

Improvement of Visual Functions and Fundus Alterations in Early Age-Related Macular Degeneration Treated with a Combination of Acetyl-L-Carnitine, n-3 Fatty Acids, and Coenzyme Q10

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Key Words

Age-related macular degeneration · Acetyl-L-carnitine · n-3 fatty acids · Coenzyme Q10 · Mitochondria · Clinical trial · Visual field · Visual acuity · Drusen

Abstract

The aim of this randomized, double-blind, placebo-controlled clinical trial was to determine the efficacy of a combination of acetyl-L-carnitine, n-3 fatty acids, and coenzyme Q10 (Phototrop[®]) on the visual functions and fundus alterations in early age-related macular degeneration (AMD). One hundred and six patients with a clinical diagnosis of early AMD were randomized to the treated or control groups. The primary efficacy variable was the change in the visual field mean defect (VFMD) from baseline to 12 months of treatment, with secondary efficacy parameters: visual acuity (Snellen chart and ETDRS chart), foveal sensitivity as measured by perimetry, and fundus alterations as evaluated according to

the criteria of the International Classification and Grading System for AMD. The mean change in all four parameters of visual functions showed significant improvement in the treated group by the end of the study period. In addition, in the treated group only 1 out of 48 cases (2%) while in the placebo group 9 out of 53 (17%) showed clinically significant (>2.0 dB) worsening in VFMD ($p = 0.006$, odds ratio: 10.93). Decrease in drusen-covered area of treated eyes was also statistically significant as compared to placebo when either the most affected eyes ($p = 0.045$) or the less affected eyes ($p = 0.017$) were considered. These findings strongly suggested that an appropriate combination of compounds which affect mitochondrial lipid metabolism, may improve and subsequently stabilize visual functions, and it may also improve fundus alterations in patients affected by early AMD.

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Introduction

Age-related macular degeneration (AMD) is a slowly progressive neurodegenerative disease of the central retina, which results in impairment of principal retinal func-

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tions. It is the most common cause of severe vision loss among people aged over 55 in the industrialized countries and as life expectancy increases, the disease is becoming an increasingly important problem [1]. The previous pathophysiologic concept on AMD assigned a primary role to the oxidative damage to the retinal pigment epithelium (RPE), photoreceptors and Bruch's membrane due to an imbalance between generation and elimination of reactive oxygen species (ROS) [2]. Epidemiological studies supported this concept showing low antioxidant diet as a risk factor for AMD [3], while higher antioxidant uptake seemed to attenuate risk for AMD [4, 5]. Based on this concept several single antioxidants or their combinations were suggested for treating AMD [6–9].

There is accumulating evidence that mitochondrial dysfunction plays a central role in the pathogenesis of several neurodegenerative diseases. Apart from being the main source of ROS, mitochondria are also the primary target of oxidative damage, which results in mutation of mtDNA and alteration of mitochondrial membranes [10, 11]. Furthermore, besides generation of ROS and reduced production of energy, mitochondrial dysfunctions also include disorders of cellular Ca^{2+} homeostasis, impairment in oxidation of fatty acids, impairment in the biosynthesis of amino acids and lipids, disorders of steroid metabolism, and in extremis, activation of apoptosis pathway [12]. These findings also suggested that mitochondria may be a potential target for early treatment of neurodegenerative diseases [13, 14].

Recent studies on AMD showed mutation of mtDNA in both photoreceptors [15] and RPE [16], suggesting that mitochondrial dysfunctions may also play a role in the development of this disease. Histopathologic studies on early AMD specimens revealed a triad of alterations in the RPE: (a) disorganization of mitochondrial membranes, (b) proliferation of peroxisomes and (c) accumulation of lipofuscin granules, each of which is related to the very high turnover rate (10–15% per day) of photoreceptor outer segments (POS) [17]. Both mitochondria and peroxisomes are key organelles of cellular lipid metabolism [18]. Alterations of these RPE organelles may result in (a) a deficit in the recycling of POS membrane lipids, primarily decosahexaenoic acid and subsequent impairment of photoreceptor renewal and functions, and (b) a deficit in the lipid catabolism and subsequent accumulation of partially degraded lipids in the RPE and in Bruch's membrane. These lipids, mainly lipofuscin and several poorly characterized lipid peroxides are responsible for the development of advanced forms (atrophic or exudative) of AMD [19].

All these findings justified the assumption that an early intervention in mitochondrial lipid metabolism, a *metabolic* rather than *simple antioxidant approach*, may be a more specific way for attenuating retinal aging and for treating early AMD. After a careful selection of mitochondriotropic (in short 'mitotropic') compounds, i.e. compounds which have particular affinity to mitochondria together with the capability of improving mitochondrial metabolism, a combination of acetyl-L-carnitine (ALC), n-3 fatty acids (n-3 FA), and coenzyme Q10 (CoQ10) was applied to test this hypothesis [20].

ALC contains carnitine and acetyl moieties, both of which have neurobiological properties. Carnitine is a fatty acid carrier through the mitochondrial inner membrane and it plays an important role in the beta-oxidation of fatty acids [21]. The acetyl moiety can be used for controlling the availability of acetyl-CoA and sustaining the activity of the respiratory chain complexes. Thus ALC modulates both membrane phospholipid and high-energy phosphate metabolism [22]. Moreover, ALC affects inner mitochondrial membrane protein composition [23].

n-3 FA, a subgroup of polyunsaturated fatty acids (PUFAs), are structural components of all cell membranes, thus playing an essential role in the regulation of both plasma and organelle membrane functions [24]. The nervous system is very rich in PUFAs, particularly in docosahexaenoic acid and eicosapentaenoic acid [25]. These fatty acids also serve as specific precursors for eicosanoids that regulate several cell and organ functions [26]. The most significant effects of n-3 FA relate to the regulation of neuronal development and maturation of the sensory systems, first of all the retina [27], as well as to the regulation of gene expression involved in both fatty acid oxidation and lipid synthesis [28, 29].

