

Oral Lutein Supplementation Enhances Macular Pigment Density and Contrast Sensitivity but Not in Combination With Polyunsaturated Fatty Acids

Ute E. K. Wolf-Schnurrbusch, Martin S. Zinkernagel, Marion R. Munk, Andreas Ebnetter, and Sebastian Wolf

Department of Ophthalmology, University Hospital Bern, Inselspital, University Bern, Bern, Switzerland

Correspondence: Sebastian Wolf, Department of Ophthalmology, University Hospital Bern, Inselspital, University Bern, CH-3010 Bern, Switzerland; sebastian.wolf@insel.ch.

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PURPOSE. It has been shown that lutein and zeaxanthin accumulate in the macula where they enhance contrast sensitivity and may reduce the risk of progression to advanced age-related macular degeneration (AMD). Furthermore, omega-3 long-chain polyunsaturated fatty acids (PUFA) might further reduce this risk. However, controversy exists regarding whether PUFA may reduce the bioavailability of lutein.

METHODS. This was a prospective 12-month, randomized, open label study evaluating the effect of supplementation with lutein, other antioxidants, and minerals on contrast sensitivity (CS) and macular pigment optical density (MPOD) in patients with age-related maculopathy. A total of 79 patients were randomized to either lutein (10 mg) and antioxidant supplement or lutein and antioxidant supplement in combination with PUFA. Patients received supplementation for a period of 6 months and were followed for a total of 12 months.

RESULTS. Serum lutein and zeaxanthin increased significantly by the first follow-up visit at 1 month, and remained elevated throughout the intervention period of 6 months in the lutein-only group but not in the lutein+PUFA group. Macular pigment optical density and CS increased significantly in the lutein-only group ($P < 0.005$) but not in the lutein+PUFA group ($P = 0.059$) compared to baseline. Best-corrected visual acuity remained unchanged during the entire study period in both groups.

CONCLUSIONS. Addition of PUFA may reduce the bioavailability of lutein and therefore lessen the beneficial effect on macular pigment and CS. This needs to be considered when prescribing lutein supplements to patients with low lutein levels. (ClinicalTrials.gov number, NCT00563979.)

Keywords: macular pigment, macular pigment, lutein, lutein, contrast sensitivity, contrast sensitivity

Macular pigment (MP) is composed of several carotenoids, mainly the two hydroxyl-xanthophylls lutein (L) and zeaxanthin (Z). It has been speculated that MP has a protective role in the development and progression of age related maculopathy (ARM).¹ Macular pigment reaches its highest concentration in the photoreceptor axons in the center of the fovea, where zeaxanthin is concentrated 2-fold higher than lutein, but in the periphery this relationship is reversed. The properties of MP include a high capacity to absorb short-wavelength blue light, and it has therefore a protective function for the fovea.² It works as a broadband filter for the macula, with an absorbance peak at 460 nm. Two advantages result: The macula's optical accuracy is enhanced,^{3,4} and the damaging photo-oxidative influence of light on the neurosensory retina is reduced. Assuming that the formation of free radicals is one cause of development of age-related macular degeneration (AMD), a high concentration of MP in the macula might be protective against light-induced oxidative damage and a low concentration increase the risk of AMD.

Mammals are not able to synthesize carotenoids but have to ingest them in food or as food supplements. Studies have shown a significant association between MP optical density

(MPOD) and lutein blood serum levels. Furthermore, there is a positive association between MPOD and dietary intake of carotenoids, either by dietary modification or by supplementation.⁵

Recently, the role of omega-3 polyunsaturated fatty acid (PUFA) for prevention of AMD has been discussed.⁶⁻⁹ High levels of docosahexaenoic acid (DHA), an omega-3 fatty acid, are present in the disc membranes of photoreceptor outer segments. The specific role of DHA in the rod outer segment membranes is yet unknown. However, it is believed that DHA and its precursor eicosapentaenoic acid (EPA) play an important role in the retina and may be involved in the development of retinal diseases. Several epidemiologic studies have investigated the effect of PUFA on the progression of AMD, but the results were inconclusive.^{6,10-12} Recently, the Age-Related Eye Disease Study 2 (AREDS2) demonstrated no beneficial effects of DHA + EPA on the progression of AMD.⁹

Nevertheless, various supplements containing high doses of PUFA are available and are widely used to prevent and slow down AMD progression.

