Macular Xanthophylls and ω-3 Long-Chain Polyunsaturated Fatty Acids in Age-Related Macular Degeneration

A Randomized Trial

Christin Arnold, Dipl-Troph; Lisa Winter, Dipl-Troph; Kati Fröhlich, PhD; Susanne Jentsch, Dipl-Ing; Jens Dawczynski, MD; Gerhard Jahreis, PhD; Volker Böhm, PhD

Importance: It has been shown that the functionality of the macula lutea depends on the nutritional uptake of lutein and zeaxanthin and that it is inversely associated with the risk of age-related macular degeneration (AMD). Additionally, ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs) may also be protective.

Objective: To investigate the effect of a 12-month intervention with macular xanthophylls and ω-3 LC-PUFAs on xanthophylls and fatty acids in plasma, antioxidant capacity, and optical density of the macular pigment of patients with nonexudative AMD.

Design: The LUTEGA study was a randomized, double-blind, placebo-controlled, parallel clinical trial that was conducted for 12 months.

Setting: University Eye Hospital and Institute of Nutrition, Friedrich Schiller University Jena, Germany.

Participants: A total of 172 individuals with nonexudative AMD.

Intervention: Individuals were enrolled and randomly divided as follows: placebo group, group 1 (a capsule containing 10 mg of lutein, 1 mg of zeaxanthin, 100 mg of docosahexaenoic acid, and 30 mg of eicosapentaenoic acid administered each day), and group 2 (same substances but twice the dose used in group 1). One hundred forty-five participants completed the study successfully.

Main Outcome Measures: Plasma xanthophyll concentrations and fatty acid profiles, optical density of the macular pigment, and antioxidant capacity in plasma (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox] equivalent antioxidant capacity and photochemiluminescence).

Results: The concentrations of the administered carotenoids in plasma as well as the optical density of the macular pigment increased significantly in the groups randomized to receive supplementary macular xanthophylls and ω-3 LC-PUFAs after 1 month of intervention and remained at this level through the end of the study. Use of the double dose resulted in a beneficial alteration of the fatty acid profile in the plasma of patients with AMD in comparison with the dose in group 1. The lipophilic antioxidant capacity in plasma was significantly elevated with the intervention.

Conclusions and Relevance: A supplement containing a fixed combination of lutein, zeaxanthin, and ω-3 LC-PUFAs during 12 months significantly improved plasma antioxidant capacity, circulating macular xanthophyll levels, and the optical density of the macular pigment.

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Author Affiliations: Bioactive Plant Products Research Group (Mss Arnold and Winter and Drs Fröhlich and Böhm) and Department of Nutritional Physiology (Dr Jahreis), Institute of Nutrition, Friedrich Schiller University Jena, and University Eye Hospital (Ms Jentsch and Dr Dawczynski), Jena, Germany. Dr Dawczynski is now with the University Eye Hospital, Leipzig, Germany.

THE ECONOMIC, POLITICAL, and technological changes of the past decades have led to a "graying" of the population in the Western world. The prevalence of cardiovascular diseases, diabetes mellitus, osteoporosis, cancer, and dementia increases. This also applies to age-related macular degeneration (AMD).[1] This malady most often affects people older than 50 years and impairs the visual performance of the central field of vision. It has been shown that the optical density of the macular pigment depends on the nutritional uptake of lutein and zeaxanthin and that it is inversely associated with the risk of AMD.[2-5] In addition, ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs) may protect against AMD.[6-8] Hence, the primary purpose of the LUTEGA study was to investigate the dose-dependent effects of a 12-month supplementation with a fixed-dose combination of lutein, zeaxanthin, docosahexaenoic acid

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Table 1. Grouping of the Patients According to the AREDS Classification System

	AREDS Classification			
Intervention	**I**	**II**	**III**	**IV**
Placebo (n = 40)	6	14	10	10
Group 1 (n = 50)	10	11	25	4
Group 2 (n = 55)	5	22	21	7
Total	**21**	**47**	**56**	**21**

Abbreviation: AREDS, Age-Related Eye Disease Study.

