

Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: the LUTEGA study

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Abstract

Background The primary objective of LUTEGA is to determine the long-term effect of a supplementation with fixed combination of lutein, zeaxanthin, omega-3-longchain-polyunsaturated-fatty-acids (O-3-LCPUFAs) and antioxidants on macular pigment optical density (MPOD) in patients with non-exudative age-related macular degeneration (AMD).

Methods The LUTEGA study is a double-blind, placebo-controlled clinical trial. 172 patients with non-exudative AMD were enrolled and randomized to three treatment arms. Supplementation included either once (dosage D1) or twice daily (dosage D2) of 10 mg L / 1 mg Z/ O-3-LCPUFAs (thereof 100 mg DHA, 30 mg EPA)/ antioxidants, or placebo (P). After best-corrected visual acuity (BCVA) test, blood sample was collected and MPOD was measured using the 1-wavelength-reflection method and recording reflection images at 480 nm (modified Visucam^{NM/FA}, Carl Zeiss Meditec, Germany). During 1 year of intervention, AMD patients were followed up after

1, 3, 6 and 12 months. 145 AMD patients (D1=50, D2=55, P=40) completed the study.

Results After 12 months of intervention, the MPOD parameters (volume, area, maxOD, meanOD) increased significantly in treatment arms D1 and D2 ($p < 0.001$). Volume of MPOD showed the highest within-group difference and increased significantly in D1 and D2, and decreased significantly in P ($p = 0.041$). Between-group comparison of absolute changes of all MPOD parameters were significantly different between D1 and P as well as D2 and P with $p < 0.001$ at end point ($t = 12$). BCVA, measured in log MAR, improved in D1 and in D2 ($p < 0.001$). After 12 months of intervention, the mean improvement in BCVA was significant in D2 ($p = 0.006$) and D1 ($p = 0.038$) compared to P.

Conclusions The supplementation of L, Z, O-3-LCPUFAs and antioxidants resulted in considerable increase in MPOD. There was no difference in accumulation of MPOD between both dosages. Thus, we believe that the used supplementation with L and Z seems to reach a saturation level in retinal cell structure. Additionally, the constant supplementation of L, Z, O-3-LCPUFAs and antioxidants in AMD patients seems to be useful, because MPOD reduces without supplementation. We conclude that the supplementation caused an increase of MPOD, which results in an improvement and stabilization in BCVA in AMD patients. Thus, a protective effect on the macula in AMD patients is assumed.

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Keywords Age-related macular degeneration · Macular pigment · Lutein · Zeaxanthin · Omega-3-LCPUFAs

Introduction

Retinal diseases with central vision loss, especially age-related macular degeneration (AMD), rise steadily through

the increasing and aging population of the western world [1, 2]. AMD is a multifactorial disease, which is associated with genetic and environmental risk factors [3, 4]. The photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaries are primarily affected in AMD [5]. Oxidative stress has been implicated in many disease processes, especially age-related disorders, and refers to cellular damage caused by reactive oxygen species (ROS) [6]. The antioxidant protective system in the macula consists of lutein (L) and zeaxanthin (Z), the key components of macular pigment (MP). The antioxidant effects of L and Z are caused by scavenging of free radicals, quenching of reactive oxygen species and preventing of lipid peroxidation [6–8]. Furthermore, MP absorbs short wavelength with a maximum of 460 nm, acts as a blue light filter and reduces chromatic aberration [9]. As a result of this, a protection of the retina from blue light induced damage can be assumed [10].

The measuring ability of the optical density of macular pigment (MPOD) in the retina is a magnificent advantage to assess the risk associated with AMD. Moreover, it is possible to determine changes of MPOD. Thus, how different dosages of targeted supplementation or certain diets affect the macula can be monitored. In-vivo measuring techniques have been evaluated for determination of MPOD [11, 12]. These techniques differ in the duration of examination, glare for the patient and demands on eye fixation behaviour. Objective methods, like autofluorescence or reflectometry methods, seem to be better suitable for clinical routine examinations, as well as for the determination of the spatial distribution among maximum of MPOD [13]. The determination of spatial distribution of MPOD provides a more complete and accurate representation of MP levels, and may enable the correlation of distribution with developing pathology [11].

Leafy green vegetables and eggs are a natural source for L and Z intake [14], whereas the daily intake of both carotenoids in the Western world is approximately 1–2 mg per day [15, 16]. Low concentrations of MP may be associated with an increased risk of AMD [17, 18]. Healthy eyes predisposed to AMD due to advanced disease on the fellow eye have significant less macular pigment than healthy eyes without such risk [19]. Furthermore, it is known that eating habits have a direct influence on MPOD. Patients with a low daily intake of fruits and vegetables especially showed a decreased macular pigment in the eye [20]. Some randomized, placebo-controlled studies on AMD patients report an improvement of visual acuity, contrast sensitivity and/or glare in correlation to macular pigment [21–23], statistically valid in some studies [23–25]. Recent studies strongly support that supplementation with macular carotenoids is probably the best method of increasing the antioxidant defences of the macula, which may be associated with a reduced risk of AMD progression [26].

The omega-3-long-chain polyunsaturated fatty acids (O-3-LCPUFAs) are another relevant group of nutrients in association with AMD. The main O-3-LCPUFA in the retina is docosahexaenoic acid (DHA), which is located as phospholipid within the membranes of retinal photoreceptor outer segments and may affect membrane permeability, fluidity and properties of lipid phase [27]. Some mediators of LCPUFAs are involved in inflammatory processes and immunomodulation [28]. Chong et al. report on associations between the consumption of fish as well as other food rich in O-3-LCPUFAs and a lower risk of AMD [29, 30]. Dietary antioxidants and minerals seem to be useful for preventing the development and progression of advanced forms in AMD. A randomised clinical trial, the Age-related Eye Disease Study 1 (AREDS 1), showed that patients with intermediate AMD treated with high dose antioxidant supplements (vitamins C and E, zinc, and β -carotene) had a 28 % reduction in the risk of progression to advanced AMD compared with placebo [31]. On the other hand, high dosages of vitamin E, zinc and β -carotene resulted in risk to diseases, like lung cancer or infection of genitourinary system [31–33], or increased mortality [34]. Thus, the dosage formulation of supplements should be adjusted to achieve a sufficient supply with antioxidants and minerals, but no unneeded risk for side effects.