CoQ10 is a lipophilic antioxidant molecule and is an integral part of both mitochondrial electron transport chain [30] and plasma membrane redox system [31]. It plays a pivotal role in cell metabolism and in cell survival [32].

Several experimental and clinical studies showed that: (a) these compounds from dietary uptake preferentially accumulate in the mitochondria of various organs [33, 34], retina included [35, 36]; (b) this accumulation was associated with functional improvement in the RPE-photoreceptor complex [20], and (c) there is a well-defined synergy between these compounds [37, 38]. Finally, a diet rich in n-3 FA and fish was associated with a lower risk of AMD in epidemiological studies [39, 40].

A previous pilot study on the efficacy of these *mitotropic compounds* reported a marked improvement of

some retinal functions in a small group of patients affected by early AMD, and this improvement was maintained by the end of the 2-year treatment period [20]. The present randomized, double-blind, placebo-controlled study is an extension of the previous study on a large series of patients affected by early AMD. The primary objective of this study was to determine how a combination of ALC, n-3 FA, and CoQ10 (Phototrop[®], Sigma-tau Health Science Ltd., Pomezia, Rome, Italy) influenced *visual functions* of patients aged 55–70 years with early AMD after 12 months of treatment compared with placebo-treated patients. The aim of the present study was also to evaluate changes in fundus alterations, as one of the secondary outcome measures.

Patients and Methods

Study Design

The study was designed as a randomized, double-blind, placebo-controlled, single-center protocol. Treatment of patients was carried out in the Department of Ophthalmology, University of Pecs (Pecs, Hungary), recruiting patients from consecutive cases of the clinical population, and the site was monitored by the staff of a Contract Research Organization (CRO): Therapeutic Management Ltd. (UK). Institutional Review Board/Ethics Committee approval was obtained.

Enrollment. A total of 100 patients were planned to be enrolled in the study, with a total of 106 patients actually enrolled and analyzed. To meet the criteria for enrollment in the study, patients (male or female) had to have a diagnosis of early bilateral AMD with best corrected visual acuity between 0.8 and 0.4 Snellen decimal chart (in the most affected eyes), be 55–70 years of age at enrollment, agree to discontinue any current vitamin regimen and provide written informed consent (see detailed inclusion and exclusion criteria in table 1).

Randomization. Patients were randomly assigned to receive either Phototrop or placebo for 12 months according to two computer-produced randomization schedules generated by statisticians at the CRO. Each schedule was balanced in blocks of 4, with 2 patients receiving Phototrop and 2 patients receiving placebo within each block. Clinic visits were scheduled every 3 months, with telephone or postal contact in between visits. Ocular examination in the screening period included an 'introductory' examination with automatic visual field analyzer to learn the correct use of these tests.

Masking. Both products were provided in yellow capsules and were indistinguishable in appearance. Allocation to the two study medications was random and masked, so neither the patient nor the investigational site staff knew which treatment each patient would receive.

Study Medication and Period. Medication consisted of 2 oral capsules per day, containing either: 100 mg of ALC, 530 mg of n-3 FA, and 10 mg of CoQ10 or an equal quantity of soy oil. The composition was calculated based on previous experiences [20]. In determining the treatment period and composition of placebo both

Table 1. Inclusion and exclusion criteria

Inclusion criteria

- 1 Have a diagnosis of early bilateral AMD
- 2 Have a visual acuity between 8/10 and 4/10 (Snellen chart decimal scale)
- 3 Be 55–70 years old and of Caucasian origin
- 4 Agree to discontinue any current vitamin regimen
- 5 Be highly motivated, alert, oriented, mentally competent and able to understand and comply with the requirements of the study, abide by the restrictions and return for all required visits
- 6 Provide written informed consent

Exclusion criteria

- 1 Late AMD (geographic atrophy or macular scarring)
- 2 Exudative retinal disease, including exudative AMD
- 3 Clinically significant corneal opacity or cataracts
- 4 Inherited retinal dystrophies or degenerative myopia
- 5 Unstable glaucoma
- 6 PVR, rhegmatogenous retinal detachment
- 7 Optic nerve disease
- 8 Active intraocular inflammatory disease
- 9 Refractive error over +4 D and –6 D
- 10 Significant cardiovascular or cerebrovascular diseases
- 11 Severe or uncontrolled hepatic, renal, pulmonary, and thyroid disease or diabetes
- 12 History of HIV infection, hepatitis B or C, or other immunosuppressive disorders
- 13 History of alcoholism, drug abuse, severe mental disorders
- 14 Were a practicing vegetarian or had an abnormal diet (<1,600 or >3,500 kcal/day)
- 15 Poor general health or unstable diseases
- 16 Known or suggested hypersensitivity to study compounds
- 17 Use of corticosteroid, phenothiazine and antimalarial drugs within 1 month prior to visit 1 or during the 12-month study period

scientific and ethical aspects were considered, i.e. the shortest period for demonstrating efficacy in the treated group and, at the same time, for creating minimal risk for an eventual worsening in the placebo group by leaving them without effective treatment.

Prior and Concomitant Therapy. All concomitant medications, including nonprescription medications, taken by the patient from 30 days prior to visit 1 up until day 360 were recorded in the case report form. Patients were not to take any medicinal product for AMD or any prohibited medications such as corticosteroid, phenothiazine and antimalarial drugs within 1 month prior to visit 1 or during the 12-month study period. There was no restriction on drugs taken previously by the patients for clinical conditions not connected with the study pathology. Any adjustments to the dosage or any new therapies for pathological events that occurred during the study were to be reported in the appropriate section of the case report form.

Treatment Compliance. Patients were instructed to take the study medication according to the prescribed dosage regimen. Study medications were dispensed to each patient at each clinic

visit. The patients were requested to return all unused medication and empty packaging to the investigator. The number of capsules that were taken was calculated from the number dispensed and the number returned or lost. The unused capsules from the previous period were collected, counted and recorded during each visit to allow the investigator to check compliance and complete drug accountability.

