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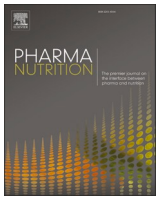

Eicosapentaenoic acid-rich omega-3 fatty acids supplementation may improve vision in dry age-related macular degeneration or Stargardt disease, as shown in MADEOS, a prospective, randomized, multicentre, double-blind, placebo-controlled pilot study☆

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ABSTRACT

Background: This study investigates the effects of eicosapentaenoic acid (EPA)-rich omega-3 fatty acids on dry age-related macular degeneration (AMD) and Stargardt disease.
Methods: MADEOS is a prospective, randomized, multicenter, double-blind, placebo-controlled pilot study, which assessed the impact of omega-3 fatty acids on best corrected visual acuity, blood omega-6/omega-3 ratio, and perceived vision and mood using a questionnaire in patients with dry AMD or Stargardt disease. Participants received either the active product (3660 mg of EPA and DHA; 14 patients) or placebo (sunflower oil; 7 patients) daily for 24 weeks. Measurements were taken at screening (Visit 1), 12 weeks (Visit 3), and 24 weeks (Visit 4). Comparisons were made within and between groups.
Results: The mean letters gained at Visits 3 and 4 were significantly different between the groups ($p=0.002$). The active group showed a mean gain of 6 ETDRS letters from Visit 1 to Visit 4 ($p=0.003$). The mean arachidonic acid/EPA ratio in the active group significantly decreased from Visit 1 (5.84 ± 1.05) to Visit 4 (1.47 ± 0.16, $p=0.002$). The questionnaire scores were similar at Visit 3 but higher for the active group at Visit 4 (9.38 ± 3.35 vs. 7.28 ± 2.36).
Conclusion: EPA-rich omega-3 supplementation may improve both objective and subjective vision in patients with dry AMD or Stargardt disease, offering a potentially simple, safe, and cost-effective approach to enhancing quality of life.

Abbreviations: AA, arachidonic acid; AMD, age-related macular degeneration; AREDS2, Age-Related Eye Disease Study Research 2; BCVA, best corrected visual acuity; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GA, geographic atrophy; LT, leukotrienes; OCT, optical coherence tomography; PG, prostaglandins; SD, standard deviation; VEGF, vascular endothelial growth factor.

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1. Introduction

1.1. Retinal degeneration

Retinal degeneration is one of the main causes of vision loss, regardless of whether it is due to an age-related condition, such as age-related macular degeneration (AMD), or a genetic condition, such as Stargardt disease. AMD is the leading cause of visual impairment and blindness in people over the age of 50 years in developed countries. The estimated number of people with this disease is 200 million worldwide and is expected to increase by more than 50 % by the year 2040 [1,2]. Among the two different forms, wet AMD is a condition where treatment options exist, including intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors. However, for dry AMD there are no treatments available at this stage to improve vision. Therefore, the need for a therapeutic intervention is fundamental.

Stargardt disease, also known as the juvenile form of macular degeneration, is the most common inherited macular dystrophy in children, with an estimated prevalence of 1 in 10000 [3]. It typically presents during the first two decades of life and often progresses to legal blindness. The severely diminished vision in these patients undoubtedly interferes with their activities of daily living and ability to function normally, with significant direct and indirect costs involved.

1.2. Omega-3 fatty acids

Omega-3 fatty acids are known to demonstrate protective effects over a range of conditions, including cardiovascular, neurological and ophthalmological diseases [4]. Over the past few decades, there has been increasing interest in the role of omega-3 fatty acids in ocular pathologies, including AMD and Stargardt disease. Several studies have demonstrated that omega-3 fatty acids, which are considered anti-inflammatory molecules, may have a protective role in inflammatory-, ischaemia-, light-,oxygen- and age-associated pathologies of the vascular and neural retinas [5]. Furthermore, resolvins, which are mediators derived from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been found to play a role in the resolution of inflammation and repair of damaged tissue [6,7]. Among the major mediators of the inflammatory response are proinflammatory eicosanoids generated from the omega-6 fatty acid arachidonic acid (AA). These mediators include pro-inflammatory prostaglandins (e.g., PGE_2) and leukotrienes (e.g., LTB_4), which can act as mediators for leucocyte chemotaxis and inflammatory cytokine production. The balance between pro-inflammatory and anti-inflammatory molecules plays a critical role for the resolution of an inflammatory response and the repair of damaged tissue.

The importance of the blood levels of specific fatty acids type, such as omega-3 and omega-6 for ocular health has recently been explored. Lower levels of particular polyunsaturated fatty acids (EPA and DHA) in either circulating blood or the retina have been associated with certain retinopathies, including AMD, Stargardt disease and diabetic retinopathy [8–10]. In one study, eyes from AMD donors exhibited significantly decreased levels of very long-chain polyunsaturated fatty acids and high omega-6/omega-3 ratios. This study emphasised the importance of monitoring the level of blood omega-6 vs. omega-3 fatty acids when conducting clinical trials on lipid supplements for the prevention and treatment of retinal diseases [8].