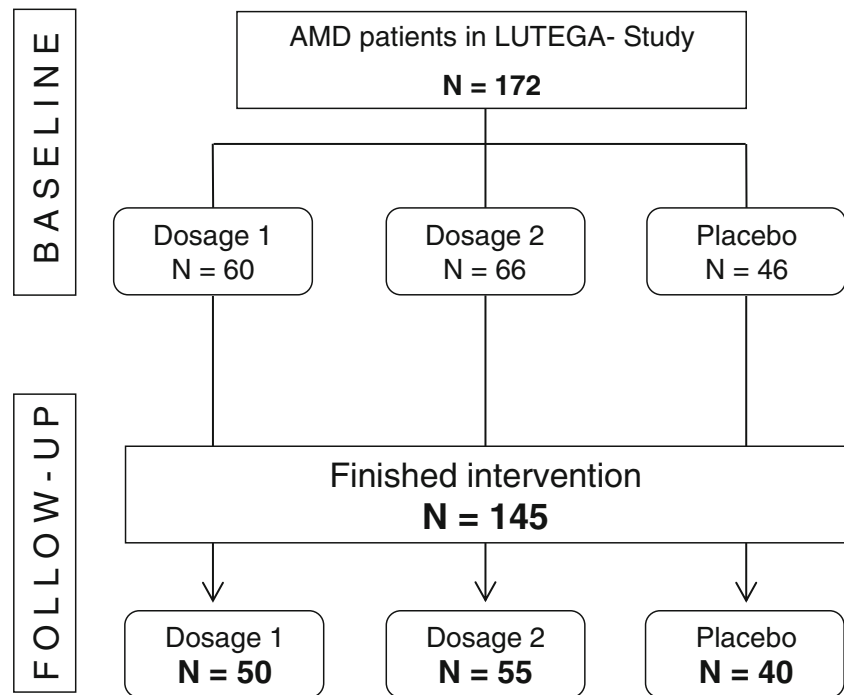
Overall, the LUTEGA study reported here is designed primarily to evaluate the effects of L, Z, O-3-LCPUFAs as well as Vitamin C, E and Zinc on macular pigment (MP) in patients with dry forms of AMD and secondarily, to study the possible functional influence on visual acuity.

Methods

The LUTEGA study is a prospective, randomized double-blind trial to determine the long-term effect of a supplementation of a fixed combination of L, Z and O-3-LCPUFA on MPOD in patients with non-exudative AMD. The study was approved by clinical ethics committee of University Hospital Jena (Germany) and conformed to the tenets of the Declaration of Helsinki. The LUTEGA study is registered at ClinicalTrials.gov (NCT00763659).

Study group

The study group was recruited at the University Hospital Jena, Department of Ophthalmology from June 2008 to August 2009. The subjects, suffering from non-exudative form of AMD at least in one eye, were classified according to the AREDS classification system [35]. They were graded using 30° digital colour fundus photographs. Only one eye of each subject was included in the trial. Subjects with central geographic atrophy, exudative forms of AMD or

Fig. 1 Flowchart showing number of subjects during the study

pronounced opacity in the intended study eye were excluded. Of 273 suitable subjects with age-related macular degeneration, 172 participated in the study. They were randomized to three treatment arms (Fig. 1).

At baseline, the mean age of the 172 subjects was 70 ± 10 years, with 94 women and 66 men. After the intervention of 1 year, 145 subjects finished the study. This study group had an mean age of 69 ± 10 years consisting of 79 women and 66 men. Detailed characteristics of body mass index, iris colour, ametropia or system diseases of the study group are shown in Table 1.

Study design

Before baseline examination was started, all patients underwent a full ophthalmologic examination, screening of medical history and blood pressure measurement. When patients fulfilled inclusion and exclusion criteria (Table 2) and had given informed consent to participate in this clinical trial, the baseline examination was performed.

After baseline examination, four more visits after 1, 3, 6 and 12 months of intervention had to be completed for the analysis. Baseline and follow-up visits included BCVA measured by Early Treatment Diabetic Retinopathy Study (ETDRS)-Charts (4 m), amsler grid, measurement of MPOD, fundus photography and autofluorescence with modified Visucam NM/FA (Carl Zeiss Meditec, Jena, Germany), slit lamp biomicroscopy, optical coherence tomography (Cirrus 4.0 OCT, Carl Zeiss Meditec, Jena, Germany), and collecting of blood samples. Colour fundus photographs of after 12 month of intervention were

classified again by AREDS classification system. All ophthalmic examinations were completed in mydriasis. The patients completed a simplified food questionnaire. Each subject was randomly assigned to one of the three treatment arms. Patients of dosage group 1 (D1) received 10 mg L (FloraGLO® Lutein, Kemin Food L.C, Des Moines, IA), 1 mg Z, 255 mg concentrated fish oil (thereof 100 mg DHA,

Table 1 Characteristics of study group at baseline and after 1 year of intervention

Characteristics	Baseline	After intervention (1 year)
Number N	172	145
Sex (m/f)	78/94	66/79
Age (years)	70 ± 10	69 ± 10
Body mass index (kg/m ²)	27.5 ± 4.3	27.8 ± 4.4
Smoking in % (N/total number)	31.4 % (54/172)	27.6 % (40/145)
Iris color		
Blue/Gray	62.8 % (108/172)	64.1 % (93/145)
Green/Hazel	12.2 % (21/172)	13.1 % (19/145)
Brown (dark)	23.3 % (40/172)	22.8 % (33/145)
Ametropia (best spherical equivalent in dpt)	0.33 ± 2.17	
Systemic diseases in % (N/total number)		
Essential hypertension	74.4 % (128/172)	71.7 % (104/145)
Diabetes mellitus, type II	12.8 % (22/172)	12.4 % (18/145)
Hypercholesterolemia	51.2 % (88/172)	46.2 % (67/145)
Treatment with lipid reducer	43.2 % (38/172)	43.2 % (33/172)