Visual Functions

The *primary efficacy variable* was visual field mean defect (VFMD), the reciprocal of the visual field mean sensitivity. A paper by Midena et al. [41] gave mean (\pm SD) sensitivity for AMD patients as 28.57 ± 1.80 dB. With 50 patients per group, this study would therefore have 80% power to detect a difference of 1.008 dB (i.e. 0.56 of the SD) in mean sensitivity between the two groups. Central visual field sensitivity was assessed using the Central 10-2 visual field program of the Humphrey Field Analyzer, model 630 (Zeiss, Germany). Standard background luminance of the instrument was 10 cd/m^2 ; the standard target size was Goldmann III. This program measures the foveal sensitivity and 68 different light sensitivity points of a circular 20° diameter retinal area centered on the fixation point. Mean defect of the central field sensitivity and foveal sensitivity measured in decibel were automatically recorded.

Two different methods were used for evaluation of changes in VFMD: (a) comparison of the *mean change* in VFMD from the baseline to 6 and 12 months in both the treated group and the placebo group, as well as between groups, and (b) comparison of the *number* of 'improved', 'unchanged' and 'worsened' cases in both the treated group and the placebo group, as well as between groups. For the latter, we determined 'physiological' long-term fluctuation of VFMD (i.e. differences between two consecutive examinations) as described by Hutchings et al. [42]. In a heterogeneous population (20 healthy subjects aged 43–65 years, mean 57 years) it was ± 1.96 dB (unpublished data). Based on these experiences we evaluated the distribution of mean changes in our trial assigning a range of ± 2.0 dB for the 'unchanged' subgroup, while higher positive values were assigned for the 'improved' subgroup and higher negative values for the 'deteriorated' subgroup.

Secondary efficacy variables were visual acuity as measured by the Snellen visual acuity chart and by the chart of the Early Treatment Diabetic Retinopathy Study (ETDRS), as well as foveal sensitivity as measured by perimetry and changes in fundus alterations. Snellen visual acuity chart (Projektor, Zeiss, Jena, Germany) with the difference between lines being 1/10 as a decimal chart was used to measure visual acuity. We also expressed visual acuity as the logarithm of the minimum angle of resolution (LogMAR) for the analyses. The ETDRS chart (General Electric F20T12-D 20W Daylight, Lighthouse Low Vision Product 36-02, Long Island City, New York, USA) has 14 lines with five letters per line. The progression of letter height from line to line is geometric. Each subject was asked to read the letters starting at the top left, working down to the smallest letter at the bottom right. They were instructed to give one reading for each letter, guessing if they were not sure. Visual acuity was assessed by scoring the number of letters read correctly. These secondary efficacy variables were compared to baseline at 3, 6, 9 months and at the end of the study in both groups, as well as between groups.

Fundus Alterations

Fundus photographs of both eyes were taken with a TOPCON Retinal Camera TRC 50ix from the central 35° area centered to the fovea at screening, after 6 and after 12 months. For digital images, the Sony 3CCD camera (model DXC950P) with 768×576 -pixel resolution for each of three colors and the Topcon Image Net System 1.53 were used. Optimal color balance and contrast were found when the software was configured with red gain set to 05 and blue gain to 00. The minimum gain adjustment was set to 136 and the maximum to 156. All images were stored as uncompressed TIF files. Digital images were examined on an IBM 15" CRT monitor (Model 6639-U3N, IBM, Armonk, New York, USA). The monitor was set at 16 bits high color and $1,024 \times 768$ -pixel resolution at 85 Hz. The monitor provided a $\times 10$ increase in image size, resulting in a total magnification, including that of the camera, of approximately $\times 25$. No image manipulation was used before or during grading. According to the previous studies these methods permitted a correct evaluation of even small fundus alterations [43, 44]. A grid consisting of three circles concentric with the center of the macula and four radial lines was superimposed onto the photographs. The diameter of the innermost circle of the grid corresponded to one third of the disc diameter (considered to be $500 \mu\text{m}$) in the fundus of an average eye, and the diameters of the middle and outer circles corresponded to 1,500 and $3,000 \mu\text{m}$, respectively. To measure drusen size, circles with diameters of 63, 125, and $250 \mu\text{m}$ were drawn using a special software (Adobe PhotoDeluxe, Adobe Inc., Calif., USA). Classification and grading of AMD were performed according to the definitions of the International Classification and Grading System (ICGS) for AMD [45].

Statistical Evaluation

Data were analyzed for all subjects who received at least one dose of the trial medications and had at least one return visit with efficacy data, i.e. analysis by intention-to-treat population. The primary unit for analysis was a patient, using the most affected eye at baseline for the analysis. Parallel analysis was also performed on the less affected (fellow) eye, with 1 patient being the primary unit for analysis.

The change in *visual functions* in both groups between the baseline values and the values at each visit was computed. As all data were nonnormally distributed, Mann-Whitney U test was used to assess the significance of the change between the two groups, with the Bonferroni-Holm adjustment for the p value in each variable group [46] even though we are aware that there has been much debate about the usefulness of this method [47]. For comparison of 'improved', 'unchanged' and 'deteriorated' cases in both treated and placebo groups, Fisher exact test was applied. In case of VFMD long-term fluctuation data were used for comparison, while in the case of the secondary efficacy variables we calculated the mean change between the baseline and 12-month visit in the placebo group, and used this data for determining 'improved', 'unchanged' and 'deteriorated' cases.

For quantitative evaluation the *drusen-covered area* (the sum area of drusen) was determined in all photos: the area of the circle with different diameters was expressed in square micrometers and multiplied by the number of drusen for each eye. All photos were evaluated by 2 different graders, the photographs were presented in random order to the separated retinal screeners, who were blind for 'treatment' or 'placebo' groups. Intergrader reliability for the detection of drusen number and drusen size in gradable photo-

Table 2. Comparison of changes in visual field mean defect of the most and less affected eyes^a

	Most affected eyes		Less affected eyes ^b	
	treated (n = 48)	placebo (n = 53)	treated (n = 43)	placebo (n = 45)
Improved or unchanged	47 (98%)	44 (83%)	43 (100%)	40 (89%)
Deteriorated	1 (2%)	9 (17%)	0 (0%)	5 (11%)
p	0.006		0.031	
Odds ratio	10.93		11.81	

^a ± 2.0 dB long-term fluctuation was applied.