1.2.1. Omega-3 fatty acids in eye diseases

Previous research has also examined the effect of omega-3 fatty acids supplementation on ocular health and visual acuity. For instance, the Age-Related Eye Disease Study Research 2 (AREDS2) involved the administration of an omega-3 formulation that was low in both EPA (650 mg per day) and DHA (350 mg per day) to patients with dry AMD for 5 years. Overall, there was no additional benefit in the reduction of the risk of developing advanced AMD by the addition of the omega-3 fatty acids to the formulation [11,12]. Gerstenblith *et al.* [13] used an omega-3 formulation that was rich in DHA (2500 mg per day) but poor in EPA (850 mg per day) for six months in patients with dry AMD but no significant changes were noted in visual acuity or retinal function.

Although the therapeutic potential of omega-3 fatty acids was not shown to be beneficial in these previous clinical studies, based on the demonstrated importance of blood omega-6/omega-3 ratios and our previous work, we believe that a higher dose of EPA may have a desirable effect, because it could maintain a lower blood omega-6/omega-3 ratio. Our research group performed previous preclinical studies in established murine models of several ocular pathologies using higher doses of EPA and DHA (Eyetas®, Eyetas Therapeutics LTD, 3660 mg per day). The findings suggested a protective effect of omega-3 fatty acids supplementation when the blood AA/EPA ratio was <2 in animal models of retinal and optic nerve diseases [14–19]. Given these encouraging preclinical findings, the present study was designed as a pilot study to investigate the potential effect of EPA-rich omega-3 fatty acids supplementation on visual acuity, the blood AA/EPA ratio, and perceived vision and mood, in patients with dry AMD or Stargardt disease. We hypothesized that when patients consumed an EPA-rich omega-3 fatty acids formulation, the blood AA/EPA ratio of the active group would be maintained below 2 and this may protect against further retinal degeneration.

2. Materials/subjects and methods

2.1. Study approval, registration and regulation

The Macular Degeneration Omega-3 Study (MADEOS) was a prospective, randomized, multicenter, double-blind, placebo-controlled study, with repeated measures for each patient. The study was designed, implemented and reported in accordance with the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice, with applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki and the national applicable legislation (ClinicalTrials.gov Number: NCT03297515). The study was conducted in four different centres, namely, Quinze-Vingts National Ophthalmology Hospital Centre, Paris, France; Centre for Clinical Trials at San Paolo Hospital, University of Milan, Italy; Excellence Eye Research Centre, University G. d'Annunzio of Chieti-Pesara, Chieti, Italy; and Department of Ophthalmology, University Vita Salute Scientific Institute of San Raffael Via Olgettina, Milano, Italy. The study was approved by separate local ethical committees in both countries (Comité de Protection des Personnes Ile de France (2017-A03176–47); Comitato Etico Milano Area 1 (13386/2019); Comitato Etico Delle Province Di Chieti e Pescara; Comitato Etico Ospedale San Raffaele, Milano (201/2018)).

2.2. Patient recruitment, consent, and inclusion

Patients only performed any of the study procedures after reading the patient information sheet and providing a written approved informed consent form. Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of our research. All patient samples and data were anonymized for the purpose of this study. General inclusion criteria for study participation included age 18–85 years; willingness to participate and provide a signed informed consent form, agree to take the randomized study investigational product for 24 weeks, and to undergo examinations at the visits (screening visit (Visit 1), baseline visit (Visit 2), at 12 weeks (Visit 3) and at 24 weeks (Visit 4)); ability to swallow soft gel capsules; and >90 % compliance with consumption of the investigational product (active) during the period between screening (Visit 1) and group allocation (Visit 2).

Inclusion criteria were designed for moderate or severe condition and were assessed at the screening visit (Visit 1). For Stargardt disease patients, there was no genetic diagnostic confirmation, and the

diagnosis was clinically based. Specific inclusion criteria for moderate dry AMD and Stargardt disease patients included: a) best corrected visual acuity (BCVA) between 35 and 55 ETDRS letters at Visit 1. For those with dry AMD, further criteria included b) a large drusen >125 μm within 1000 μm from the centre of the fovea or c) geographic atrophy (GA) present but >300 μm away from the centre of the fovea. For those with moderate Stargardt disease, a further criterion was GA present but measuring <2000 μm in diameter anywhere. For severe dry AMD and Stargardt disease patients, the specific inclusion criteria included BCVA between 21 and 34 ETDRS letters at Visit 1. For those with dry AMD, further criteria included a) GA potentially involving the fovea but <2500 μm in diameter or b) any size GA but >200 μm from the centre of the fovea anywhere. For those with severe Stargardt disease, a further criterion was GA present but measuring <2500 μm in diameter anywhere.

Both eyes were assessed in all the study visits. The study eye that fulfilled all the eligibility criteria was used for statistical analysis. If both eyes met the eligibility criteria, the eye with the best BCVA at screening was used for the placebo and active group. The exclusion criteria of the study are discussed in the Supplementary Data 1. Patients who were recruited attended screening, baseline and follow-up visits, as described in Supplementary Table 1.

2.3. Sample size, randomization and product administration

The randomization procedure was performed after Visit 1 and before Visit 2 with patients who fulfilled inclusion criteria and were given a randomization number according to a specific procedure and was centrally generated to ensure that the investigational product assignment was unbiased.