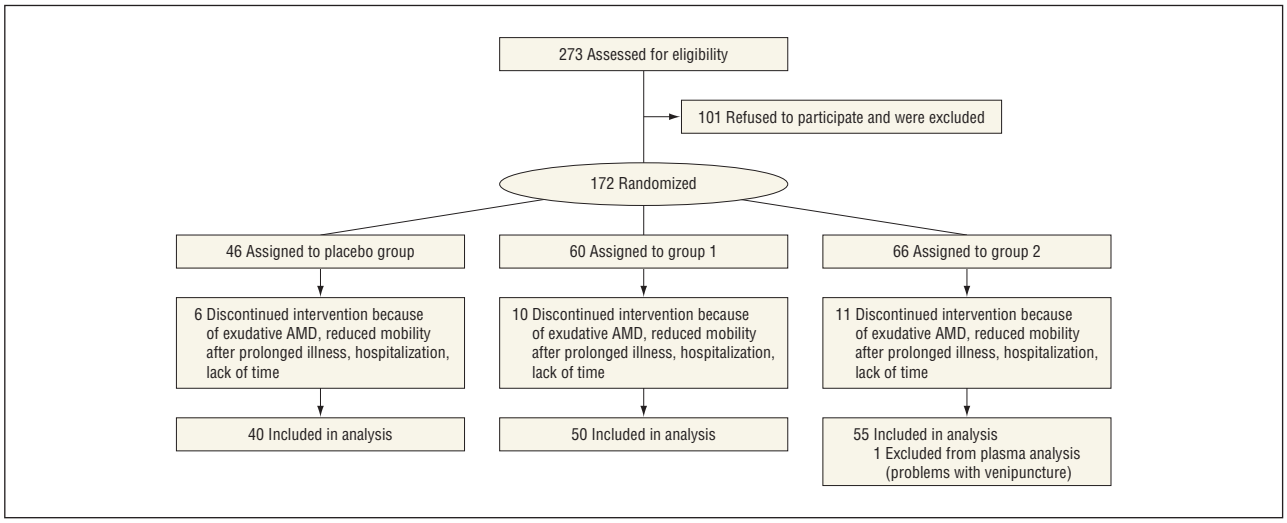

Figure 1. Number of participants per group and reasons for withdrawal. AMD indicates age-related macular degeneration.

(DHA), and eicosapentaenoic acid (EPA) on the optical density of the macular pigment in patients with nonexudative AMD (dry form of the disease). The present analysis reports on the alteration of the concentrations of lutein and zeaxanthin and of blood lipids, as well as the antioxidant capacity in plasma and the fatty acid profile in blood samples. Furthermore, the effect of the intervention on the optical density of the macular pigment is described.

METHODS

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving humans were approved by the ethics committee of the Friedrich Schiller University Jena, Jena, Germany. Written informed consent was obtained from all patients.

PARTICIPANTS

Patients from the local population were classified according to the Age-Related Eye Disease Study (AREDS) classification system (**Table 1**).[9] Their eyes were graded using 30° digital color fundus photographs. Only 1 eye of each patient was included in the trial for macular pigment measurements. Patients with central geographic atrophy, exudative forms of AMD, or pronounced opacity in the intended study eye were excluded. Two hundred seventy-three patients with nonexudative AMD were asked to participate in the study; 172 agreed and 145 completed the study successfully (**Figure 1**). One patient originally randomized to group 2 was excluded from plasma analysis because of problems with venipuncture. Participants (79 women, 66 men) (**Table 2**) aged 50 to 93 years were instructed to abstain from dietary supplements containing carotenoids and fish oil during the study period.

Sample size was determined using the Lehr formula, and the random allocation sequence was generated with a random number generator (SPSS; SPSS Inc). Masking was done by an independent scientist who did not have contact with the study participants. Participants, care providers, and those assessing outcomes were masked using sequential numbering. The care provider (S.J.) enrolled the participants and assigned them to an intervention. Allocation was concealed using numbered blisters containing the capsules. All participants were assigned to ingest 2 capsules each day: the placebo group ingested 2 placebo capsules; group 1, 1 placebo and 1 supplement capsule; and group 2, 2 supplement capsules according to the corresponding number on the blister.

STUDY DESIGN

The study was randomized, double-blind, placebo-controlled, and parallel and was carried out at the study center in Jena between May 27, 2008, and October 12, 2010. The administered supplement was provided as capsules by Novartis Pharma GmbH (**Table 3**).

Each participant was randomly assigned to one of the 3 study groups: group 1 (n = 50) received a capsule containing 10 mg of lutein, 1 mg of zeaxanthin, 100 mg of DHA, and 30 mg of EPA each day; group 2 (n = 55) received twice the dose of group 1 each day; and the placebo group (n = 40) received capsules with an equal composition of ingredients but without any of the substances being investigated. Placebo and active treat-

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Table 2. Characterization of the Participants in the 3 Study Groups

Characteristic	Mean (SD)		
	Placebo (n = 40)	Group 1 (n = 50)	Group 2 (n = 54)[a]
Total (N = 144)			
Age, y	68 (9)	69 (11)	70 (9)
Weight, kg	77 (13)	79 (15)	80 (16)
Height, m	1.68 (0.08)	1.68 (0.09)	1.68 (0.09)
BMI	27.1 (3.9)	27.9 (4.9)	28.2 (4.1)
TC level, mg/dL	222.6 (41.0)	219.2 (36.2)	217.6 (56.8)
Triglycerides level, mg/dL	122.9 (60.9)	150.9 (109.1)	159.1 (83.9)
LDL-C level, mg/dL	137.2 (37.2)	130.3 (30.5)	132.8 (44.6)
HDL-C level, mg/dL	56.9 (12.0)	57.4 (15.4)	53.1 (14.3)
LDL-C:HDL-C ratio	2.54 (0.91)	2.43 (0.86)	2.66 (1.18)
Women (n = 78)	(n = 21)	(n = 29)	(n = 28)
Age, y	71 (9)	71 (11)	71 (9)
Weight, kg	71 (12)	77 (16)	74 (13)
Height, m	1.62 (0.06)	1.63 (0.06)	1.62 (0.06)
BMI	26.9 (4.2)	28.7 (5.7)	28.1 (4.8)
TC level, mg/dL	228.2 (34.9)	211.5 (28.6)	229.2 (35.3)[b]
Triglycerides level, mg/dL	123.4 (68.2)	127.4 (55.4)	173.6 (90.8)
LDL-C level, mg/dL	142.0 (29.3)	127.2 (27.3)	139.6 (33.3)
HDL-C level, mg/dL	58.3 (11.0)	57.2 (13.0)	56.7 (14.9)
LDL-C:HDL-C ratio	2.54 (0.76)	2.35 (0.78)	2.70 (1.39)
Men (n = 66)	(n = 19)	(n = 21)	(n = 26)
Age, y	66 (9)	66 (12)	69 (9)
Weight, kg	84 (12)	82 (12)	86 (17)
Height, m	1.75 (0.04)	1.75 (0.09)	1.74 (0.08)
BMI	27.2 (3.7)	26.8 (3.2)	28.2 (3.2)
TC level, mg/dL	216.4 (47.0)	229.8 (43.1)	205.1 (71.9)
Triglycerides level, mg/dL	122.4 (53.6)	183.4 (151.5)	143.5 (74.4)
LDL-C level, mg/dL	131.9 (44.8)	134.7 (34.6)	125.7 (53.9)
HDL-C level, mg/dL	55.4 (13.2)	57.6 (18.6)	49.3 (12.7)
LDL-C:HDL-C ratio	2.54 (1.08)	2.53 (0.97)	2.61 (0.92)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