^b Data were modified by adding 0.5 to each value in the less affected eyes for odds ratio computing.

graphs was excellent (kappa values 0.85 and 0.80, respectively). As all data were nonnormally distributed, these values were evaluated by Wilcoxon signed-rank test within a group, while Mann-Whitney U test was used to assess the significance of the change between the two groups. In addition, the ratio of drusen-covered area at 12 months to screening was determined, and the distribution of improved (ratio <1), unchanged (ratio = 1), and deteriorated (ratio >1) cases was evaluated by Fisher exact test.

Data were entered into a computerized database, and statistical calculations were performed using a commercially available statistical package (Statistica 6.0, Statsoft, Inc. Tulsa, Okla., USA); the significance level was $p < 0.05$ in all statistical analyses. All data are indicated as mean \pm SD.

Results

A total of 106 patients were randomized to the study: 51 to the treated group and 55 to the placebo group. The majority of patients completed the study (94% in the treated group and 96% in the placebo group). Overall 5 patients (3 from the treated group and 2 from the placebo group) interrupted study medication, of which 2 patients (1 from the treated group and 1 from the placebo group) had no postbaseline efficacy data and 3 (2 in the treated group and 1 in the placebo group) were due to adverse effects (not related to treatment). The mean age of patients in the treated group was 63.5 ± 2.45 and 63.0 ± 2.95 years in the placebo group. The majority of patients were female (67%), with similar percentages of males and females being seen between treatment groups and between the two age groups. Only 15.1% of patients smoked tobacco and 18.9% of patients consumed alcohol, with the incidence between treatment groups being similar. All patients achieved compliance levels $\geq 80\%$.

Visual Functions

When VFMD of the *most affected eyes* was considered (fig. 1a), in the treated group there was an improvement after 6 months from baseline (0.76 ± 2.03 dB), which was maintained by the end of the study (0.77 ± 2.57 dB). In the placebo group, there was an improvement after 6 months (0.69 ± 2.39 dB), followed by deterioration after 12 months (-0.31 ± 3.70 dB). However, none of these changes proved to be significant between the treated and the placebo group.

We also performed the same set of analyses for the *less affected (fellow) eyes* (fig. 1b). In the treated group there was an improvement after 6 months (0.49 ± 1.80 dB) and 12 months (0.53 ± 2.36 dB) as compared to the baseline. In the placebo group there was a slight worsening after 6 months (-0.01 ± 1.41 dB) and further worsening at the end of the study (-0.39 ± 1.52 dB). There was a statistically significant difference between the two groups after 6 months ($p = 0.023$) and at the end of the study ($p = 0.004$) in favor of the treated group (with the Bonferroni-Holm-adjusted $p < 0.025$ considered significant).

Comparison of changes in VFMD are summarized in table 2. In this examination ± 2.0 dB was applied as a range for long-term fluctuation (i.e. 'unchanged'). When the *most affected eyes* were considered, in the treated group 47 out of 48 cases (98%) were 'improved' or 'unchanged', and 1 (2%) 'deteriorated'. In the placebo group 44 cases out of 53 (83%) were 'improved' or 'unchanged' and 9 (17%) 'deteriorated'. The difference between treated and placebo groups was significant ($p = 0.006$, odds ratio: 10.93). Comparison of changes in VFMD of the *less affected eyes* showed similar results. In the treated group all eyes were 'improved' or 'unchanged' (100%), and no eyes 'deteriorated'. In the placebo group 40 cases from 45 (89%) were 'improved' or 'unchanged' and 5 (11%) 'de-