The final number of enrolled participants was 37 and a randomization list was produced by the coordinating centre (AIBILI - Association for Innovation and Biomedical Research on Light and Image, Portugal), using a validated system that randomly assigns study arms to randomization numbers in the specified ratio 2:1 and according to severity stratification (moderate or severe). The study was double-blinded, nor the patients neither the clinical investigators had information about the assignment of the two investigational products (placebo vs active), and the randomization and assignment were performed by the coordinating centre (AIBILI). The investigational products were delivered masked to the clinical sites.

Each active supplement of soft gel capsules (1200 mg coloured bovine gelatine with vanilla in each) contained 750 mg of EPA and 165 mg DHA (Eyetas®, Eyetas Therapeutics LTD), as well as 7.5 mg of D-α-tocopherol, which was used as an antioxidant. The placebo capsules were identical in appearance to the active supplements; however, they contained the same volume of sunflower oil and D-α-tocopherol instead. The patients were dispensed a run-in investigational product (active) at screening that they consumed daily until Visit 2. The purpose of having the period between Visit 1 and Visit 2 (10–14 days), was to evaluate patients' ability to swallow the capsules, to estimate those with high compliance (>90 %), and to monitor for any signs of adverse events. The patients were advised to take 4 capsules of the investigational product per day (3660 mg of EPA and DHA) for 24 weeks and the capsules were dispensed to the respective groups at Visit 2 and Visit 3. Compliance was estimated at Visits 2, 3, and 4 by counting the capsules returned by each patient. Patients with low compliance (below 70 %) over the study period (from Visit 2 to Visit 4) were excluded from the primary analysis. The allowed window for Visit 3 and Visit 4 was ± 10 days. A reminder call was scheduled prior to the visits and to check on any adverse events. Another call was placed 30 days after Visit 4 to check for any adverse events. Patients were advised that if they experience any significant adverse events to report them and to terminate the supplementation. Patients who successfully completed the study through Visit 4 were included in the data analysis.

Table 1
Summative information on the clinical characteristics of the patients included in the study.

Clinical characteristics	
Number of patients recruited in the study	37
Number of patients included in the analysis	21
Patients with AMD (%)	Total (38.10 %, N=8) Moderate AMD (14.29 %, N=3) Severe AMD (23.81 %, N=5)
Patients with Stargardt disease (%)	Total (61.90 %, N=13) Moderate Stargardt disease (47.62 %, N=10) Severe Stargardt disease (14.29 %, N=3)
Patients included in the placebo group (%)	Total (33.33 %, N=7) AMD (42.86 %, N=3) Stargardt disease (57.14 %, N=4)
Patients included in the active group (%)	Total (66.67 %, N=14) AMD (35.71 %, N=5) Stargardt disease (64.29 %, N=9)
Patient sex (%)	Female (42.90 %, N=9); male (57.10 %, N=12)
Patient age (mean ± SD)	60.0 ± 2.3 years Range 25–85 years
Comorbidities (%)	Arterial hypertension (19.05 %, N=4) Hypercholesterolemia (14.29 %, N=3) Type II diabetes mellitus (9.52 %, N=2) Cardiac arrhythmias (4.76 %, N=1) Dyspepsia (4.76 %, N=1)

2.4. Measurements

The type of measurements performed at each visit are summarised in Table 1. Clinical assessments for both eyes were performed at each study visit. During Visit 1, refraction, BCVA, optical coherence tomography (OCT), fundus autofluorescence, slit lamp, vital signs and finger prick blood test were performed. Visit 2 served for assigning the patients into randomized groups and to exclude those who could not swallow large capsules; however same measurements were taken as Visit 1, excluding the blood test. During Visit 3 and Visit 4, capsules accountability was noted (to estimate compliance) and refraction, BCVA, OCT, fundus autofluorescence, slit lamp, vital signs, finger prick blood test and completion of the questionnaire were performed.

The primary variable of the study was the number of ETDRS letters gained from BCVA measurements from Visit 1 to Visit 3 and Visit 4. Three of the clinical sites used the non-electronic ETDRS chart and one site used an electronic chart. The secondary variables were a) the blood levels of AA and EPA, expressed as the AA/EPA ratio, and b) the questionnaire score on perceived vision and subjective mood.

Blood was collected from all patients at Visit 1, Visit 3 and Visit 4 using a finger prick, the blood samples were collected on Whatman filter paper and stored at −20°C until analysis. Gas chromatography was used to determine the blood levels of AA and EPA, as previously described by Prokopiou *et al.* [14–16]. Briefly, samples' supernatants were collected and dried using an analytical evaporator, the samples were redissolved in n-hexane and analysed using a gas chromatography flame-ionization detector.

A questionnaire (Supplementary Data 2) on perceived vision and mood was administered at Visit 3 and Visit 4. This instrument was created for the current study. It included 7 questions that asked patients to compare their initial (at screening) perceived vision and mood with those perceived on Visits 3 and 4. The items were rated on a Likert scale from 0 to 4. A total scale score was obtained for each patient, where high scores indicated a positive change in perceived vision and mood. The purpose of designing this questionnaire was for simplicity and patients' convenience; thus, avoiding the long and complex questionnaires that

have already been published and previously used.