TABLE 1. Ingredients of Supplements

	VitaluxPlus	VitaluxOmega
Lutein	10 mg	10 mg
Vitamin C	60 mg	60 mg
Vitamin E	20 mg	20 mg
Niacin, vitamin B ₃	10 mg	10 mg
Copper	0.25 mg	0.25 mg
Zinc	10 mg	10 mg
Zeaxanthine	1 mg	1 mg
Omega-3 fatty acid (DHA/EPA)	-	160 mg (130 mg)

In the current study we have investigated the effects of lutein/zeaxanthin supplementation as well as supplementation with lutein/zeaxanthin in a fixed combination with PUFA.

MATERIALS AND METHODS

Study Participants

Consecutive patients who were referred to the outpatient department of the Department of Ophthalmology, Inselspital, University Bern, for AMD were recruited between July 2007 and June 2008 for this prospective study. Inclusion criteria were age over 50 years with early or intermediate AMD.¹³ Exclusion criteria were other eye disease in the study eye and opacities of optical media precluding fundus photography. Only one eye of each patient was included in the study. If both eyes were eligible for the study, the eye with more advanced AMD changes was included.

Study Design and Procedures

This was a 12-month, randomized, open label study evaluating the effect of supplementation with lutein and other antioxidants and minerals on contrast sensitivity (CS) and MPOD in patients with AMD. Eligible patients were randomized in a 1:1 ratio to either lutein and other vitamins (VitaluxPlus; Novartis, Basel, Switzerland) or lutein, omega-3, and other vitamins (VitaluxOmega, Novartis). The antioxidants and minerals in VitaluxPlus and VitaluxOmega are shown in Table 1. Both drugs were commercially available at the time of the study.

All patients received supplementation for a period of 6 months and were followed for a total of 12 months. Examinations were scheduled at baseline, month 1, and months 3, 6, 7, 8, 9, and 12. At each visit a comprehensive ocular examination with best-corrected visual acuity (BCVA) using Early Treatment Diabetic Retinopathy Study (ETDRS) charts, CS using the Pelli-Robson charts, dilated binocular ophthalmoscopy, color fundus photography (FF 450 plus; Carl Zeiss Meditec, Jena, Germany), and MPOD measurement were performed. At each visit the empty blisters from the study medication were collected and a pill count was performed to ensure compliance with the study medication.

Additionally, serum concentrations of lutein and zeaxanthin were measured. Contrast sensitivity was assessed using the total contrast sensitivity score (CS score) ([number of letters-3] × 0.05). Macular pigment optical density measurements were obtained with the modified confocal scanning laser ophthalmoscope¹⁴ (mpHRA; Heidelberg Engineering, Heidelberg, Germany) using autofluorescence images obtained at two wavelengths based on pioneering work of Delori et al.¹⁵ According to previous work,¹⁶ we quantified MP densities by calculating a macular pigment density (MPD) map and comparing foveal and parafoveal autofluorescence at 488 and 514 nm. Density maps were obtained by subtracting the log