SI conversion factors: to convert HDL-C, LDL-C, and TC to millimoles per liter, multiply by 0.0259; to convert triglycerides to millimoles per liter, multiply by 0.0113.

[a] One patient originally randomized to group 2 was excluded from analysis.

[b] Significant difference in relation to group 1, unpaired *t* test, $P < .05$.

ment capsules were not outwardly distinguishable from each other. At study entry (baseline) and after 1, 3, 6, and 12 months, ophthalmologic examinations were carried out, including measurement of the optical density of the macular pigment, a standardized visual acuity test (Early Treatment Diabetic Retinopathy Study), Amsler grid, slitlamp biomicroscopy, and fundus photography. Fasting blood samples were obtained at baseline and 1, 6, and 12 months, and plasma samples were stored at –80°C.

ANALYSIS OF CAROTENOIDS

Carotenoids were extracted under subdued light with n-hexane containing 0.1% butylhydroxytoluene as described previously[10] and were measured via normal-phase high-performance liquid chromatography with a UV/visual spectroscopy detector (445 nm; Merck-Hitachi) using an amino phase column (250 mm × 4 mm, 5 μm; Separation Service Berlin). The mobile phase consists of n-hexane/propan-2-ol (98:2, volume/mass), the flow was set at 1.5 mL/min, and the column temperature was set at 40°C. A sample volume of 100 μL was injected. The concentrations of lutein and zeaxanthin were quantified by 5-point calibration curves of standards. Astaxanthin served as the internal standard. The concentrations of the stock solutions were checked periodically and were calculated from the specific extinction coefficients (2550 for lutein in ethanol at 445 nm and 2480 for zeaxanthin in ethanol at 450 nm).[11]

Table 3. Composition of the Supplement

Ingredient	Per Capsule, mg
Carotenoids	
Lutein, free	10
Zeaxanthin	1
ω-3 Fatty acids	
Concentrated fish oil	255
DHA	100
EPA	30
Vitamins	
C	60
E	20
Trace elements	
Zinc	10
Copper	0.25
Energy value	
Protein	120
Carbohydrates	70
Fat	320
kcal/kJ	3.6/14.7

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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FATTY ACID ANALYSIS

Lipids from plasma were extracted by a mixture of methanol and chloroform as described previously.[12] Briefly, lipids were extracted and saponified with methanolic sodium hydroxide and methylated with methanolic boron trifluoride. The resulting fatty acid methyl esters (FAMEs) were isolated by thin-layer chromatography, dissolved in n-hexane, and stored at –20°C. Analysis was performed by a gas chromatography–flame ionization detector (GC-17A; Shimadzu) using a Durabond 225 mass spectrometry column (60 m × 0.25 mm × 0.25 μm). Hydrogen was used as the carrier gas. The gas chromatography method has been described.[13] Fatty acid profiles for 4- to 26-carbon moieties were measured and expressed as the percentage of the total peak area of all FAME (%FAME). Standard FAME was used for identification of fatty acids by means of their specific retention times.

ANTIOXIDANT CAPACITY

The plasma of 30 participants (10 per group) was randomly selected to determine the influence of the intervention on the hydrophilic and lipophilic antioxidant capacity.

TROLOX EQUIVALENT ANTIOXIDANT CAPACITY

For the determination of the antioxidant capacity, 2 versions of the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay were conducted. This assay is based on the absorbance of the radical cation 2,2'-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS) at approximately 730 nm.[14,15]

Samples for the hydrophilic version (H-TEAC) were prepared as follows. After a strong acidic hydrolysis with hydrochloric acid, a saponification with methanolic sodium hydroxide, and a precipitation of proteins with metaphosphoric acid, antioxidants were extracted by a mixture of methanol and water (1 + 1). At that time, 20 μL of the extract was transferred into a microplate. After addition of 200 μL of the test-specific ABTS solution, the absorbance was recorded after 1 minute.

