justified to more fully investigate the role of saffron and saffron-based therapies as treatments for AMD.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licencing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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