TABLE 2. Demographics and Baseline Characteristics

Characteristics	Lutein, <i>n</i> = 40	Lutein+Omega, <i>n</i> = 39	<i>P</i> Value
Age, y			
Mean (range)	75.2 (54-88)	72.5 (54-88)	>0.05
Sex, <i>n</i> (%)			
Male	18 (45)	13 (39)	
Female	22 (55)	26 (61)	
BMI			
Mean (range)	25 (16-36)	25 (18-32)	>0.05
Early AMD ¹³	22	18	
Intermediate AMD ¹³	18	21	
VA, ETDRS letters			
Mean (SD)	79.7 (±7.4)	78.6 (±10.5)	>0.05
Range	53-94	40-95	
CS score			
Mean (SD)	1.286 (±0.245)	1.227 (±0.273)	>0.05
MPOD D.U.			
Mean (SD)	0.543 (±0.192)	0.564 (±0.205)	>0.05
Lutein serum, μg/mL			
Mean (SD)	0.147 (0.076)	0.163 (0.117)	>0.05
Zeaxanthin serum, μg/mL			
Mean (SD)	0.025 (0.011)	0.025 (0.012)	>0.05

BMI, body mass index; VA, visual acuity; lutein, lutein serum levels; zeaxanthin, zeaxanthin serum levels.

images digitally. The central MPD (MPD_c) is indicated in optical density units (D.U.) and was calculated within a 1° diameter circle centered on the fovea. All quantitative analyses were done by the software provided by the manufacturer of the scanning laser ophthalmoscope. Assessment and evaluation of respective imaging technique have previously been described in detail.^{14,15}

The primary objective of the study was to evaluate the effect of supplementation on CS and MPOD after 6 months. Secondary outcome measures were the change of CS, MPOD, BCVA, and serum concentrations of lutein and zeaxanthin over the time period of 12 months. Statistical analyses were conducted with SPSS software (SPSS, Inc., Chicago, IL, USA). In order to compare both treatment arms, data were analyzed using repeated measures ANOVA. Paired *t*-test was used to evaluate changes from baseline to the primary endpoint within one treatment arm. The level of significance was set at 0.05.

Prior to any study procedures, written informed consent was obtained from each patient after an extensive discussion about the purpose of the study and all the risks associated with participation. The research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. This study is registered at ClinicalTrials.gov (NCT00563979).

RESULTS

A total of 79 patients were randomized to the two treatment groups (lutein, *n* = 40; lutein+omega, *n* = 39). Demographics of included patients are summarized in Table 2. Six subjects were smokers, 23 subjects were ex-smokers, and 50 subjects had never smoked. All patients had medium or large drusen or AMD pigmentary abnormalities. The two treatment arms were

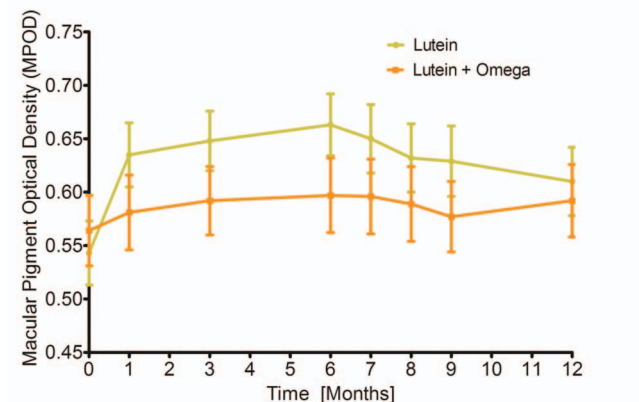
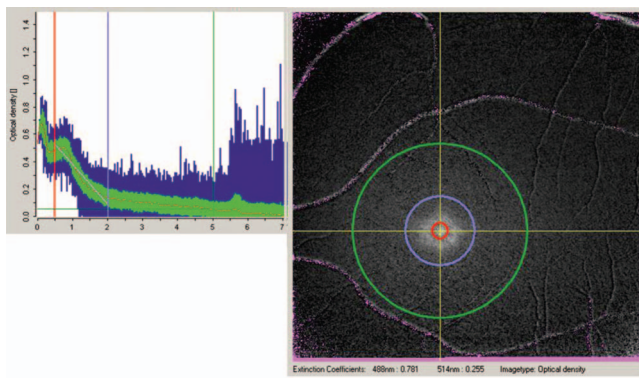