Sample preparation for the analysis of the lipophilic antioxidant capacity (L-TEAC) initially includes the precipitation of proteins with ethanol. Antioxidants were extracted 3 times with n-hexane, and 100 μL of the extract and 600 μL of the aqueous test-specific ABTS solution were mixed in a tube. The solution was transferred into a cuvette and centrifuged for phase separation. Absorbance was measured 2 minutes after mixture[16]; α-tocopherol served as the calibration standard.

PHOTOCHEMILUMINESCENCE

Photochemiluminescence is based on the photochemical generation of free radicals as described previously.[17] This method was carried out using a new prototype of a system (Photochem; Analytik Jena AG). Reagent 1 and reagent 2 of the test kit were mixed with 25 μL of the photosensitizer and 25 μL of the sample (extraction identical to L-TEAC). α-Tocopherol was used as the calibration standard.

MACULAR PIGMENT

The optical density of the macular pigment was determined by a 1-wavelength reflection method that was developed by the Department of Ophthalmology, University Hospital Jena. For the examinations, a fundus camera (Visucam; Carl Zeiss Meditec) was used. The principle of the method is based on the assumption of local and spectral selectivity of the macular carotenoids lutein and zeaxanthin, as described previously.[18,19]

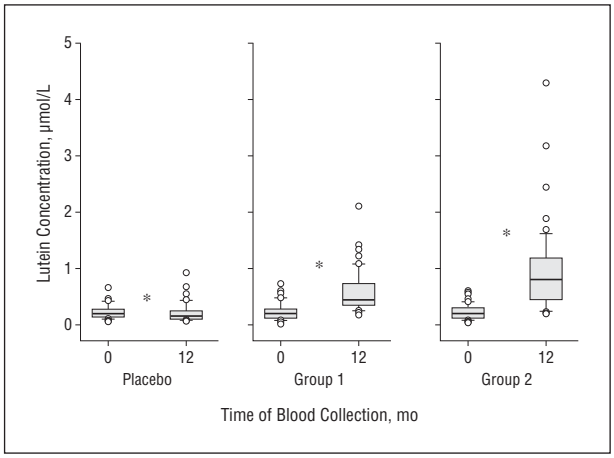

Figure 2. Concentrations of lutein in plasma at baseline (0) and at study's end (12 months) within the study groups. *Significant differences ($P < .05$, paired *t* test, natural logarithm-transformed values). The box and whisker plots show the 25th and 75th percentiles, the median (rule within the box), and the 10th and 90th percentiles (whiskers); outliers are symbolized by the circles.

BLOOD LIPIDS

Triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol in plasma were determined by enzymatic methods using an autoanalyzer (Architect C16000; Abbott Diagnostics). These procedures were performed according to the methods of the Institute of Clinical Chemistry and Laboratory Medicine, Friedrich Schiller University Jena.

STATISTICAL ANALYSIS

Data were analyzed using commercial software (SPSS, version 18.0; SPSS, Inc). Values are reported as mean (SD). The Kolmogorov-Smirnov test was applied to check on gaussian distribution, and the Levene test was used to evaluate the homogeneity of the variances. If the variances were not homogeneous, the values were transformed adequately before they were subjected to the tests. The influence of supplementation was tested with the general linear model procedure (repeated measurements). Unpaired 2-tailed *t* test was used to assess the differences between the groups. Correlations between variables were calculated using the Pearson or Spearman correlation. The level of significance was $P < .05$; $.05 < P < .10$ was considered as an indicator of a trend toward significance.

RESULTS

CAROTENOIDS

At baseline, the 3 study groups had comparable contents of lutein and zeaxanthin in their plasma (**Figure 2**). The concentrations of the administered carotenoids in plasma increased significantly in both active treatment groups after 1 month of supplementation compared with baseline (single dose; lutein: 0.22 [0.15] vs 0.60 [0.32] μmol/L, zeaxanthin: 0.045 [0.039] vs 0.060 [0.047] μmol/L) (**Figure 3**). The values reached a plateau after 1 month of intervention in both treatment groups. The

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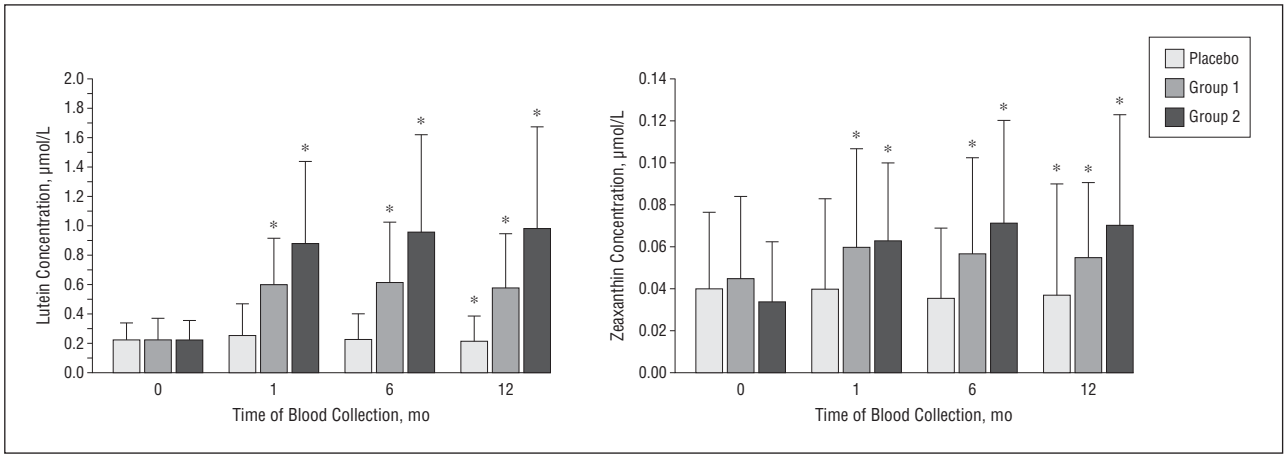