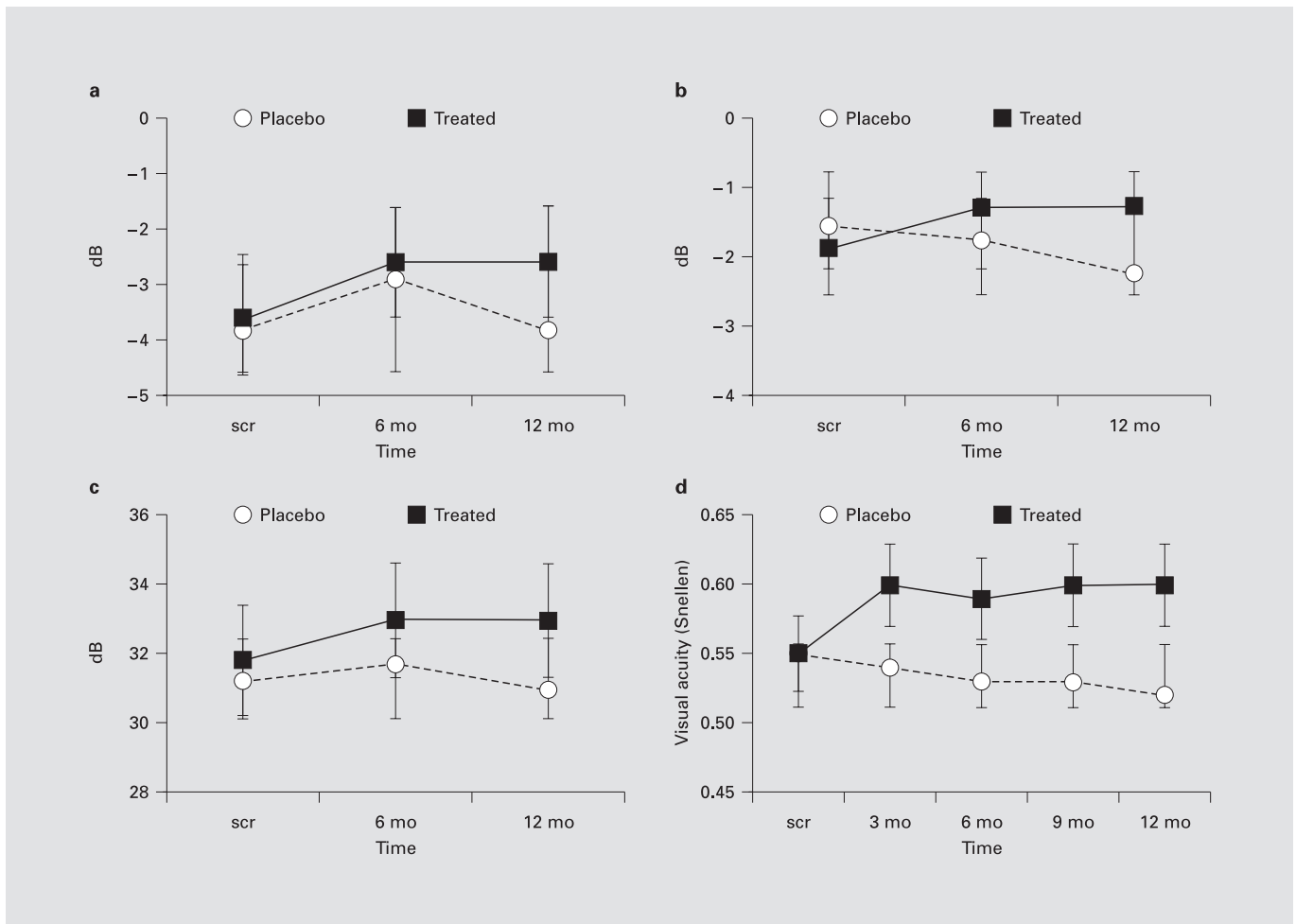


Fig. 1. Mean changes in VFMD of the most affected eyes (a) and the less affected eyes (b), as well as mean changes in foveal sensitivity (c), and in visual acuity (Snellen chart) of the most affected eyes (d). Visual functions maintained the initial improved levels in the treated group, while they deteriorated in the placebo group by the end of the observation period (scr = screening, mo = months).

teriorated'. The difference between treated and placebo groups was significant ($p = 0.031$, odds ratio: 6.61).

The mean change in foveal sensitivity for the *most affected eyes* is shown in figure 1c. In the treated group there was an improvement in foveal sensitivity at 6 months and at 12 months, while in the placebo group, an initial improvement in foveal sensitivity was seen after 6 months followed by a worsening after 12 months. No significant difference was found between groups at the end of the study (Mann-Whitney U test $p > 0.05$). However, in the treated group 33 (69%) eyes were 'improved' or 'unchanged' and 15 (31%) 'deteriorated'. In the placebo group 26 (49%) cases were 'improved' or 'unchanged' and 27 (51%) 'deteriorated'. The difference between treated

and placebo groups was statistically significant ($p = 0.035$, odds ratio: 2.29) (table 3).

The mean change in visual acuity using the Snellen chart for the most affected eyes is shown in figure 1d. An improvement was found in the treated group after 3 months and it was maintained by the end of the study. In the placebo group, a deterioration in visual acuity was seen by the end of the study period. A statistically significant difference was found between the two groups after 3 months in results (0.05 ± 0.15 vs. 0.03 ± 0.19 , Mann-Whitney U test $p = 0.012$, with the Bonferroni-Holm-adjusted $p < 0.0125$ considered significant). At the same time LogMAR parameters also showed similar results (-0.004 ± 0.15 vs. 0.003 ± 0.18 , Mann-Whitney U