Table 2 Inclusion and exclusion criteria

Inclusion criteria

- Ages between 50 and 95 years
- Patients with all dry forms of AMD (nonexudative form) in at least one eye
- No lutein, zeaxanthin or omega-3 fatty acid supplementation within the last 6 months

Exclusion criteria

- Marked RPE proliferations or neovascularisation in study eye, (i.s.e.)
- Subretinal hemorrhages, (i.s.e.)
- Central geographic atrophy, (i.s.e.)
- Missing fixation, (i.s.e.)
- Subjects with optic nerve disease (neuropathy, atrophy, papilledema); (i.s.e.)
- Unstable glaucoma as defined by intraocular pressures greater than 25 mmHg, (i.s.e.)
- History of retina-vitreous surgery, (i.s.e.)
- Advanced cataract, (i.s.e.)

i.s.e: in the study eye

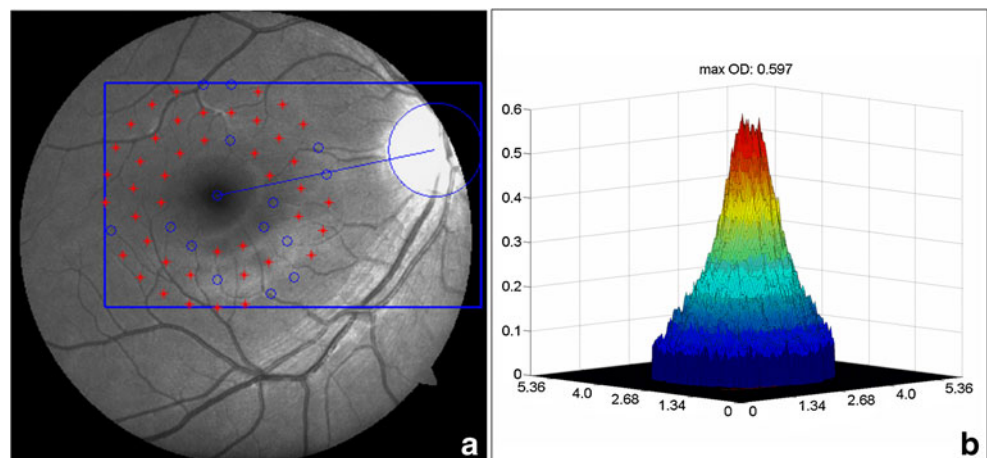
30 mg EPA) and antioxidants (60 mg vitamin C, 20 mg vitamin E, 10 mg zinc, 0,25 mg copper); and dosage group 2 (D2) received 20 mg L, 2 mg Zeaxanthin Z, 500 mg concentrated fish oil (thereof 200 mg DHA, 60 mg EPA) and antioxidants (120 mg vitamin C, 40 mg vitamin E, 20 mg zinc, 0,5 mg copper). The third group received placebo capsule (P).

Measurement of optical density of macular pigment (MPOD)

MPOD was measured using the 1-wavelength-reflection method recording reflection images at 480 nm by a modified fundus camera (VISUCAM^{NM/FA}, Zeiss Meditec, Jena, Germany). The method was described previously by Schweitzer et al. [36, 37]. The 1-wavelength reflection

method uses the local and spectral selectivity of macular pigment (MP). Local selectivity means that MP is detectable only in a certain foveal region. Spectral selectivity means that MP absorbs blue light at wavelengths of < 530 nm, maximally at 460 nm. In this method, the fundus is illuminated by blue light of one wavelength at 480 nm near the absorption maximum of MP. In this way, the macula appears dark with increasing absorption of MP in the recording reflection image. Furthermore, retinal vessels also appear dark and exudates exhibit an increased reflection. The optical density of MP results from the logarithmic ratio of virtual fundus reflection below the macular pigment (reference area) to the very low macula reflection. Individual vignetting in the reflection images is corrected by a shading function. In this way, the approximation of a paraboloid was used to simulate the reflective underground below the macular pigment. For this, an algorithm finds nodes in the structure less image regions in the reference area around the macula (Fig. 2a). The logarithmic ratio of every pixel results in the distribution of MPOD (Fig. 2b). In this distribution, four parameters were separated: maximal optical density (max OD); mean optical density over all pixels (mean OD); the sum of density at all pixels, referred to as “volume”; and the area in which the OD exceeded a threshold. Optical density is measured in optical density units (ODU), the volume in ODU·square degrees (deg²), and the area in deg². The influence of stray light of the examined subject’s crystalline lens is compensated from the age of 45 years for mean OD, max OD and volume of MP, and was also previously described, as was the reproducibility and comparing to two-wavelength-autofluorescence method [36]. The reproducibility was determined in ten examinations of macular pigment in the same eye of three subjects at the age of 47, 61, and 78 years, and was lower than 6 % for all MPOD parameters. The comparison of two-wavelength-autofluorescence method (HRA I) and one-wavelength-reflection method revealed a strong correlation ($R=0.924$)

Fig. 2 Principle for determination of nodes for shading function (*stars*—used, *rings*—excluded) in reflection image at 480 nm (**a**) and distribution of MPOD (**b**): volume 1.671 ODU·deg², area 6.725 deg², max OD 0.597 ODU, mean OD 0.248 ODU



by 19 AMD patients (mean age 74.9 ± 8.8 years). They were suffering from cataract or had intraocular lens (IOL) implants. No correction for stray light was applied in pseudophakic eyes [36].