Finally, safety evaluations included the assessment of any adverse events that might have been experienced by the patients during the visits. In cases of serious adverse events, the patient would be considered an "early termination" and encouraged to attend the clinical site for a last visit with the same procedures conducted at Visit 4.

2.5. Statistical analyses

SPSS software (version 25.0) was used for the statistical analyses. Descriptive statistics were obtained for all variables (continuous and categorical). Shapiro–Wilk tests were used to assess the normality of the continuous variables BCVA, AA/EPA ratio and questionnaire score (a $p>0.05$ showed normality) and thus indicated the appropriate statistical tests (parametric or nonparametric). More specifically, a) for BCVA, the Mann–Whitney test was used to determine significant differences in BCVA values between groups (placebo vs. active) at screening. Between-group comparisons were performed for the ETDRS letters gained from screening to 12 weeks (Mann–Whitney test) and from screening to 24 weeks (independent samples *t* test). The chi-square test was used to examine between-group differences in the percentages of patients gaining/not gaining more than 5 ETDRS letters from screening to 24 weeks. A gain of >5 letters is indicative of a gain in an ETDRS line. Within-group comparisons of BCVA at screening with BCVA at 12 weeks and with BCVA at 24 weeks were performed (paired samples *t* test for placebo and Wilcoxon signed-rank test for active). In addition, b) for AA/EPA, Mann–Whitney tests were used to examine differences in the AA/EPA ratio between groups (at Visit 1, Visit 3 and Visit 4). Within each group, Wilcoxon signed-rank tests were used to determine significant changes in the AA/EPA ratio from Visit 1 to Visit 3 and to Visit 4. Friedman's test was used to verify significant differences in the AA/EPA ratio among the three visits. Furthermore, c) for the questionnaire on perceived vision and mood, the score was calculated for both timepoints (Visit 3 and Visit 4). Within-group comparisons were made to test for differences in the questionnaire score from Visit 3 to Visit 4 (paired samples *t* test for placebo; Wilcoxon signed-rank test for active). Between-group comparisons of the score were made at Visit 3 (Mann–Whitney test) and Visit 4 (independent samples *t* test), to examine differences between the active and placebo groups. Only patients with valid values at Visit 1 and Visit 4 were considered for all statistical analyses. Missing values due to discontinuations or other types of censoring were not imputed.

3. Results

3.1. Subjects' clinical characteristics

We initially intended to recruit a total sample size of 60 patients (for both AMD and Stargardt disease). However, due to the COVID-19 pandemic, patient recruitment and follow-up were very challenging; therefore, only 37 patients entered the study. The recruitment period took place from May 2019 to February 2020. The last patients' visits were performed during September 2020. Nine patients were excluded after recruitment following a detailed evaluation of their ophthalmological examinations that were not fulfilling the inclusion criteria. Two patients were excluded due to low treatment compliance <70 % and 3 patients due to loss during follow-up visits. In addition, two patients were excluded following analysis of the blood fatty acids; one patient from the placebo and one patient from the active group who were found to have significantly high levels of EPA and AA/EPA <2 (an indication that these patients were most probably taken omega-3 fatty acids supplementation, which was part of the exclusion criteria). Therefore, the final number of patients included in the study was 21; of those, 7 patients were included in the placebo group and 14 patients in the active group (Supplementary Figure 1). To ensure higher statistical power, the analysis used the merged sample of AMD and Stargardt patients, although some comparisons were made between the AMD and Stargardt disease patients only for indicative purposes (data not shown due to small sample size). Severity of disease was noted only for patients' clinical characteristic purposes and was not used for further analysis. The study included 9 females (42.9 %) and 12 males (57.1 %), of whom 4 females and 3 males were in the placebo group and 5 females and 9 males were in the active group. The study participants including both diseases had a mean age of 60.0 ± 2.3 years (range: 25–85 years). Details on patients' clinical characteristics are shown in Table 1.

3.2. Between- and within-group BCVA comparisons

Based on the normality results, various parametric or non-parametric tests were performed. First, there was no significant difference in the BCVA between the patients in the active group and the patients in the placebo group at Visit 1 ($p=0.387$; Mann-Whitney test); in other words, the two groups demonstrated equivalent visual performance before any treatment. Between- and within-group comparisons were performed on the ETDRS letters gained from Visit 1 to Visit 4. Within the active group, the mean BCVA increased from 40.93 ETDRS letters at Visit 1 to 46.93 ETDRS letters at Visit 4, i.e., a mean gain of 6 ETDRS letters, and the median gain was 10 ETDRS letters; this difference was statistically significant ($p=0.003$; Wilcoxon signed-rank test). In contrast, within the placebo group, the change in the BCVA between Visit 1 and Visit 4 was not significantly different ($p=0.838$; paired samples t-test); the mean BCVA decreased slightly, from 44.86 to 44.71, respectively (a loss of 0.15 ETDRS letters), while the median decreased from 48 to 45 (a loss of 3 ETDRS letters). In addition, a between-group comparison of BCVA at Visit 4 showed a statistically significant difference between the active and placebo groups in terms of the mean ETDRS letters gained (a difference of 6.15 ETDRS letters; $p=0.002$, independent samples t-test). Moreover, 57 % of the patients (8 out of 14) in the active group gained more than 5 ETDRS letters, as opposed to 0 % of patients in the placebo group. The difference between groups in terms of patients gaining more than 5 ETDRS letters was statistically significant (chi-square=6.464, $p=0.011$).