FIGURE 1. Change of macular pigment optical density (MPOD) during the study period. Representative mean macular pigment density profile (top left). The green area represents standard deviation, and blue bars represent extreme values at the respective radius. Corresponding MPOD map after digital processing of the 488- and 514-nm image (top right). Change of MPOD over the course of 1 year for patients treated with lutein only and with lutein in combination with omega-3 long-chain polyunsaturated fatty acids (lutein+omega) (ANOVA, $P < 0.05$, $n = 79$).

FIGURE 2. Change of contrast sensitivity (CS) (mean \pm SEM) over the course of 1 year for patients treated with lutein only and with lutein in combination with omega-3 long-chain polyunsaturated fatty acids (lutein+omega) (ANOVA, $P < 0.05$, $n = 79$).

drastically decreased after cessation of the supplementation after 6 months in the lutein group, whereas no changes were observed in CS in the lutein+omega group (Fig. 2). The differences between the groups were significant (ANOVA, $P < 0.01$).

Plasma levels of lutein (Fig. 3) and zeaxanthin (Fig. 4) showed changes only in the lutein group. Values in the lutein+omega group remained unchanged during the study period. The differences between the groups were significant for the plasma concentrations of lutein as well as for zeaxanthin (ANOVA, $P < 0.01$).

The BCVA letter score remained unchanged during the entire study period in both groups (baseline L: 80 ± 7 ETDRS letters, L+O: 79 ± 11 ETDRS letters; 6 months L: 79 ± 7 ETDRS letters, L+O: 80 ± 11 ETDRS letters; 12 months L: 81 ± 5 ETDRS letters, L+O: 80 ± 10 ETDRS letters).

DISCUSSION

Lutein and zeaxanthin as nutritional complements have been extensively investigated in biomedical and nutritional research, as they selectively accumulate in the macula. The characteristic yellow aspect of the macula is due to the presence of MP composed of lutein and zeaxanthin, which absorb blue light and prevent peroxidation of lipids in the retina.¹⁷ Besides other factors, MP density is currently under investigation as a predictive factor in the development of AMD.^{1,18-21} Although there is growing evidence that oxidative damage contributes to the development of AMD^{1,22,23} and that MP is protective against ARM,^{18,24,25} this still remains unproven and considerable controversy exists.²⁶ Previous studies have shown a positive correlation between lutein intake and MPOD.²⁷⁻³⁰ Substitution of lutein with a lutein dose of 10 mg or above resulted in a significant increase in serum levels of lutein and MPOD.³¹

Recently, the role of PUFA in the pathophysiology of AMD has been discussed. Since the retina contains high concentrations of DHA, it has been speculated that supplementation with PUFA may help to prevent progression of AMD.^{6,10,32,33} However, these epidemiologic studies have been used only to generate hypothesis and cannot show whether supplementation of PUFA leads to a significant effect on the progression of AMD. For this, prospective randomized clinical trials would be necessary.

Here, we show that supplementation of omega-3 PUFA with lutein leads to significantly lower plasma lutein levels when

comparable with respect to baseline characteristics (Table 2). The first patient visit took place in July 2007, and the last study visit took place in June 2009.

During the first 6 months of the study, all patients took daily supplementation of 10 mg of lutein with or without omega-3 fatty acids. No subject developed any systemic or ocular disorders during the study period.

The MPOD levels were 0.54 ± 0.19 D.U. for the lutein group (L) and 0.56 ± 0.21 D.U. for the lutein+omega group (L+O) at baseline. The MPOD levels increased after 6 months of supplementation to 0.66 ± 0.18 and 0.60 ± 0.22 D.U., respectively. The increase in the lutein group was highly significant ($P < 0.005$), whereas the change in the lutein+omega group failed to be significant ($P = 0.059$). ANOVA for repeated measurements revealed a significant difference between the two groups ($P < 0.01$) at the primary endpoint. The MPOD levels over the entire study period of 1 year showed a relatively slow decrease (Fig. 1). The differences between the groups were significant (ANOVA, $P < 0.01$).