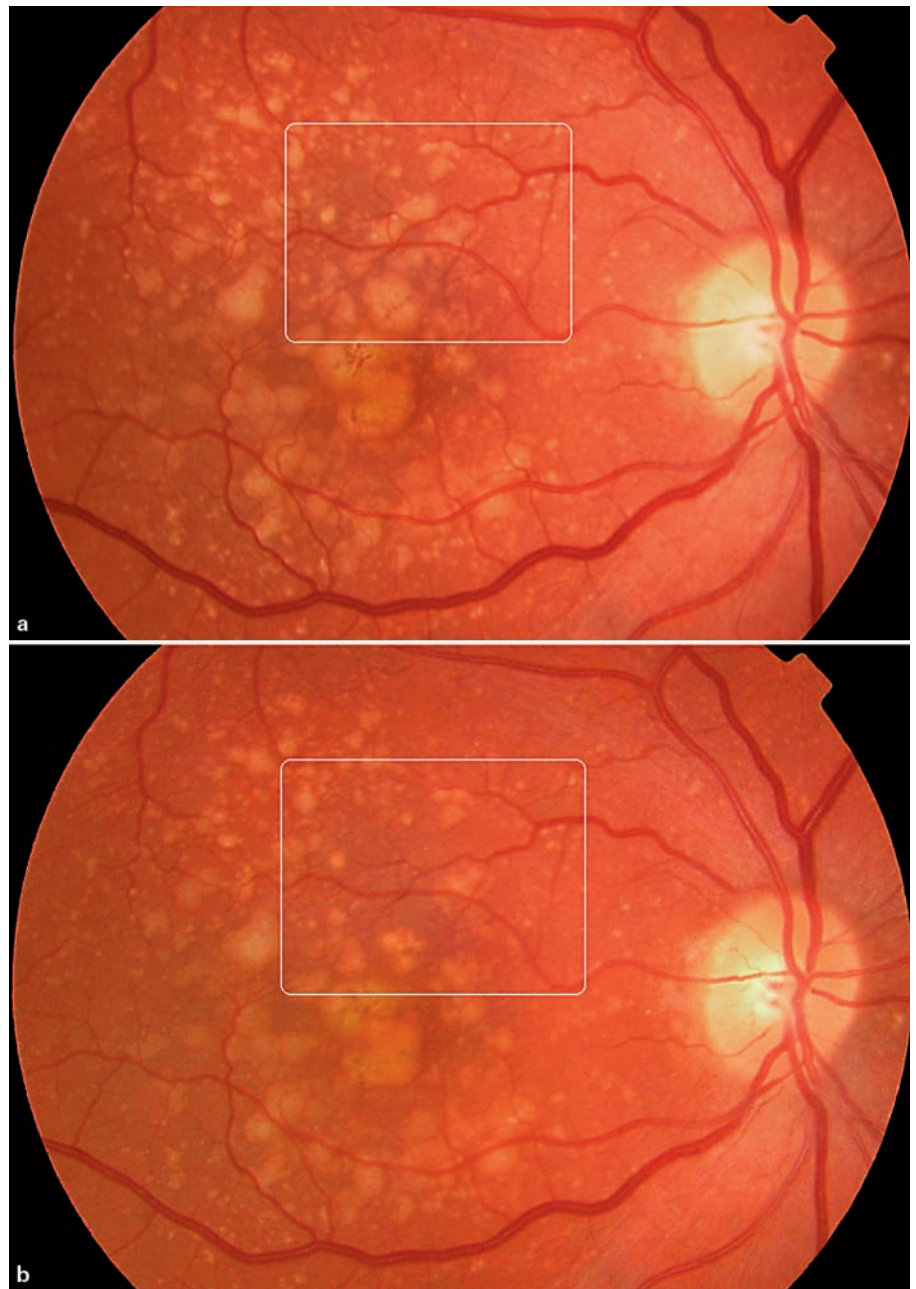


Fig. 2. Fundus alterations at screening (**a**) and after 12 months' treatment with Phototrop (**b**). Evident reabsorption of drusen can be verified in the whole affected area, particularly in the insert (69 years, female).

Table 3. Comparison of changes in secondary efficacy variables of the most affected eyes

	Foveal sensitivity		Snellen		LogMAR	
	treated (n = 48)	placebo (n = 53)	treated (n = 48)	placebo (n = 53)	treated (n = 48)	placebo (n = 53)
Improved or unchanged	33 (69%)	26 (49%)	37 (77%)	29 (55%)	36 (75%)	29 (55%)
Deteriorated	15 (31%)	27 (51%)	11 (23%)	24 (45%)	12 (25%)	24 (45%)
p	0.035		0.015		0.027	
Odds ratio	2.29		2.78		2.48	

test $p = 0.011$, with Bonferroni-Holm-adjusted $p < 0.0125$ considered significant). This improvement was maintained, although not statistically significant, by the end of the study (0.009 ± 0.23 vs. -0.014 ± 0.23). The assessment of visual acuity in the most severe eye using the ETDRS chart shows a similar trend to that seen with the Snellen chart, however there was no significant difference between groups at different time points. Comparing mean change in the visual acuity by Snellen chart between baseline and 12 months, in the treated group 37 (77%) cases were 'improved' or 'unchanged' and 11 (23%) cases 'deteriorated'. In the placebo group 29 (55%) eyes out of 53 were 'improved' or 'unchanged' and 24 (44%) 'deteriorated'. The difference between the treated and placebo groups was statistically significant ($p = 0.015$, odds ratio: 2.78). Change in visual acuity expressed in LogMAR also resulted in a statistically significant difference. In the treated group 36 (75%) eyes were 'improved' or 'unchanged', and 12 (25%) eyes 'deteriorated'. In the placebo group 29 (55%) were 'improved' or 'unchanged' and 24 (45%) 'deteriorated' ($p = 0.027$, odds ratio: 2.48) (table 3).

In the less affected eyes changes of secondary efficacy variables between the baseline and different time points were not significant (Mann-Whitney U test, $p > 0.05$), nor did Fisher's exact test reveal any differences between treated and placebo groups.

Fundus Alterations

Both eyes of each patient were photographed. However, 3 of the most affected eyes were excluded from further evaluation (2 from the treated group, 1 from the placebo group), as well as 4 of the less affected eyes (all from the placebo group) due to poor quality of the photograph. Furthermore, 7 of the less affected eyes (2 from the treated group and 5 from the placebo group) were not evaluated due to visual acuity better than 0.8, i.e. they did not enter into the inclusion criteria of this trial. Overall 92 eyes from the treated group (46 from the most affected subgroup and 46 from the less affected subgroup), and 96 eyes from the placebo group (52 from the most affected group and 44 from the less affected subgroup) were involved for evaluating fundus alterations. This was a homogeneous group of early AMD, and almost all cases corresponded to the criteria of the first and second grade of AMD. Only 8 out of 92 eyes in the treated group, and 4 out of the 96 eyes in the placebo group showed fundus alterations corresponding to the third grade of AMD.

Since AMD is a slowly progressive disease, it is unlikely to detect any fundus changes at 1-year follow-up,

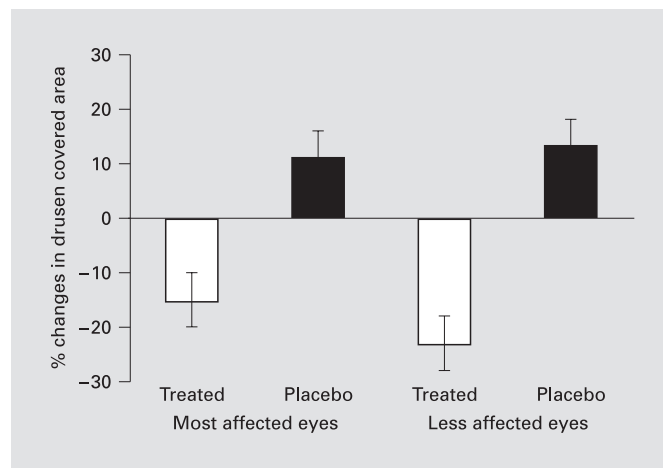


Fig. 3. Comparison of changes in the drusen-covered area (ratio of 12-month values to baseline) showed statistically significant differences between 'treated' and 'placebo' groups of both the most affected eyes and the less affected eyes (0.85 ± 0.39 vs. 1.11 ± 0.65 , $p = 0.045$ and 0.77 ± 0.43 vs. 1.13 ± 0.77 , $p = 0.017$, respectively).

but through the use of ICGS we were able to quantify these changes in fundus alterations. In addition, in several cases we could even reveal an improvement with ophthalmoscopy in the treated group (fig. 2).