Similar between- and within-group comparisons were performed on the ETDRS letters gained from Visit 1 to Visit 3. In the active group, the mean BCVA significantly increased from 40.93 ETDRS letters at Visit 1 to46.36 ETDRS letters at Visit 3, i.e., a mean gain of 5.43 ETDRS letters, and the median number of ETDRS letters gained was 8.5 ($p=0.002$; Wilcoxon signed-rank test). The mean BCVA for the placebo group slightly increased from 44.86 to 46.14 between Visit 1 and Visit 3, although the difference was not statistically significant (1.28 ETDRS letters gained; $p=0.108$; paired samples *t* test), and the median remained the same (48.00). Furthermore, the mean letters gained between the two groups (placebo vs. active) at Visit 3 were significantly different (a difference of 4.15 ETDRS letters; $p=0.042$, Mann–Whitney test), showing that the treatment resulted in a higher gain of letters on average from Visit 1 to Visit 3 than the placebo.

All the above results indicate that 24 weeks of supplementation with an EPA-rich formulation resulted in a gain of ETDRS letters for the patients in the study, whereas there was no improvement for the patients who did not receive the treatment.

The detailed results regarding the BCVA are presented in Table 2 and Fig. 1 (1 A for mean and 1B for median differences).

3.3. Between- and within-group comparisons of the AA/EPA ratio

The difference in the AA/EPA ratio was tested at different time points (from Visit 1, to Visit 3 and Visit 4) within each group and between the two groups (placebo vs. active). A detailed analysis of the AA/EPA ratio is presented in Table 2, and a graphical presentation of the results is shown in Fig. 2. Examination within groups showed that for the active group, the mean AA/EPA ratio decreased significantly from Visit 1 (5.84) to Visit 3 (1.50) and from Visit 1 to Visit 4 (1.47); similar

Table 2
The mean (standard deviation; SD) and median of best corrected visual acuity (BCVA) at each visit for both the placebo and the active group. P values from the statistical tests are also presented.

BCVA		Placebo group	Active group	P value
Visit 1	**Mean (SD)**	44.86 (9.21)	40.93 (9.18)	0.387[a]
	Median	48.00	35.00	
Visit 3	**Mean (SD)**	46.14 (9.99)	46.36 (9.51)	0.042[a]
	Median	48.00	43.50	
	Mean letters gained (since Visit 1)	1.28	5.43	
		P = 0.108[b]	P = 0.002[b]	
Visit 4	**Mean (SD)**	44.71 (8.28)	46.93 (9.18)	0.002[a]
	Median	45.00	45.00	
	Mean letters gained (since Visit 1)	-0.15	6.00	
		p = 0.838[c]	p = 0.003[c]	
AA/EPA		**Placebo group**	**Active group**	**P value**
Visit 1	**Mean (SD)**	5.67 (0.61)	5.84 (1.05)	0.837[a]
	Median	5.67	5.81	
Visit 3	**Mean (SD)**	5.52 (1.92)	1.50 (0.23)	0.001[a]
	Median	5.78	1.53	
		p = 0.499[b]	p = 0.002[b]	
Visit 4	**Mean (SD)**	5.37 (2.12)	1.47 (0.16)	0.007[a]
	Median	6.50	1.47	
		p = 0.866[c]	p = 0.002[c]	

[a] Between-group comparisons (placebo vs active groups)
[b] Within-group comparisons (Visit 1 to Visit 3)
[c] Within-group comparisons (Visit 1 to Visit 4)

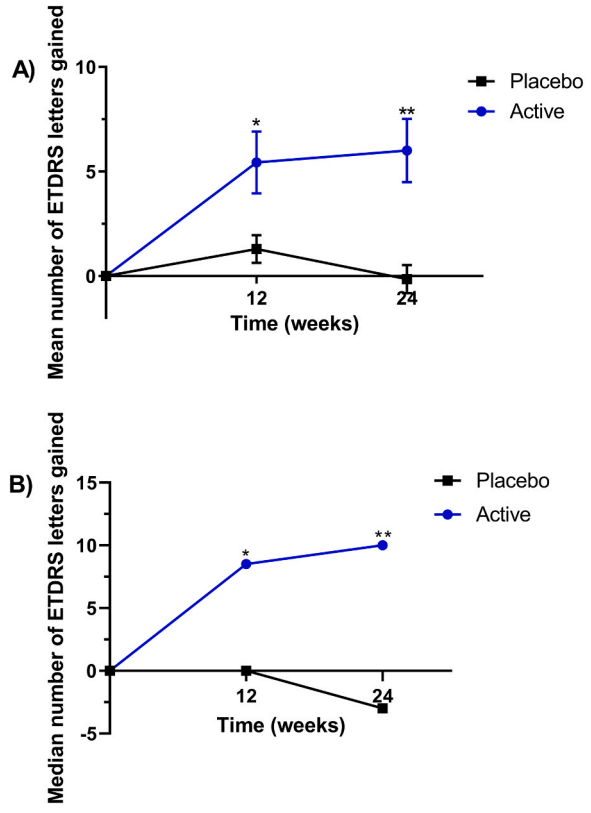

Fig. 1. A) Comparison of the mean number of ETDRS letters gained in the placebo vs. active groups at Visit 3 (12 weeks) and Visit 4 (24 weeks). B) Comparison of the median number of ETDRS letters gained in the placebo vs. the active group at Visit 3 (12 weeks) and Visit 4 (24 weeks). The differences between the two groups were statistically significant at Visit 3 and Visit 4 (*p<0.05, **p<0.01).