The CS score was 1.29 ± 0.25 for the lutein group and 1.23 ± 0.27 for the lutein+omega group at baseline. Contrast sensitivity increased to 1.69 ± 0.22 in the lutein group ($P < 0.01$) and remained unchanged in the lutein+omega group after 6 months (1.30 ± 0.25 ; $P = 0.086$). ANOVA for repeated measurements revealed a significant difference between the two groups ($P < 0.01$) at the primary endpoint. The CS score

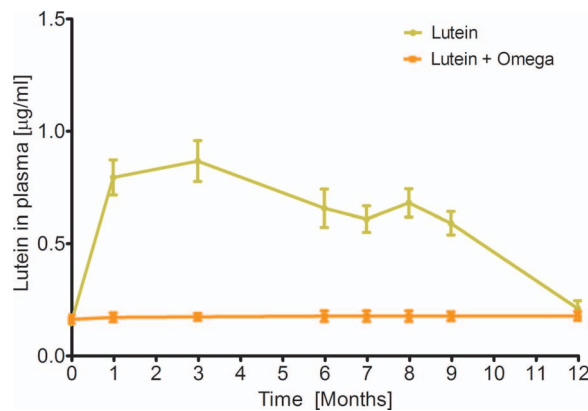


FIGURE 3. Change of serum lutein concentration (mean \pm SEM) during the study period. The change of the lutein concentration in plasma was significantly different (ANOVA, $P < 0.05$) between the treatment arms ($n = 79$).

compared to lutein intake without supplementation of PUFA during the first year of treatment. Furthermore, the difference in lutein levels observed between the lutein-only and the lutein+PUFA group mirrors the difference in CS and MP density. There is evidence that the nature of fatty acids can directly affect intestinal lutein absorption.³⁴⁻³⁶ Some lipids, such as phosphatidylcholine, have been suggested to strongly retain the hydrophobic lutein in the mixed micelles, thereby suppressing its intestinal uptake.³⁷

It has been shown that monounsaturated fatty acids (MUFA), such as olive oils, increase the intestinal uptake of lutein, whereas PUFA-rich fats reduce the uptake of lutein.^{34,38,39}

Previous studies have related serum lipid levels to the MP density in the retina; a recent study showed that oxidized low-density lipoprotein (LDL) was negatively correlated with MPOD, suggesting that increase in oxidized LDL could reduce MPOD.⁴⁰ Likewise, it has been reported that high-density lipoprotein (HDL) is the preferential carrier of the MP carotenoids in plasma.⁴¹ Another recent study has shown that MP is positively correlated with serum total cholesterol⁴² but negatively correlated with serum triglycerides.⁴³ Although these reports show a correlation between MP and/or lutein with serum lipid levels, there are few data on serum levels of lutein after combined supplementation with specific lipids. Similar mechanisms may explain the decreased serum levels of lutein in our cohort, as VitaluxOmega contains DHA (100 mg) and EPA (30 mg) in concentrated fish oil (251 mg total).

Our results seem to contradict the findings of AREDS2, in which lutein + zeaxanthin supplementation led to similar serum levels as lutein + zeaxanthin and DHA + EPA after 1 year.⁹ However, we have to note that our study extended to only 1 year, whereas AREDS2 presents 5-year results. Considering the influence of lipids on the intestinal absorption and thus the bioavailability of lutein, this may be due to differing suspension formulas of DHA and EPA; whereas in the AREDS2 trial DHA + EPA was given in ethyl ester form as ROPUFA 75 n-33, we used VitaluxOmega, which contains DHA and EPA as components of concentrated fish oil. As such, differences in lipid composition may have led to unequal conditions for intestinal lutein absorption. Another relevant factor may be a common single nucleotide polymorphism of beta-carotene 15,15'-monooxygenase 1, which seems to influence the plasma level of carotenoids after dietary carotenoid/lutein intake.^{44,45} Neither AREDS2 nor our study analyzed this polymorphism, so