When the *most affected eyes* were compared, the difference in the drusen-covered area between screening and 12 months was statistically not significant in the treated group ($p = 0.091$), or in the placebo group ($p = 0.6184$). However, comparisons of changes in the drusen-covered area (ratio of drusen area at 12 months to drusen area at screening) showed a statistically significant difference between treated and placebo groups (0.85 ± 0.39 vs. 1.11 ± 0.65 , $p = 0.045$). This means that the drusen-covered area decreased by 15% in the treated group, while it increased by 11% in the placebo group as compared to the screening (fig. 3).

When the *less affected eyes* were compared, the difference in the drusen-covered area between screening and 12 months was statistically significant ($p = 0.0003$) in the treated group, but not ($p = 0.587$) in the placebo group. However, as in the case of the most affected eyes, comparisons of changes in drusen-covered area showed a statistically significant difference between treated and placebo groups (0.77 ± 0.43 vs. 1.13 ± 0.77 , $p = 0.017$). This means that the drusen-covered area decreased by 23% in the treated group and increased by 13% in the placebo group as compared to the screening (fig. 3).

Table 4. Distribution of changes in the drusen-covered area

	Most affected eyes		Less affected eyes	
	treated (n = 46)	placebo (n = 52)	treated (n = 4)	placebo (n = 44)
Improved or unchanged	38 (83%)	39 (75%)	42 (91%)	31 (70%)
Deteriorated	8 (17%)	13 (25%)	4 (9%)	13 (30%)
p	0.25		0.01	
Odds ratio	1.58		4.40	

When the *distribution of changes in the drusen-covered area* of the most affected eyes was considered, the drusen-covered area improved or was unchanged in 38 (83%) and deteriorated in 8 (17%) out of 46 cases in the treated group, while in the placebo group it improved or was unchanged in 39 (75%) and deteriorated in 13 (25%) out of 52 cases ($p = 0.2525$; odds ratio: 1.58). When the less affected eyes were considered, in the treated group the drusen-covered area improved or remained unchanged in 42 (91%), and deteriorated in 4 (9%) out of 46 cases, while in the placebo group 31 (70%) were improved or unchanged and 13 (30%) out of 44 cases had deteriorated ($p = 0.0112$, odds ratio: 4.40) (table 4).

Discussion

Improvement was found in each of the four parameters of *visual functions* in the most affected eyes of early AMD patients taking Phototrop. It is particularly important that VFMD (the primary efficacy variable), visual acuity and foveolar sensitivity (secondary efficacy variables) showed statistically significant differences in changes comparing treated with placebo groups. To our knowledge, this is the first clinical study that demonstrated a *treatment-related improvement of visual functions in early AMD*. In addition, in the treated group only 2% showed clinically relevant (>2.0 dB) worsening in VFMD while in the placebo group that was 17% by the end of the study period. From a clinical point of view these are very important findings, as they could mean that every year 17 cases of visual impairment may develop from each 100 cases of untreated early AMD and that this impairment could almost completely be prevented by Phototrop treatment.

In previous studies visual functional tests have been found to be useful for detection of early AMD as they were found to be altered even in the asymptomatic fellow

eyes [48, 49]. Several studies also suggested that the measurement of central visual field sensitivity is the most suitable method for early diagnosis and for measuring treatment efficacy in studies for AMD [50, 51]. Our present experience is in full accordance with these observations. Mean sensitivity or mean defect of the central visual field is a simple, fast and noninvasive method for routine examination and at the same time, it is likely to be a better indicator of macular function compared to visual acuity. Furthermore, automatic field analysis is a more objective method compared to visual acuity measurement particularly in early AMD where good visual acuity may be associated with a worsening in the quality of vision probably due to the mosaic-like damage of the retina [41].

Comparison of the most and less affected eyes showed another important finding. In evaluating the results of the most affected eyes both primary and secondary variables showed a statistically significant treatment effect between baseline and final visits. This implies that the treatment had a potential to prevent further deterioration. However, among the less affected eyes direct improvement in the primary efficacy variable could be detected by paired comparisons of the change in VFMD between baseline and all time points. The Fisher exact test could also clearly demonstrate prevention of further worsening, although no detectable improvement was found in the secondary efficacy variables. These findings seem reasonable taking into consideration that the less affected eyes had better baseline visual function, and that they responded better to this treatment. These findings further urge early diagnosis and treatment of AMD.

In addition to improvement in the treated group and worsening in the placebo group, the time course of functional changes in each group showed important characteristics. In the treated group all four visual functions maintained the initial improved levels to the end of the observation period. In the placebo group, all visual func-

tions worsened by the end of the observation period, although some of them showed initial improvement (likely 'placebo effect'). These observations confirmed our preliminary results, which showed a similar time course of functional changes in both treated and placebo groups [20].

This clinical trial also showed that improvement in *fundus alterations* can be achieved after 1 year of treatment with Phototrop. The drusen-covered area decreased in the treated group while it increased in the placebo group, and the difference between the treated and the placebo groups became statistically significant in favor of the treated group by the end of the study. Improvement in fundus alterations, particularly reabsorption of some drusen, is a well-known clinical phenomenon, which may occur spontaneously, or it may be enhanced by low-dose laser treatment [52]. However, in our study the comparison of treated and placebo groups showed a statistically significant difference in favor of the treated group, suggesting a *treatment-related reabsorption of the drusen*. Comparison of treatment efficacy in fundus alterations of subgroups showed a more marked improvement if the less affected eyes were evaluated. These findings are in full agreement with the changes in visual functions found in this trial, i.e. the less affected eyes showed a more marked treatment-related improvement compared to the most affected eyes. We may speculate that this difference may come from an increased treatment efficacy in the *less damaged* RPE/photoreceptor and Bruch's membrane. This could be another element suggesting that early diagnosis and treatment are essential for successful treatment of AMD.