This report presents the four parameters: maximum OD in ODU, mean OD in ODU, volume in ODU·deg² and area in deg². MPOD examination was repeated three times and averaged, in all parameters at baseline and follow-up visits. At first, a dependence of AREDS classification on MPOD at baseline is represented. Furthermore, the change of MPOD over 1 year of intervention is described.

Measurement of visual acuity

The monocular best-corrected visual acuity (BCVA) was measured by ETDRS-charts at a distance of 4 m. The visual acuity was converted into log minimal angle of resolution (log MAR). The visual acuity of 1.0 is related to log Mar 0.0 and a number of 55 reading letters.

In addition to the mean BCVA at baseline, a dependence of AREDS classification on absolute reading letters is represented. Furthermore, the change in absolute reading letters over 1 year of intervention is described.

Statistical analysis

Statistical analysis was performed with IBM® SPSS® 20.0 software. Values are reported as means and standard deviation. Gaussian distribution was evaluated by Kolmogorov–Smirnov test and homogeneity of variances was evaluated by Levene’s test. Unpaired Student’s t-test or non-parametric Mann–Whitney-U test were used to determine the differences between the groups. The influence of intervention in the three treatment arms was tested with the general linear model (GLM) procedure for repeated measurements (one-way variance analysis). For detailed analysis between the five single time points of the study, paired Students t-test or non-parametric Wilcoxon-test was used. Furthermore, the mixed GLM procedure for repeated

measurements was used to determine influence of age (covariate) or AREDS stages from stage I to stage IV (between-subjects factor) on measured parameters of MPOD and BCVA over intervention time points. A two-tailed $p < 0.05$ was considered the level of significance.

Results

Baseline

Most of the subjects were assigned to AREDS stage III (40.1 %, 69 of 172 subjects) at baseline. Subjects of AREDS stage IV (26 of 172 subjects) suffered from exudative AMD or central atrophy of the fellow eye. Mean values of volume and area decreased between subgroups of AREDS I to AREDS III at baseline (Fig. 3a). Max and mean MPOD of AREDS subgroups ranged around total mean values. The area of MPOD significantly decreased in AREDS III with an area of 5.790 deg², compared with an area of 6.984 deg² in AREDS I ($p = 0.009$, Table 3). The variation of measured values of all cases of the four MPOD parameters was very strong (Table 3).

The mean visual acuity at baseline was 0.13 ± 0.16 log Mar or 48.7 ± 8.7 absolute reading letters. The BCVA declined with increasing AREDS classification (Fig. 3b). There was a statistically significant difference in absolute reading letters between AREDS I (54.4 ± 5.0) and AREDS III (45.83 ± 9.1) as well as AREDS IV (44.2 ± 10.6) ($p < 0.001$).

Results after 1 year of intervention

The data sets of 145 AMD patients, which finished the intervention, were used for results and statistical analysis. MPOD values were measured at baseline ($t = 0$) and after 1, 3, 6 and 12 months of intervention. All MPOD parameters (absolute max OD, mean OD, volume, area and absolute change from baseline of max OD, mean OD, volume, area) already increased significantly in both treatment arms (D1

Fig. 3 Baseline characteristics of AMD patients according to AREDS stages. Area of MPOD (in deg²), unpaired Student’s t-test, * $p < 0.05$ (a); BCVA (in absolute reading letters), Mann–Whitney-U-test, $p < 0.05$ (b); white circle—outlier, white diamond—extreme value

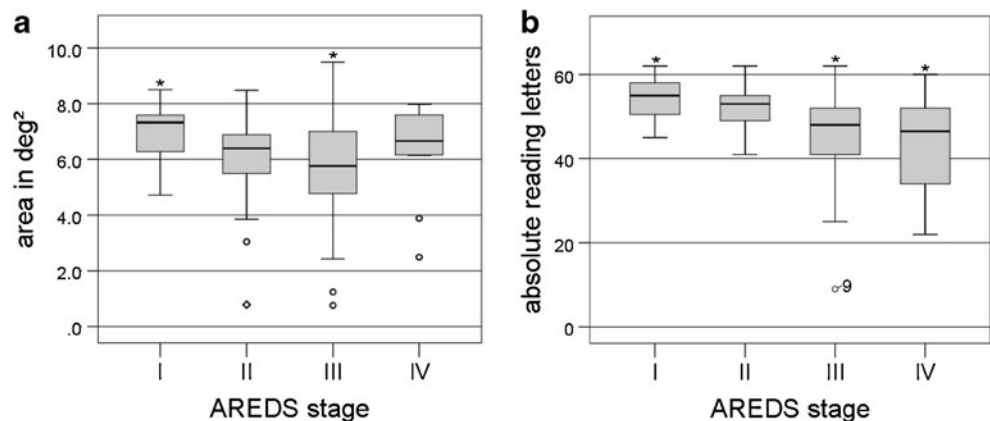


Table 3 Baseline characteristics in AMD patients for mean values of MPOD parameters in dependence of AREDS stages

	AREDS stages				All cases
	I	II	III	IV	
N	24	53	69	26	Total (N=172)
Mean OD	0.214±0.047	0.231±0.038	0.223±0.041	0.226±0.058	0.225±0.044
Max OD	0.556±0.101	0.581±0.085	0.546±0.097	0.569±0.143	0.562±0.103
Volume	1.501±0.457	1.463±0.457	1.306±0.513	1.458±0.619	1.404±0.509
Area	6.984±1.156 *	6.271±1.419	5.790±1.875 *	6.333±1.720	6.187±1.720

Mean values of mean and max OD are given in ODU, of volume in ODU·deg², and of area in deg²

Values are mean ± SD; N: number of cases

Unpaired Student's t-test, **p*<0.05

and D2) after the first month of intervention and after all other time points (*t*=3, 6, 12 months; Fig. 5a–d). The highest within-group difference from baseline to end point (*t*=12) was determined for relative volume of MP in D2 with 28.4 % and D1 with 20.0 % (Fig. 4) as well as absolute volume of MP in treatment arm D1 (from 1.41±0.46 to 1.64±0.54 ODU·deg²; *p*<0.001) and in D2 (from 1.41±0.47 to 1.68±0.51 ODU·deg²; *p*<0.001). Volume of MP decreased significantly in P from 1.46±0.53 to 1.43±0.53 ODU·deg² with *p*=0.022 (*t*=12, Table 4). While the volume is a suitable parameter to evaluate the spatial distribution, the maximum OD is characteristic of the central MPOD. Maximum OD raised significantly in D1 (from 0.581±0.096 to 0.625±0.096 ODU) and in D2 (from 0.555±0.088 to 0.606±0.089 ODU), with *p*<0.001.