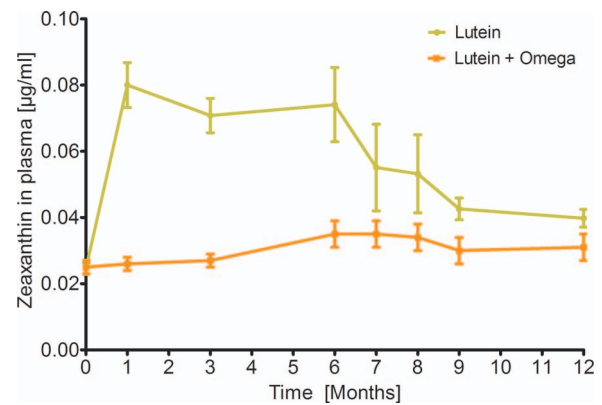


FIGURE 4. Change of serum zeaxanthin concentration (mean \pm SEM) during the study period. The change of zeaxanthin was significantly different (ANOVA, $P < 0.05$) between the treatment groups ($n = 79$).

the response may vary due to divergent genetic backgrounds of the study populations.

It may be argued that because the AREDS formulation in primary analyses did not further reduce risk of progression to advanced AMD, there is generally no need for supplementation with lutein. However, the study population in AREDS2 has been reported to be a well-nourished cohort, and in subgroup analysis of subjects with the lowest dietary intake of lutein + zeaxanthin, lutein + zeaxanthin demonstrated a protective effect for progression to advanced AMD. And although the primary analyses demonstrated no beneficial effect of lutein/zeaxanthin in the treatment of advanced AMD in AREDS2, the post hoc analyses suggested that lutein and zeaxanthin may well play a role to reduce the risk of AMD progression, whether given with or without beta-carotene.⁴⁶ Furthermore, it has been shown that lutein leads to improved CS, however, without increase in visual acuity, and thus may improve patients' quality of life.^{5,47} As such, lutein supplementation may still be beneficial for a subgroup of patients suffering from ARM.

Our study has some limitations that need to be considered. Firstly, although we found a striking difference in lutein level between patients supplemented with VitaluxPlus and VitaluxOmega, we cannot conclusively define which lipid components may have led to decreased serum lutein levels in the omega group. Another limitation is that we have not measured serum levels of PUFA in our study. We have no data on bioavailability of the ingredients of either drug; therefore we cannot exclude that the bioavailability of lutein and zeaxanthin in the VitaluxOmega is reduced. However, since Vitalux with a combination of lutein + zeaxanthin and DHA + EPA is commercially available, the information on the reduced effect on MP changes may be important. Further investigations are necessary to clarify the interaction of lipid supplementation with intestinal lutein uptake. Additionally, further studies are needed to investigate the serum levels of the PUFA. Also, genetic polymorphisms may be another influencing factor worth studying in this context. Furthermore, because of the lack of placebo control there are no data with which to compare the active treatments. Taken together, serum lutein levels and MPOD are increased after supplementary intake of lutein. However, strikingly, serum lutein levels and MPOD were not increased after supplementation of lutein with concentrated fish oil containing DHA and EPA. This suggests that supplementation with specific fatty acids in lipids may largely prevent intestinal lutein uptake and therefore abolish the positive effects of lutein supplementation. This needs to be

taken into account when prescribing lutein supplementation for patients with AMD. Our findings further support the outcome of AREDS2 showing that intake of DHA + EPA has no beneficial effect for treatment of AMD.⁹ Therefore, we see very little reason to add DHA + EPA to the AREDS or other supplement formulations.

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