Figure 3. Alteration of the concentrations of lutein and zeaxanthin in the plasma of patients with age-related macular degeneration during a 12-month study. Values are mean (SD). *Significant differences within the groups in relationship to baseline (0), ($P < .05$, general linear model procedure [repeated measurements], natural log–transformed values).

concentrations of lutein and zeaxanthin did not change significantly after that time (Figure 3). Consumption of the double dose (group 2) resulted in significantly higher concentrations of lutein in plasma in comparison with the single dose, but these were not twice the concentrations observed with the single dose.

Neither the age nor sex of the patients had an influence on the initial plasma concentrations of lutein and zeaxanthin. However, sex had a significant influence on the alteration of lutein throughout the study. Women in group 2 responded to supplementation with significantly higher concentrations of lutein in the plasma compared with men (data not shown).

Patients within the lowest body mass index (BMI) category (18-24; calculated as weight in kilograms divided by height in meters squared) tended to have higher concentrations of lutein in plasma. Further statistical analysis did not identify consistent associations between BMI and concentrations of lutein in the plasma within the present study.

Some participants did not respond or responded only slightly to supplementation with an increase of lutein or zeaxanthin concentrations in plasma. No response was identified for 2 patients in group 1 and 2 patients in group 2. At 12 months, their lutein levels were well below (6%-32%) the baseline values. A slight increase (up to 50%) was found for 15 patients (9 in group 1 and 6 in group 2). Furthermore, there were 53 patients (29 in group 1 and 24 in group 2) who responded to supplementation with up to 500% higher lutein levels at 12 months in comparison with baseline and 32 patients (10 in group 1 and 22 in group 2) with an increase of more than 500% (up to 2108%). The initial lutein values correlated positively with the values at study end in both active treatment groups (data not shown).

FATTY ACIDS

Fatty acids were analyzed at baseline, 1 month, and 12 months (**Table 4**). The 3 groups started the study with mostly comparable values. Significantly different baseline values were assessed for the sums of ω-3 fatty acids, ω-6 fatty acids, saturated fatty acids, monounsaturated fatty acids, and PUFAs between the groups.

Within the placebo group, the profile of all fatty acids present remained constant during the study period. In the plasma lipids of group 1, the intervention resulted in a significant decrease of arachidonic acid (AA); EPA, DHA, and the sum of ω-3 fatty acids increased significantly. These alterations were also observed in group 2, but here the values of PUFA increased and the levels of saturated fatty acids and monounsaturated fatty acids decreased significantly. After 12 months of intervention, group 2 showed significantly higher EPA and DHA levels than group 1 or the placebo group. This was reflected by significant changes in the calculated sums and ratios (eg, EPA + DHA, AA: EPA) compared with group 1 and the placebo group. The intervention in group 1 did not lead to noticeable changes in comparison with the placebo.

ANTIOXIDANT CAPACITY

Plasma from 30 participants was randomly selected to determine the alteration of lipophilic and hydrophilic antioxidant capacity. The intervention did not lead to detectable alterations of the H-TEAC in the plasma of patients with AMD. In contrast, the L-TEAC increased significantly in group 1 and group 2, with no changes observed in the placebo group. Data from the photochemiluminescence assay confirmed the observations of the L-TEAC assay for placebo and group 2 but did not reflect the changes measured for group 1 (**Figure 4**).

MACULAR PIGMENT

Complete data were available for 145 patients (placebo, 40; group 1, 50; group 2, 55). The optical density of the macular pigment is illustrated in **Figure 5**. As the diagram shows, the optical density of the macular pigment increased significantly in group 1 and group 2, whereas the levels in the placebo group remained relatively constant. Ingestion of the double dose of the supplement (group 2) did not lead to a significantly higher optical density of the macular pigment compared with group 1.