The MPOD values at baseline showed a high inter-individual variability in the study population. To compare between-group differences the absolute change of MPOD parameters (Δ mean OD, Δ max OD, Δ volume, Δ area) was determined at time points of intervention *t*=1, 3, 6 and 12. The highest between-group difference was determined in volume of MP between D2 (Δ volume: 0.265±0.151

ODU·deg²) and P (Δ volume: -0.031±0.082 ODU·deg²) after 12 months of intervention (*t*=12; *p*<0.001; Table 5, Fig. 5b). Only Δ mean OD tended to result in a between-group difference of the active treatment arms D1 (Δ mean OD: 0.016±0.015 ODU) and D2 (Δ mean OD: 0.025±0.022 ODU, *p*=0.049; Fig. 5c). In this, the changes in the area of macular pigment were nearly the same in both treatment arms, with 0.662 deg² in D1 and 0.700 deg² in D2 (Table 5, Fig. 5d).

Best-corrected visual acuity (BCVA) measured in log MAR with ETDRS charts improved significantly in both treatment arms D1 (from 0.134±0.17 to 0.104±0.18 log MAR, *p*=0.001) and D2 (from 0.104±0.14 to 0.064±0.16 log MAR, *p*<0.001). There was no significant within-group difference detected in the P arm (from 0.129±0.16 to 0.127±0.16 log MAR, *p*=0.681; Fig. 6, Table 6). Between-group comparison at the end point of intervention (*t*=12) indicated a trend to improvement of BCVA in log MAR between D2 and P (*p*=0.063, Table 6). An improvement of BCVA measured in reading letters was significant in treatment arm D2 (2.02±3.1 letters, *p*=0.006) and D1 (1.46±2.8 letters, *p*=0.038) compared to P (0.08±2.8 letters). There was no significant difference in BCVA between D1 and D2 (Table 6).

Using the general linear model (GLM) for repeated measurements, there was no significant effect of covariate of age on change in MPOD parameters in one of the treatment arms (lowest *p* value found for max OD in D2 with *F* [2; 212]=1.940, *p*≥0.190; full data not shown). Furthermore, there was no progression in AMD stages for the intended study eye in any treatment arm during the intervention time period of 1 year. All colour fundus photographs of study eyes were classified again after 12 months of intervention by AREDS classification system, and resulted in same stages as at baseline examination.

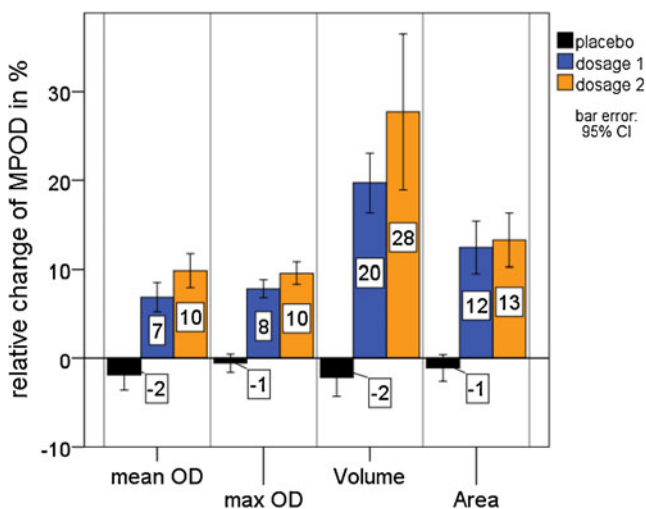


Fig. 4 Relative change of MPOD parameters at end point (*t*=12)

Discussion

The LUTEGA study demonstrated that a dietary supplementation among AMD patients with L, Z, O-3-LCPUFAs and

Table 4 Within-group differences between start and end point of intervention for MPOD

MPOD parameter		Placebo		D1		D2	
		Mean	p value	Mean	p value	Mean	p value
Mean OD	<i>t</i> =0	0.227	0.019	0.236	< 0.001	0.227	< 0.001
	<i>t</i> =12	0.223		0.252		0.252	
Max OD	<i>t</i> =0	0.577	0.325	0.581	< 0.001	0.555	< 0.001
	<i>t</i> =12	0.574		0.625		0.606	
Volume	<i>t</i> =0	1.456	0.022	1.412	< 0.001	1.41	< 0.001
	<i>t</i> =12	1.425		1.677		1.725	
Area	<i>t</i> =0	6.322	0.267	6.027	< 0.001	6.12	< 0.001
	<i>t</i> =12	6.272		6.689		6.82	

Mean values of mean and max OD are given in ODU, of volume in ODU-deg², and of area in deg²

Paired student's *t*-test, *p*<0.05

antioxidants over 1 year resulted in a considerable increase in MPOD compared to placebo. Moreover, both study groups D1 and D2 revealed stabilization as well as an improvement in visual acuity during the one-year supplementation.