observations were made for the median AA/EPA ratio (5.81, 1.53 and 1.47 at Visits 1, 3 and 4, respectively). Friedman's test additionally showed a highly significant difference in the AA/EPA ratio between the three time points for the active group (chi-square=18.167, p<0.001). In contrast, for the placebo group, the mean AA/EPA ratio did not change significantly from Visit 1 (5.67) to Visit 3 (5.52) or to Visit 4 (5.37); a similar trend was observed for the median values (5.67, 5.79 and 6.50 at Visits 1, 3 and 4 respectively). Notably, there was no significant change in the AA/EPA ratio from Visit 3 to Visit 4 in the active group.

Mann–Whitney tests were used to examine the differences between the two groups at each visit: at Visit 1, the two groups had similar AA/EPA ratios, with nonsignificant differences. However, at Visit 3, there was a highly significant difference between the two groups (p=0.001), with the active group having a lower AA/EPA ratio than the placebo group. Similarly, at Visit 4, there was a significant difference between the two groups (p=0.007); again, the active group had a lower AA/EPA ratio than the placebo group.

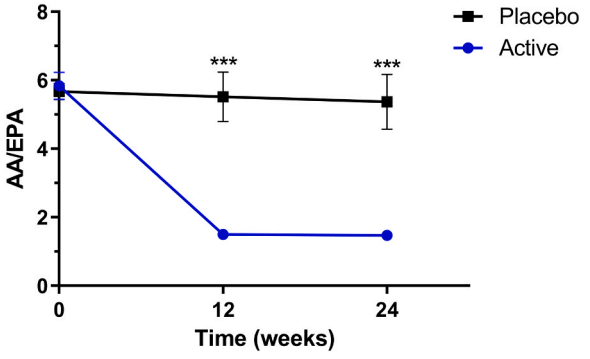

Fig. 2. Comparison of the AA/EPA ratio in the active vs. placebo groups at Visit 1, Visit 3 and Visit 4. There was a statistically significant decrease in the AA/EPA ratio between Visit 1 and Visits 3 and 4 for the active group (***p<0.001).

Table 3
The 7-item questionnaire is shown along with the mean (standard deviation; SD) and median of the total questionnaire score and the minimum and maximum values. P values of the statistical tests are also presented.

Questionnaire questions				
Q1: How would you describe your vision compared to your initial visit?				
Q2: How would you describe your ability to read/see words compared to your initial visit?				
Q3: How would you describe your overall eye function compared to your initial visit?				
Q4: How do you feel in general compared to your initial visit?				
Q5: How satisfied are you with the amount of energy you have for everyday activities compared to your initial visit?				
Q6: How has your interest or pleasure in doing things changed compared to your initial visit?				
Q7: In what way have your thoughts about the future changed compared to your initial visit?				
	Total questionnaire score	**Placebo group**	**Active group**	**P value**
Visit 3	**Mean (SD)**	9.33 (2.42)	9.23 (3.14)	0.751[a]
	Median	9.00	8.00	
	Minimum - Maximum	7.00–13.00	7.00–17.00	
Visit 4	**Mean (SD)**	7.28 (2.36)	9.38 (3.35)	0.161[a]
	Median	7.00	9.00	
	Minimum - Maximum	5.00–12.00	4.00–16.00	
		$p = 0.093$[b]	$p = 0.964$[b]	

[a] Between-group comparisons: Comparing placebo vs active groups
[b] Within-group comparisons: Comparing Visit 3 to Visit 4

3.4. Results from the questionnaire on perceived vision and mood

3.4.1. Within-group comparisons (Visit 1 to Visits 3 and 4) and between-group comparisons (placebo vs. active groups) of the total questionnaire scores for perceived vision and mood

The content and face validity of the questionnaire were assessed by a panel of experts (academics and ophthalmologists) and confirmed. The reliability of the questionnaire was evaluated at both timepoints, with measures similar to those used in other studies [20,21]. Cronbach's α was close to 1 at both timepoints (0.801 at Visit 3 and 0.843 at Visit 4), corrected item-total correlations were satisfactory (ranging from 0.32 to 0.84), while Cronbach's α values did not increase if any item was deleted. The above verified the internal consistency and reliability of the questionnaire.