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Table 4. Fatty Acid Distribution in Plasma Lipids at Baseline and After 1 and 12 Months of Intervention

Fatty Acid	% FAME, Mean (SD)[a]		
	Placebo (n = 40)	Group 1 (n = 50)	Group 2 (n = 54)[b]
AA			
0	5.92 (1.37)	5.68 (1.24)	5.62 (1.32)
1 mo	5.85 (1.18)	5.60 (1.17)[c]	5.78 (1.42)[c]
12 mo	5.90 (1.32)	5.45 (0.94)[c]	5.58 (1.31)[c,d]
EPA(t)			
0	0.81 (0.64)	0.66 (0.37)	0.72 (0.33)
1 mo	0.78 (0.39)	0.86 (0.68)[c]	0.97 (0.45)[c]
12 mo	0.75 (0.37)	0.68 (0.28)	0.96 (0.37)[c,e]
DPA			
0	0.41 (0.12)	0.37 (0.08)	0.39 (0.07)
1 mo	0.40 (0.10)	0.38 (0.09)	0.40 (0.08)
12 mo	0.41 (0.10)	0.36 (0.07)[f]	0.39 (0.07)
DHA			
0	1.49 (0.55)	1.39 (0.47)	1.35 (0.43)
1 mo	1.45 (0.41)	1.68 (0.64)[c]	1.89 (0.50)[c,e,f]
12 mo	1.50 (0.54)	1.62 (0.42)[c]	2.00 (0.43)[c,d,e,f]
Sum ω-3 FA(t)			
0	3.25 (1.20)	2.94 (0.87)[f]	2.98 (0.77)
1 mo	3.15 (0.88)	3.46 (1.36)[c]	3.77 (0.92)[c,f]
12 mo	3.25 (0.97)	3.19 (0.77)[c]	3.87 (0.80) [c,e,f]
Sum ω-6 FA			
0	34.97 (4.40)	33.61 (5.18)	32.16 (3.93)[f]
1 mo	34.87 (4.27)	33.59 (5.42)	32.80 (3.83)[f]
12 mo	34.68 (3.75)	33.28 (4.68)	32.85 (4.06)
SFA			
0	32.90 (2.47)	33.52 (2.40)	34.05 (1.84)[f]
1 mo	33.05 (2.29)	33.51 (2.79)	33.78 (1.98)
12 mo	32.87 (2.06)	33.62 (2.16)[f,g]	33.43 (1.81)[c]
MUFA			
0	26.55 (3.62)	27.74 (3.33)	28.59 (3.60)[f]
1 mo	26.77 (3.10)	27.27 (3.73)	27.58 (3.13)[c]
12 mo	27.02 (3.12)	27.83 (3.44)	27.80 (3.59)[c]
PUFA			
0	38.78 (4.70)	37.15 (5.23)	35.73 (4.27)[f]
1 mo	38.59 (4.49)	37.64 (5.71)	37.14 (3.97)[c]
12 mo	38.49 (4.01)	37.01 (4.79)	37.29 (4.41)[c]
EPA + DHA(t)			
0	2.30 (1.12)	2.05 (0.80)	2.07 (0.70)
1 mo	2.23 (0.76)	2.54 (1.27)[c]	2.85 (0.87)[c,e,f]
12 mo	2.26 (0.86)	2.31 (0.63)[c]	2.96 (0.72)[c,e,f]
EPA + DPA + DHA(t)			
0	2.71 (1.18)	2.42 (0.85)	2.46 (0.75)
1 mo	2.63 (0.82)	2.92 (1.34)[c]	3.25 (0.94)[c,e,f]
12 mo	2.67 (0.92)	2.66 (0.68)[c]	3.35 (0.77)[c,e,f]
Sum ω-6:sum ω-3(t)			
0	11.73 (3.32)	12.43 (4.13)	11.36 (2.60)
1 mo	11.82 (3.31)	10.74 (3.28)[c]	9.16 (2.29)[c,e,f]
12 mo	11.55 (3.43)	11.02 (3.08)[c]	8.78 (1.84)[c,e,f]
AA:EPA(t)			
0	9.44 (4.49)	10.9 (5.46)	9.11 (3.64)
1 mo	9.54 (5.21)	8.69 (3.67)[c]	6.76 (2.46)[c]
12 mo	9.86 (5.43)	9.12 (3.64)	6.61 (2.81)[c,e,f]
AA:(EPA + DPA + DHA)(t)			
0	2.42 (0.78)	2.56 (0.81)	2.41 (0.65)
1 mo	2.43 (0.82)	2.16 (0.65)[c]	1.86 (0.48)[c,e,f]
12 mo	2.42 (0.78)	2.17 (0.62)[c]	1.73 (0.51)[c,d,e,f]

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FAME, fatty acid methyl ester; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; sum ω-3 = α-linolenic acid + EPA + DPA + DHA; sum ω-6 = linoleic acid + γ-linolenic acid + eicosadienoic acid + AA + dihomo-γ-linolenic acid.

[a] Values are mean (SD) of percentage of total FAME; (t) indicates that, for statistical analysis, the data were natural log–transformed because of variance heterogeneity.

[b] One patient originally randomized to group 2 was excluded from plasma analysis (problems with venipuncture).

[c] Significant difference compared with baseline (0) (general linear model, repeated measurement), $P < .05$.

[d] Significant difference compared with 1 month (general linear model, repeated measurement), $P < .05$.

[e] Significant difference compared with group 1 (unpaired *t* test), $P < .05$.

[f] Significant difference compared with the placebo group (unpaired *t* test), $P < .05$.

[g] Significant difference compared with group 2 (unpaired *t* test), $P < .05$.