The composition of dietary supplement in the LUTEGA study was initially based on the Age-related Eye Disease Study (AREDS) formulation. Main AREDS components are β -carotene, Vitamin C and E, as well as Zinc and Copper [31]. In the current formulation used in the LUTEGA study, β -carotene was replaced by L and Z, since these two types of carotenoids are present in the human macula. This approach was also described by the AREDS2 research group in the development of the AREDS2 formulation [38]. Moreover, β -Carotene has been reported to increase the risk of lung cancer in smokers and ex-smokers [32]. In addition, O-3-LCPUFAs were added to the LUTEGA formulation. Dietary intake of Omega-3-LCPUFAs indicated a positive effect on early and late AMD [27, 30, 39–41]. Concentration of Vitamin C and E as well as zinc were decreased in the LUTEGA formulation compared to AREDS, to benefit from their positive antioxidant qualities, but to avoid possible side effects when exceeding a certain dosage level. High dosages of 400 I.U. Vitamin E have been associated with an increased mortality rate [34] and an elevated risk of lung carcinomas [33]. High dosages of zinc have been linked to deposits in drusen, an increased infection rate within the genitourinary system, decreased high-density lipoprotein cholesterol and an upset stomach [31, 42]. Overall,

supplementation within the LUTEGA study focused on AREDS elements updated by L and Z and O-3-LCPUFAs, as well as a balanced dosage. Comparing the study design the formulation of the AREDS2 and the LUTEGA are nearly the same; only the individual amounts of the nutrients were varied. Moreover, LUTEGA investigated two dosage groups with same formulation against placebo, whereas AREDS2 investigated three different compositions of supplements (L, Z/ O-3-LCPUFAs/ L, Z, O-3-LCPUFAs) against placebo [38].

Macular pigment optical density

The macular pigments L and Z are postulated to improve the effects of chromatic aberration and to protect the central retina by absorbing blue and ultraviolet light supporting a neutralization of reactive oxygen species (ROS) [6, 43, 44]. The neutralization of ROS is concluded from the presence of L and Z metabolites found in the blood and in the retina [45–47]. High levels of ROS are considered as biomarkers for oxidative stress and of age-related changes. Within the macula Z is the highest concentration within the foveal part, whereas L dominates within the parafoveal periphery [48, 49]. Therefore, L may protect the more peripheral located rods from high development of ROS, and Z may protect the more central cones within the macula [50].

AMD is a multi-factor disease. Among other factors, inflammation as well as oxidative stress are discussed as

Table 5 The absolute change of MPOD parameters at end point (*t*=12) in AMD patients and between-group comparison

	Δ mean OD	Δ max OD	Δ volume	Δ area
Absolute change at end point (<i>t</i> =12)				
P (<i>N</i> =40)	-0.004 \pm 0.011	-0.003 \pm 0.175	-0.031 \pm 0.082	-0.050 \pm 0.278
D1 (<i>N</i> =50)	0.016 \pm 0.015	0.044 \pm 0.018	0.265 \pm 0.151	0.662 \pm 0.395
D2 (<i>N</i> =55)	0.025 \pm 0.022	0.050 \pm 0.025	0.315 \pm 0.130	0.700 \pm 0.408
Between-group comparison (<i>t</i> =12)				
P versus D1	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001
P versus D2	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> <0.001
D1 versus D2	<i>p</i> =0.049	<i>p</i> =0.534	<i>p</i> =0.213	<i>p</i> =0.949