The correlation between visual functions and fundus alterations in AMD is somewhat controversial. In a small series of patients affected by early AMD there was no significant difference in sensitivity between drusen and non-drusen areas in each patient [53]. In another study visual field defects were significantly correlated with the area of atrophy, but not with the area of drusen. These results suggested that deficits of retinal sensitivity in patients with early AMD could be attributed to alteration of photoreceptor function associated with RPE atrophy, but not with drusen [54]. However, recent studies showed that an increase in drusen number and increasing drusen confluence reduced central visual field sensitivity and macular recovery function as well as some selected spatial frequencies of spatiotemporal contrast sensitivity in early AMD [41]. In another study, using short wavelength automated perimetry, patients with soft drusen had significantly lower sensitivity than those without, and the sensitivity was

also reduced in those eyes with fellow eyes having a sight-threatening complication of AMD [51]. Finally, a most recent study showed that the time course of dark adaptation was prolonged in early AMD. Furthermore, in a high proportion of patients with visual loss from AMD in one eye, the fellow eye showed abnormal dark adaptation [49].

Morphological studies also suggested a correlation between RPE/photoreceptor and Bruch's membrane alterations in AMD. Morphometric studies on RPE revealed alterations of mitochondrial membrane structures, especially damage to cristae, significantly more marked in early AMD as compared to age-matched controls [55]. Most recently, immunohistochemical studies revealed several drusen-associated structural and molecular abnormalities of retinal cells: shortening and deflection of photoreceptor inner and outer segments, abnormalities in the synaptic terminals of photoreceptor cells, and an increase in intermediate filament protein immunoreactivity within Müller glial cells. The structural and molecular abnormalities observed in both photoreceptors and Müller glial cells were confined to retinal regions directly overlying and immediately adjacent to both hard and soft drusen. Moreover, all these abnormalities were also found over small subclinical drusen [56]. Ongoing studies from our group are dedicated to quantify correlations between functional impairment and fundus alterations in AMD and to establish a functional classification of AMD adapted to the criteria of ICGS, which is based purely on fundus alterations.

As both RPE and photoreceptor cells are postmitotic cells, improvement of visual function in the treated group most likely comes from restoration of certain *cellular (metabolic) processes* responsible for impaired visual functions. Previous studies in heart and brain showed that dietary supplementation with a blend of n-3 FA and carnitine/ALC was associated with (a) extensive changes in the activities of key enzymes in the mitochondria [33], (b) improved recovery of mitochondrial energy metabolism [57], (c) increased n-3 FA content of mitochondrial membranes [58], and subsequent improvement in mitochondrial Ca^{2+} flux and Ca^{2+} -dependent processes [34], as well as (d) improvement of lipid metabolism [59, 60]. Taking these findings together with our findings we assume that beneficial effects of Phototrop on *visual functions* in early AMD may come from the synergetic action of ALC, n-3 FA, and CoQ10 on mitochondrial functions. Photoreceptors are among the most metabolically active cells in the body and require the functional support of the RPE for the exchange of metabolites and energy neces-

sary for the regeneration of photopigment, and for the renewal of shed photoreceptor disc membranes. These mitotropic compounds may help to preserve and/or restore membrane structure and function of mitochondria and subsequently may improve RPE/photoreceptor metabolism and either prevent or attenuate early AMD. This mode of action is essentially different from the effects of antioxidant vitamins and lutein. Furthermore, they have different indications, as mitotropic compounds seem to be effective for treating early AMD, while antioxidants seem to be more effective for preventing late complications of AMD [8, 9]. Thus, at a first glance, the metabolic and antioxidant approaches are alternative and/or complementary treatments for AMD depending on the severity of the disease.

There is accumulating evidence that alterations of the RPE/photoreceptor complex, besides impairment in visual functions, may also play a primary role in the development of fundus alterations seen in early AMD [61]. Studies analyzing the composition of drusen suggested that at least three cell-mediated mechanisms are responsible for Bruch's membrane alterations including drusen formation: (1) Discharge of partially metabolized lipid-containing materials by RPE in Bruch's membrane either diffusely or forming drusen [62]; the lipids were derived from long-chain PUFAs normally found in POS, providing support for the cellular origin of the lipids [63, 64]. (2) Arrest of cholesterol uptake from plasma by saturation of RPE metabolism, likely due to mitochondrial dysfunction [65]. (3) Deposition of proteins derived from RPE activated by local or systemic inflammations [66–68].

However, in AMD at least three drusen-mediated mechanisms have been established which may be responsible for functional and morphological alterations in RPE/photoreceptors associated with drusen [56]: (1) Drusen contains molecules that have a cytotoxic effect on RPE and photoreceptors, such as oxidatively modified proteins, cholesterol, apolipoproteins, several other poorly characterized lipid peroxides and glycoproteins including advanced glycation end products. Most recently, amyloid beta has been identified in substructural components of drusen. (2) Drusen impairs the normal exchange of ions and metabolites between choroidal blood supply and the RPE/photoreceptors by establishing a physical barrier to diffusion. (3) Displacement of RPE/photoreceptors by encroaching drusen damages their structural integrity.

All these findings justify the assumption that a bidirectional mechanism works in the pathophysiology of fundus alterations in early AMD: (a) a metabolic disorder of

the RPE/photoreceptor complex responsible for drusen formation, and (b) these drusen may further damage RPE/photoreceptor structure and function. Taking together these findings and our experiences on treatment-related *improvement in fundus alterations* we may suppose that beneficial effects of mitotropic compounds on fundus alterations are likely due to the restoration of certain metabolic processes of the RPE/photoreceptor complex involved in both visual functions and in turnover of Bruch's membrane.

However, alterations of Bruch's membrane may also include thickening of the basement membrane of RPE (lamellar deposits), and thickening of the basement membrane of the choriocapillary. These alterations are ophthalmoscopically not visible. In addition, drusen are three-dimensional alterations, and we were able to detect only two-dimensional changes. These limitations have to be taken into consideration when evaluating the effect of the treatment on these structures.

In conclusion, the results of this clinical trial suggest that treatment of early AMD with a combination of ALC, n-3 FA, and CoQ10 may improve both visual functions and fundus alterations likely by improving the metabolism of the photoreceptor/RPE/Bruch's membrane complex. Although our results have immediate clinical significance for treating early AMD, further studies are certainly needed to support this hypothesis, but first of all, to learn more on the pathophysiology of AMD.

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