The total score on the 7-item questionnaire was obtained for each patient at Visit 3 and Visit 4. The questionnaire questions and all the results (descriptive and statistical tests) appear in Table 3. The mean score was similar between the two groups at Visit 3 (9.23 in the active group, 9.33 in the placebo group), but it was higher for the active group than for the placebo group at Visit 4 (9.38 vs. 7.28). Although the difference between groups at Visit 4 was not statistically significant, the within-group tests showed that the decrease in perceived vision and mood among the patients in the placebo group compared to the active group was marginally significant (at the 10 % level) from Visit 3 to Visit 4. Therefore, the results imply a consistently increased level of perceived vision and mood among the patients in the active group, as opposed to a decrease in perceived vision and mood level from Visit 3 to Visit 4 for the placebo patients.

3.5. Safety results

For the duration of the study, the patients were monitored for any signs of adverse events and the clinical investigators were placing phone calls prior to the visits to check on the patients' safety. The results showed that the administration of omega-3 fatty acids was safe and well-tolerated at the dosage regimen. No major adverse effects were reported that were directly related to the administration of omega-3 fatty acids. Two patients in the active group experienced unusual episodes, namely, left facial nerve paralysis and atrial fibrillation, and were treated accordingly; both of these patients also suffered from arterial hypertension prior to their enrolment in the study. Interestingly, none of the patients in the active group developed exudative AMD during the study period and follow up, but one patient from the placebo group had one of his eyes progressed to exudative AMD.

4. Discussion

4.1. Changes in visual acuity

In the current pilot study, the efficacy of EPA-rich omega-3 fatty acids supplementation was investigated in patients with two different ophthalmological conditions associated with progressive visual impairment that can eventually lead to blindness, i.e., dry AMD and Stargardt disease. To date, there is no specific treatment to improve vision for these conditions, only supportive measures. The primary objective of our study was to provide evidence of significant differences between the placebo and the active group with regards to the number of ETDRS letters gained following 24 weeks of treatment. Indeed, within the active group, a vision improvement was observed by an increase in the number of letters gained (6 ETDRS letters) relative to the placebo group (a loss of 0.14 ETDRS letters). This positive effect was also noted following 12 weeks of treatment; therefore, this finding may indicate that treatment with omega-3 fatty acids can be beneficial even at 12 weeks.

Moreover, we grouped the patients into those who gained >5 letters versus those who did not, in both the active and placebo groups since 5 letters make up a complete line on ETDRS chart. We found that more than half of the patients (57 %) in the active group gained > 5 letters at Visit 4, as opposed to none in the placebo group. Similarly to the previous set of data, this finding is of great significance since it seems to correlate the effect of treatment with an improvement in vision.

As an additional type of analysis, we grouped the patients depending on their condition and observed that patients with AMD gained a higher number of letters than Stargardt patients following treatment. Although the study had a small sample size and the results are indicative and should be treated with caution, the trend appears to favour patients with AMD in terms of treatment outcome.

4.2. Fatty acids levels

The AA/EPA ratio notably decreased in the active group from Visit 1 up to Visit 4, as opposed to the placebo group, in which the ratio remained the same. Examination of systemic biomarkers (including the levels of AA and EPA) when conducting therapeutic trials could be a good indication of disease progression and treatment success [8,22,23]. However, further large-sized studies are required to better establish the relationship between the level of certain fatty acids and the progression of such pathologies. Perhaps, lipidomic analysis of specific isolated cell types could provide insight on additional markers of disease

progression.

4.3. Questionnaire scores

Furthermore, the results from the questionnaire scores implied that subjective vision and mood was improved from 12 weeks to 24 weeks in the active group; thus, a longer duration of treatment may have a positive outcome on how patients perceive vision. In addition, the mean score of the questionnaire at Visit 4 was higher for the active group than for the placebo group, indicative of a positive correlation of the treatment with improvements in perceived vision and mood. This finding may suggest that the longer the treatment lasts the more the beneficial effects, thus long-term supplementation with omega-3 fatty acids may be desirable. Future studies could include more complex questionnaires, i.e. the National Eye Institute Visual Functioning Questionnaire – 25, to further assess patients' vision.

4.4. Safety data

For the duration of the study, the patients were monitored for any signs of adverse effects. Overall, omega-3 fatty acids supplementation (3660 mg EPA and DHA) was shown to be well tolerated. The fact that omega-3 fatty acids oral administration is not associated with significant adverse events provides a safe and well-tolerated approach to patients that suffer from vision loss. In addition, it is important to note that oral administration of omega-3 fatty acids can positively affect both eyes simultaneously, compared to intravitreal injections, which are associated with pain and other complications [24,25]. Moreover, omega-3 fatty acids may not only provide protection in eye diseases, but are also well-known to have other health benefits, including cardiovascular protective actions, as well as effects in diabetes, cancer, Alzheimer's disease, dementia, depression, and neurological development [26–28].

4.5. Limitations of the study

A major limitation of this study is the small sample size of the patients enrolled and participated. However, additional larger multicentre clinical studies are encouraged to confirm these results, in which patients could be further divided depending on their condition (e.g., AMD vs. Stargardt disease) and disease severity (mild vs. moderate vs. severe). Future studies could also involve a preventative approach for newly diagnosed patients with mild disease to investigate the effect of omega-3 fatty acids in averting vision loss. Furthermore, no comparisons were performed of the outcomes by patients' clinical characteristics (age, sex, or comorbidities) due to the small sample size; however, this is a point for consideration for future studies. Additional study limitations include the use of electronic and non-electronic ETDRS charts when assessing visual acuity, which resulted in combining data from both chart types, as well as the fact that treatment adherence was relied upon pill counts only and may not have been a realistic representation of the actual adherence.