Values are mean \pm SD; *N*: number of cases

Non-parametric Mann–Whitney-U-test, *p*<0.05

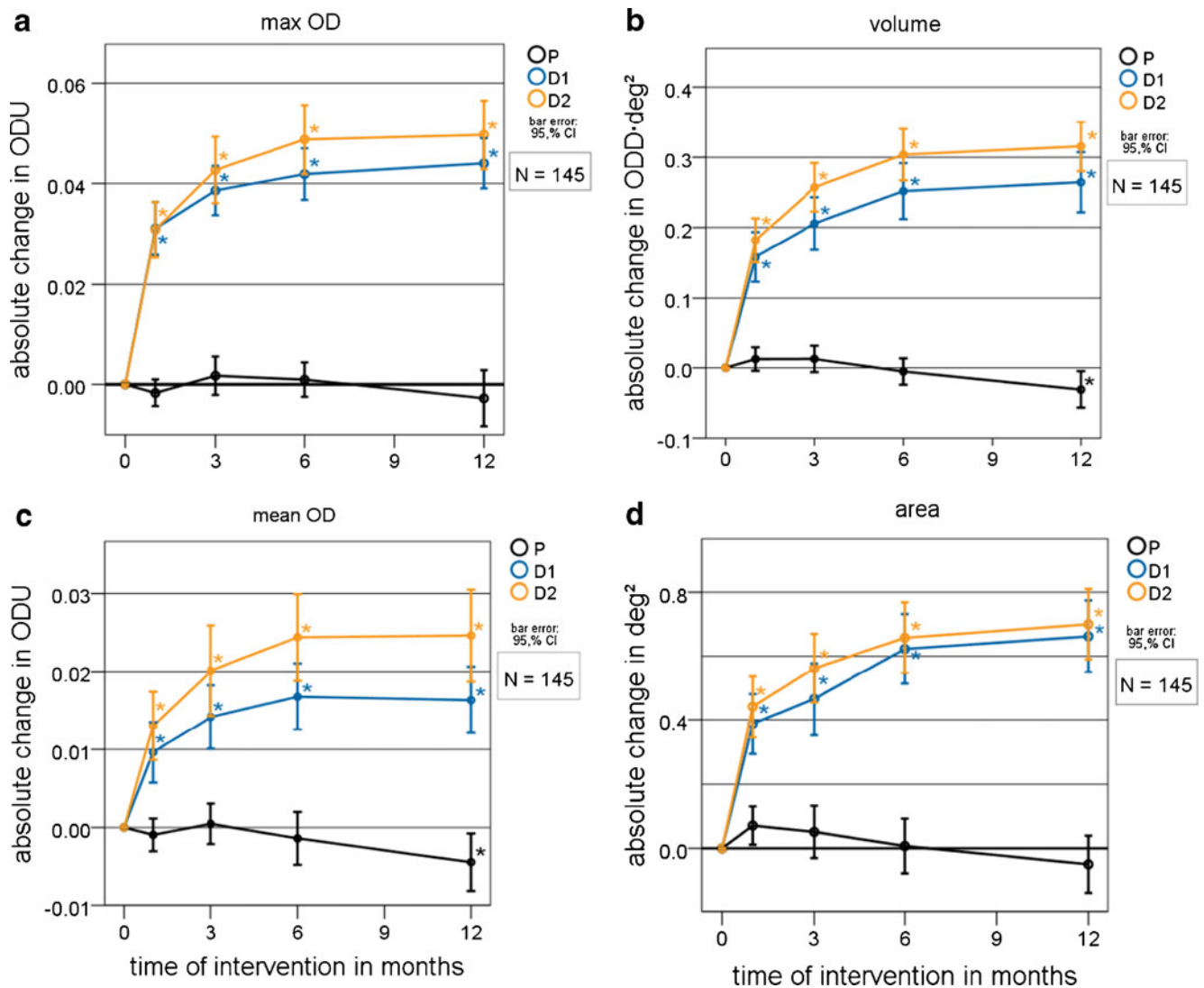


Fig. 5 Absolute change of MPOD parameters max OD (a), volume (b), mean OD (c), area (d). * significant differences within the groups in relation to $t=0$ ($p < 0.05$, General linear model procedure (repeated measurements))

two important key factors affecting the development and progression of AMD. There is growing evidence that L and Z may provide a benefit in the diabetic treatment of AMD, influencing either its progression [14, 51–55] or even minimize the potential risk of an AMD development [24, 56, 57]. The age dependency of MPOD in healthy subjects is inconsistent. A study comparing five MPOD measurement techniques could not find any age dependence on MPOD in healthy subjects [58]. Nolan et al. reported on age dependence on MPOD in healthy subjects, and in a subgroup of this cohort with confirmed family history of ARM, MPOD values were significant lower [59]. Studies comparing MPOD in AMD patients with age-matched controls report reduced MPOD values in AMD patients [36, 60, 61]. Consistently, low levels of L and Z in the macula are hypothesized as a pathogenic risk factor for AMD [62]. Furthermore, AMD patients regularly consuming nutritional

supplements containing L had an average higher macular pigment level compared to AMD patients not consuming these supplements [25]. Baseline characteristics of the LUTEGA study show reduced MPOD values with progression in AMD (AREDS stage I to III), especially in volume and area of macular pigment. There seems to be a lack of the protective substances in advanced AMD. Furthermore, a study of 1,585 women of the CAREDS study suggested that variation in genes related to the metabolism of carotenoids, O-3-LCPUFAs and a maculopathy related to the absence of macular pigment is related to variation in the measured MPOD [63]. Thus, the strong variations of measured MPOD values at baseline in the LUTEGA study population could be justified.

We assume that AMD patients benefit from the uptake of L and Z, since both macula pigments have the potential to downregulate ROS by filtering blue-light and UV-light, thereby minimizing oxidative stress in the retinal cells.

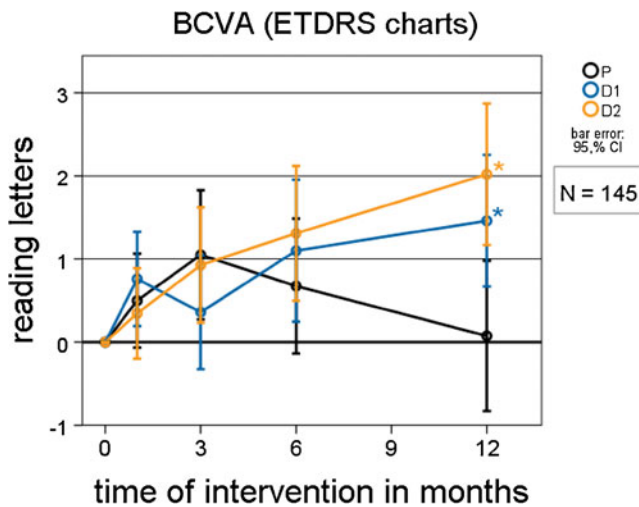


Fig. 6 Change of BCVA in reading letters (measured with ETDRS-chart). * significant differences within the groups in relation to $t=0$ ($p < 0.05$, General linear model procedure [repeated measurements])

Intervention by the supplementation of L, Z, O-3-PUFAs and other antioxidants in this study revealed a highly significant enrichment in all MPOD parameters for both treatment arms, and a significant decrease in MPOD volume and mean MPOD for the placebo arm. The highest change in number resulted from analyzing the MPOD volume, indicating the impact of its measurement. Furthermore, measurement of the MPOD volume provides a detailed three-dimensional distribution, which supplies best possible information on MPOD changes over time. Sharing the easily interpreted graphic image of how their MPOD is re-established may support patient’s compliance. In addition, it may also provide more insight and a deeper understanding on how the human MPOD is rebuilt under conditions of supplementation.

The carotenoids L, Z and meso-zeaxanthin (MZ) are found in the retina as macular pigments.

Because MZ is not derived from the diet and is not detected in the plasma, it has been proposed that L is most likely isomerized to MZ in the retina via the migration of a double bond [64]. The significant enrichment of MPOD in the treatment arms of the presented study is considered to result from the supplementation elements L and Z. Currently published studies also reported that MPOD significantly increased with supplementation for over 1 year with L [65], L and DHA [66] as well as different groups of L and Z composition [23] in subgroups with less than 36 AMD patients. Distinct from these studies, Z, EPA and antioxidants were additionally supplied in the LUTEGA study in every active treatment arm. Furthermore, LUTEGA revealed a very fast significant increase of MPOD after the first month of intervention and a saturation level after 6 months in both treatment arms, as well as a significant decrease of volume and mean OD of macular pigment in the placebo group.

The analyses of L, Z, LCPUFAs, blood lipid status and antioxidant capacities of blood samples of LUTEGA participants were reported by Arnold et al. [67]. In contrast to the plasma concentration of L, there was no significant difference in the accumulation of MPOD between both dosages D1 and D2 applied. Supplementation of L and Z seems to reach a saturation level in retinal cells. The steady state of the spatial distribution after 6 months of MPOD may indicate that L and Z uptake is limited to a specific anatomical structure, which we assume to probably be the Henle fibers. Thus, supplementation with L and Z is suggested to be a matter of a constant long-term intake rather than dosage.