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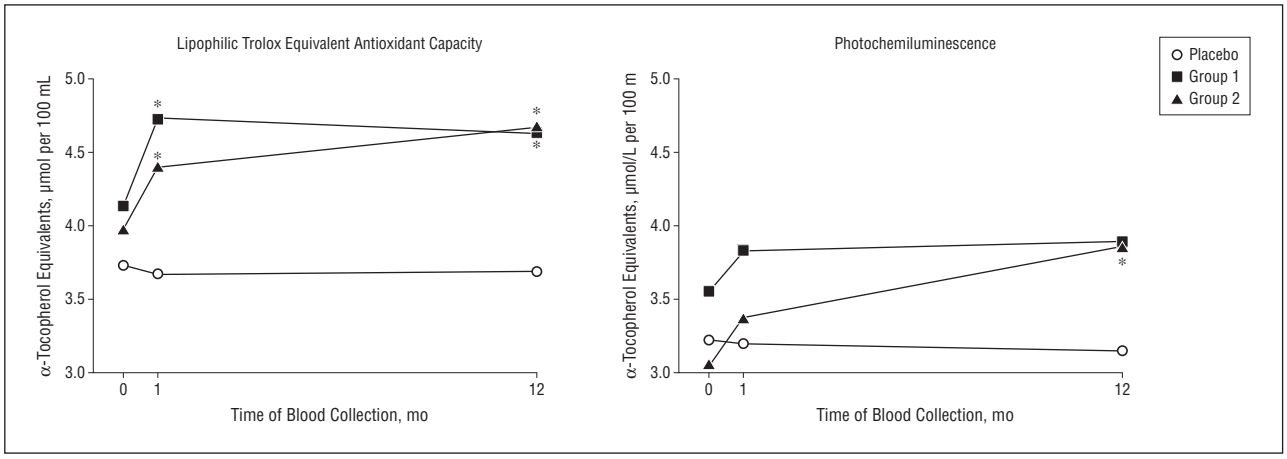

Figure 4. Alteration of the lipophilic antioxidant capacity in plasma during 12 months of intervention. *Significant differences within the groups in relation to baseline (0) ($P < .05$, general linear model; repeated measurements). Trolox indicates 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

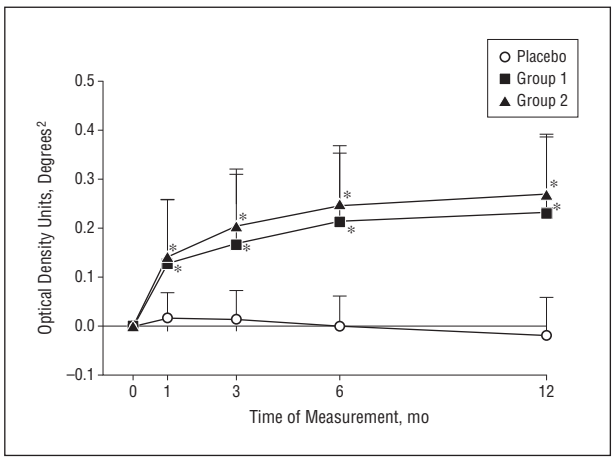

Figure 5. Alterations of the optical density of the macular pigment in patients with age-related macular degeneration during the study. Values are mean (SD). *Significant differences within the groups in relation to baseline (0) ($P < .05$, general linear model procedure; repeated measurements).

However, the values in both treatment groups differed significantly from those in the placebo group at all measured times (data not shown).

BLOOD LIPIDS

In the placebo group, total cholesterol levels decreased significantly after 12 months, and triglycerides tended to decrease in group 2 ($P = .06$). No relationships between blood lipid values and plasma concentrations of lutein and zeaxanthin were found.

COMMENT

The results of this trial show that supplementation with lutein and zeaxanthin significantly increased the concentration of both macular xanthophylls in the plasma of patients with AMD as described previously.[2,20,21] The circulating levels of lutein and zeaxanthin reached a plateau after 1 month of intervention in both treatment groups compared with baseline. Such a plateau has been described in studies investigating the plasma kinetics of lutein and zeaxanthin[21-23] and may reflect an unknown mix of absorption and disposition factors.[24] The present analysis demonstrates that women responded to the supplement with significantly higher concentrations of lutein in plasma compared with men. The EPIC (European Investigation into Cancer and Nutrition) study also reported significantly higher total carotenoid levels in women than in men.[25] The authors reporting on the EPIC study discussed sex-specific differences in intake, absorption, and metabolism responsible for this result. But this does not explain why only women in group 2 in the present study exhibited higher lutein levels than men. Different sample sizes in statistical subgroup analysis may have led to this inhomogeneity of the results.

The BMI has been established as a further risk factor for AMD.[26] The present analysis showed that patients within the lowest BMI category (18-24) tended to have higher plasma concentrations of lutein. Further statistical analysis did not lead to consistent associations between BMI and concentrations of lutein in the plasma. This may be the result of smaller sample sizes in subgroup analysis; therefore, the relationship between BMI and AMD remains speculative in the present study. However, our findings are confirmed by the available data insofar as it has been reported that changes in macular optical density do not vary with BMI.[27] Furthermore, different classes of carotenoids differ in their association with BMI. For instance, β-carotene, but not lutein or lycopene, concentrations in plasma are inversely correlated with BMI in obese individuals.[28] This may reflect faster removal from plasma to other body pools (adipose tissue) than in nonobese people, or it may suggest a different degree of tissue specificity for these carotenoids.[28]