4.6. Consistency of results

Our results are in agreement with additional studies that have evaluated the role of diet in retinal degeneration. In a meta-analysis and systematic review of prospective cohort studies, Zhu *et al.* [29] demonstrated that fish consumption could reduce the incidence of AMD; specifically, they found a significant dose response relationship between fish consumption and the risk of AMD. To examine the association of omega-3 dietary intake (from fish sources) with the incidence of late-stage AMD, the AREDS2 Research Group estimated nutrient and food intake from a validated food frequency questionnaire in AREDS participants [12]. The obtained data indicated that people consuming the highest levels of EPA and EPA + DHA omega-3 fatty acids had a 50 % reduced likelihood of disease progression (from bilateral drusen to central GA), indicating a clear correlation between dietary lipid intake and the progression of AMD. Recently, Dziedziak *et al.* [30] reviewed the effects of dietary antioxidants in AMD and glaucoma patients and concluded that for AMD, carotenoids and omega-3 fatty acids were sufficient for preventing neurodegeneration.

The role of a wider range of omega-3 fatty acids in the diet was investigated in relation to disease severity in 18 family members who were genetically verified to have Stargardt disease and 26 family members without the associated gene mutation [9]. Red blood cell membrane and adipose lipid EPA and DHA levels were correlated with disease phenotypic severity based on the BCVA, dilated fundus examinations and fundus photography. When red blood cell lipids were analysed, an inverse relationship was identified between disease severity and both EPA and DHA levels. When the adipose lipids were analysed, a significant inverse relationship was detected between the phenotype and the level of EPA, but there was no significant correlation between the phenotype and the level of DHA. Promisingly, dietary factors appear to influence the severity and possibly the progression of human macular dystrophies. The findings of these studies suggest that the type of omega-3 fatty acids and the specific dose used, play significant roles in disease progression and therapeutic outcome in each patient.

An evident example being the AREDS2 trial, where the omega-3 fatty acids formulation used was low in both EPA (650 mg per day) and DHA (350 mg per day), therefore the protective effects of these fatty acids perhaps were not evident in patients with dry AMD [12]. In our study, the amount of omega-3 fatty acids (3660 mg of EPA and DHA) was much higher compared to the AREDS2 study, which aligned with our initial hypothesis, that omega-3 fatty acids and especially EPA, may demonstrate beneficial effects at a high enough dose. The emphasis is given to EPA, for several reasons; a) high levels of DHA are usually incorporated in the retina [31], whereas retinal EPA is low or undetectable, perhaps due to its greater metabolism by b-oxidation in the brain [32]; however, we have previously demonstrated an increase of retinal EPA following treatment with the specific omega-3 fatty acids formulation [14–16]; b) EPA competes with AA and significantly inhibits AA oxygenation *in vitro*, thus reducing activity in the inflammatory pathways involving prostaglandins D, E and F [33,34]. Therefore, not only there is an increase in blood and retinal EPA, as a direct consequence from the oral administration, but there could also be a reduction in the AA levels, due to the nature of the competition with EPA. This possibly could further decrease the AA/EPA ratio, to the desirable range.

As a final note, numerous studies have been reported in both AMD and Stargardt disease patients involving stem cell therapy, gene treatments, various pharmacological approaches and dietary supplementation [11,35–39]. While some of these studies included comparison of treatment groups with placebo controls, no placebo-controlled study with an oral supplement has shown significant improvement in vision, (i.e. increase in visual acuity), highlighting the significance of this pilot study.

5. Conclusion

Ocular pathologies, including dry AMD and Stargardt disease, lack a therapeutic approach for preventing disease progression in terms of vision loss. However, this pilot study demonstrated that omega-3 fatty acids (3660 mg; EPA and DHA, Eyetas®, Eyetas Therapeutics LTD) may provide a mean to improve objective and subjective vision or prevent further vision loss, in patients with moderate to severe dry AMD or Stargardt disease. The encouraging results of this study may suggest that EPA-rich omega-3 fatty acids supplementation has a positive impact on the quality of life of patients experiencing these conditions. Future multicentre studies with larger sample sizes are encouraged to further evaluate the potential of omega-3 fatty acids as a therapeutic intervention in dry AMD and Stargardt disease patients.

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CRediT authorship contribution statement

Ekatherine Prokopiou: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Panagiotis Kolovos:** Methodology, Formal analysis, Data curation. **Haritini Tsangari:** Methodology, Formal analysis, Data curation. **Saddek Mohand-Said:** Investigation, Data curation. **Luca Rossetti:** Investigation, Data curation. **Leonardo Mastropasqua:** Investigation, Data curation. **Francesco Bandello:** Investigation, Data curation. **Tassos Georgiou**: Supervision, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Tassos Georgiou has patents related to the use of omega-3 fatty acids in eye diseases.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phanu.2024.100400.

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