Omega-3-long-chain polyunsaturated fatty acids

The eye is highly enriched with O-3-LCPUFAs, and specifically, high levels of DHA are found within the retina. DHA and EPA influence gene expression, retinal cell differentiation

Table 6 Comparison between start and end point of intervention as well as between-group comparison for BCVA

Comparison start/ end*		log MAR		Change in reading letters
		Mean ± SD	p value	
Placebo (N=40)	t=0	0.129±0.16	0.681	0
	t=12	0.127±0.16		0.08±2.8
Dosage 1 (N=50)	t=0	0.134±0.17	0.001	0
	t=12	0.104±0.18		1.46±2.8
Dosage 2 (N=55)	t=0	0.104±0.14	< 0.001	0
	t=12	0.064±0.16		2.02±3.1
Between-group comparison**		t=0	t=12	t=12
Placebo vs. dosage 1		p=0.895	p=0.526	p=0.038
Placebo vs. dosage 2		p=0.43	p=0.063	p=0.006
Dosage 1 vs. dosage 2		p=0.338	p=0.232	p=0.354

*Non-parametric Wilcoxon-test, $p < 0.05$; **Non-parametric Mann-Whitney- U-test, $p < 0.05$

and survival [68]. They act as potent regulators of retinal vascular function, cell survival, inflammation and energy balance. DHA status has been discussed to affect retinal cell signalling mechanisms involved in phototransduction, to enhance activation of membrane-bound retinal proteins and to contribute to rhodopsin regeneration. O-3-LCPUFAs are a source of Resolvins and Neuroprotectins, which have been shown to resolve chronic inflammation and to protect against oxidative stress [27, 69–71]. Thus, besides the maintenance of photoreceptor cell membrane structure and function, O-3-LCPUFAs downregulate pathways involved in retinal neovascularization, inflammation and angiogenesis, further supporting the beneficial effect of O-3-LCPUFAs supplementation in AMD patients. Supplementation with high dosages of vitamin C and E as well as zinc has been reported to reduce the progression of AMD [31]. The main characteristic of vitamin C and E is the anti-oxidative potential assisting in cell protection against oxidative stress. Thus, we assume that these elements, also present within the LUTEGA formulation, may add a positive effect on the overall AMD development.

In summary, AMD patients may benefit from a constant supplementation of L and Z and O-3-FA as well as antioxidants, which may on the one hand result in an elevated MPOD, protecting the macula against the development of high level ROS. On the other hand, anti-inflammatory, neuro-protective and anti-apoptotic pathways may be supported by O-3-LCPUFAs and the anti-oxidative vitamins C and E, as well as zinc. Oxidative stress and inflammation may lead to apoptosis of the photoreceptors, and thus are discussed as a critical impact factor for the risk and development of AMD.

Visual acuity

In the LUTEGA study, patients with supplementation of L, Z, O-3-LCPUFAs and antioxidants showed a significant improvement in BCVA for both treatment arms D1 and D2 after 12 months of intervention. We conclude that the supplementation caused an improvement or stabilization in visual acuity as well as a significant increase of MPOD. Currently published studies only reported a trend toward improvement in BCVA [22, 23, 65].

In concordance, the Veterans LAST study (placebo-controlled Lutein and Antioxidant Supplementation Trial) revealed an improvement of visual function by supplementation of L alone or in combination with a broad spectrum of antioxidants in predominantly male AMD patients [25]. The Zeaxanthin and Visual Function Study (ZVF) investigate Z according to visual function, and demonstrated a visual benefit by improving of central vision parameters compared to treatment of L [50]. Depletion of O-3-LCPUFA, and particularly of DHA, has been associated with an impairment of the development of visual acuity, a decrease in the amplitude of the electroretinogram, and an impairment to

learn visual discrimination in animals. In humans, O-3-LCPUFA deficiency leads to blurred vision and peripheral neuropathy; these symptoms are reversed with adequate supplementation [70, 72]. Thus, a protective effect on the macula in AMD patients may be assumed.

Progression of AMD

The AREDS formulation of antioxidant vitamins C (500 mg), E (400 I.U.), and β -carotene (15 mg), and minerals, zinc (80 mg) with copper (2 mg), reduced the 5-year risk of developing advanced AMD in eyes with intermediate AMD by 25 % [31]. The Veterans LAST study did not find any remarkable progression in AMD stages during intervention of 1 year with 10 mg L or 10 mg L added with antioxidants, vitamins and mineral [25]. Similarly to the LAST study, there was no progression in AMD stages in the LUTEGA study population during the intervention time. For risk estimations of progression in AMD and intervention with L, Z, O-3-LCPUFAs, and antioxidants, there are high numbers of AMD patients and long intervention time periods necessary, like the study design of ARED study 2 [38].

Based on the first year LUTEGA study data, patients with a non-exudative AMD should consider a supplementation with L, Z, O-3-LCPUFAs and antioxidants such as those used in this study. Due to equal benefits between a once or twice daily supplementation with 10 mg Lutein (L), 1 mg Zeaxanthin (Z), 255 mg concentrated fish oil (thereof 100 mg DHA, 30 mg EPA) and antioxidants (60 mg vitamin C, 20 mg vitamin E, 10 mg zinc, 0.25 mg copper), the once daily dosage is recommended for a long term-basis dietary AMD supplementation to support improved patient compliance. The parameter volume of MPOD allows a detailed observation of macular pigment distribution in macular area and had the highest sensitivity to determine changes during this intervention period. Both dosage groups benefit in visual acuity under this supplementation. Further investigations are necessary to evaluate supplementation of L, Z and O-3-LCPUFAs on dependency of the different AREDS stages and their visual acuity.

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