Individual differences in the response to supplementation with macular xanthophylls have been described.[20,29,30] Patients in the present study who did not respond with increased plasma concentrations of lutein and zeaxanthin exhibited higher mean initial values (2.5-fold) in comparison with the other patients. Therefore, the lack of response was not caused by malabsorption. The missing response to the administered supplement seems rather to be the result of a higher intake of macular xanthophylls through the diet or unknown meta-

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bolic effects. However, poor adherence by a few patients cannot be excluded. Although these unclear factors make the results difficult to interpret, the observation that the initial lutein values correlated positively with the values at the study's end confirms similar findings.[22,31]

The available data suggest a relationship between the concentrations of HDL-C and lutein in plasma.[32] This is based on the findings that macular xanthophylls are incorporated into plasma lipoproteins,[33] predominantly in HDL-C.[34] However, in the present study, no correlation between the contents of HDL-C and the concentrations of lutein in the plasma of patients could be established, even though it has been described previously.[30] No effect of the supplement on other blood lipid values was observed within this study.

The optical density of the macular pigment increased significantly in both treatment groups and reached a plateau comparable to the circulating levels of both administered macular xanthophylls. In contrast to the results of the plasma carotenoid analysis, ingestion of the double dose of the supplement did not lead to a significantly higher optical density in group 2 compared with group 1. This led to the assumption that the dose in group 1 (10 mg of lutein per day) is effective to improve the optical density of the macular pigment and that the double dose did not provide additional benefit. A risk assessment of oral lutein indicated that up to 20 mg per day of lutein is safe.[35] Despite the fact that the evidence for this dosage level is strong, the lower dose should be preferred considering the present findings.

Studies demonstrated that the mean concentrations of lutein in plasma decreased after cessation of the supplement,[22] whereas the macular pigment remained constant when intervention ceased.[29] These findings suggest a slow turnover of carotenoids in the retina and the sustainability of supplementation.[21] Further studies are necessary to verify this hypothesis.

In both treatment groups, the intervention resulted in a significant decrease of AA over the full study period, and EPA, DHA, and the sum of ω-3 fatty acids increased significantly. The double dose was also potent enough to elevate the values of PUFAs and to decrease saturated fatty acids and monounsaturated fatty acids significantly. At the study's end, patients in group 2 exhibited significantly higher EPA and DHA levels than did patients in group 1 or the placebo group. The resulting improvement of calculated sums and ratios (eg, EPA + DHA, AA:EPA) compared with group 1 and the placebo group is beneficial, since the pathogenesis of AMD is linked to atherogenesis,[36] and several studies[37] showing the antiatherogenic effect of LC-PUFAs are available. The additional anti-inflammatory potential of these fatty acids[38] may provide further benefit for patients with AMD. Furthermore, it has been shown that use of ω-3 LC-PUFAs decreases cardiovascular risk factors.[39] Because group 1 findings did not differ significantly from those of the placebo group, the doses are considered too low. However, intake of ω-3 LC-PUFAs is related to a decreased risk of progression to geographic atrophy.[40]

The hydrophilic antioxidant status of the patients was not influenced by the intervention, which may be the result of the composition of the administered capsules. The products contained 60 mg (group 1) and 120 mg (group 2) of vitamin C with no additional hydrophilic antioxidants. The reason for the unmodified antioxidant capacity seems to be the sufficient supply of vitamin C in Germany (mean, 131 mg/d)[41] and the excretion of excessive vitamin C doses by the kidneys.

The supplement was potent enough to increase the lipophilic antioxidant capacity in the plasma of the patients in both treatment groups, whereas the values of the placebo group remained constant throughout the study. Because an insufficient antioxidative status is linked to AMD,[42] the data of the present analysis may suggest a beneficial effect of the intervention on the course of the disease. However, because the available data on the antioxidant status of patients with AMD are rather limited, interpretation of the present analysis remains speculative.

In conclusion, the LUTEGA study demonstrates that 12-month supplementation with a combination of lutein, zeaxanthin, and ω-3 LC-PUFAs significantly elevated plasma antioxidant capacity and circulating macular xanthophyll levels, as well as the optical density of the macular pigment. Therefore, such supplementation may be beneficial for AMD patients. Two major limitations of the study need to be acknowledged. The first limitation concerns the relatively short study period in relation to the insidious course of the disease; the second limitation concerns the limited scope of the study population. Hence, the results of the AREDS 2 (4000 participants, 5-6 years' follow-up) on the effect of oral supplementation of macular xanthophylls and/or ω-3 LC-PUFAs on the progression to advanced AMD are awaited. Because the single dose is as effective as the double dose, there seems to be no need to use the higher dosage. No adverse or other unintended effects were noted in any of the observed groups in the present study; however, harmful effects associated with high-dose antioxidant supplementation cannot be ruled out (especially for smokers). With this, and the limitations of the study, no general implications for clinical practice can be given. According to Evans,[43] trials evaluating different categories of individuals (healthy people at risk for AMD and those with early, intermediate, or late stages of the disease) are required, since it seems likely that the potential protective effects of macular xanthophylls and ω-3 LC-PUFAs depend on the stage of the disease.

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Correspondence: Volker Böhm, PhD, Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Strasse 25-29, D-07743 Jena, Germany (Volker.Boehm@uni-jena.de).
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