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Original article

Fluorescence lifetime imaging ophthalmoscopy and the influence of oral lutein/zeaxanthin supplementation on macular pigment (FLOS) – A pilot study

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SUMMARY

Background & aims: Oral lutein (L) and zeaxanthin (Z) supplementation enhances macular pigment optical density (MPOD) and plays a protective role in the development of age-related macular degeneration (AMD). Fluorescence lifetime imaging ophthalmoscopy (FLIO) is a novel in vivo retinal imaging method that has been shown to correlate to classical MPOD measurements and might contribute to a metabolic mapping of the retina in the future. Our aim was to show that oral supplementation of L and Z affects the FLIO signal in a positive way in patients with AMD.

Methods: This was a prospective, single center, open label cohort study. Patients with early and intermediate AMD received oral L and Z supplementation during three months, and were observed for another three months after therapy termination. All visits included measurements of clinical parameters, serum L and Z concentration, MPOD measurements using heterochromatic flicker photometry, dual wavelength autofluorescence imaging, and FLIO. Correlation analysis between FLIO and MPOD were performed.

Results: Twenty-one patients completed the follow up period. Serum L and Z concentrations significantly increased during supplementation (mean difference 244.8 ng/ml; 95% CI: 81.26–419.9, and 77.1 ng/ml; 95% CI: 5.3–52.0, respectively). Mean MPOD units significantly increased (mean difference 0.06; 95% CI: 0.02–0.09; at 0.5°, 202; 95% CI: 58–345; at 2°, 1033; 95% CI: 288–1668; at 9° of eccentricity, respectively) after three months of supplementation with macular xanthophylls, which included L and Z. Median FLIO lifetimes in the foveal center significantly decreased from 277.3 ps (interquartile range 230.2–339.1) to 261.0 ps (interquartile range 231.4–334.4, $p = 0.027$). All parameters returned to near-normal values after termination of the nutritional supplementation. A significant negative correlation was found between FLIO and MPOD ($r^2 = 0.57$, $p < 0.0001$).

Conclusions: FLIO is able to detect subtle changes in MPOD after L and Z supplementation in patients with early and intermediate AMD. Our findings confirm the previous described negative correlation between FLIO and MPOD. Macular xanthophylls seem to contribute to short foveal lifetimes.

This study is registered at ClinicalTrials.gov (identifier number NCT04761341).

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Abbreviations: FLIO, Fluorescence Lifetime Imaging Ophthalmoscopy; MP, Macular Pigment; AMD, age-related macular degeneration; MPOD, macular pigment optical density; L, Lutein; Z, Zeaxanthin.

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1. Introduction

Macular pigment (MP) consists mainly of two carotenoids, the xanthophylls lutein (L) and zeaxanthin (Z), as well as meso-zeaxanthin and the oxidative metabolites which selectively accumulate in the foveal region of the retina [1]. Carotenoids cannot be synthesized de novo by mammals and thus have to be obtained

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solely through dietary sources [2]. It has been speculated that MP has an ocular protective function through its ability to filter phototoxic blue light radiation as well as via its antioxidant activity [3]. The antioxidant properties have led to the hypothesis that carotenoids may have a protective role against the development and progression of age-related macular degeneration (AMD), the most common cause of blindness in the elderly worldwide [4,5]. This theory has been further reinforced by epidemiological studies demonstrating that patients with lower serum carotenoid concentrations and macular pigment optical density (MPOD) measurements are associated with a higher risk of developing AMD [6]. On the other hand, previous studies have shown that nutritional supplementation and diets rich in carotenoids reduces the risk of progression to advanced AMD by influencing MP concentrations [7]. The Age-Related Eye Disease Study (AREDS) 2 supplement formulation, consisting of 10 mg of L and 2 mg of Z among several other micronutrients, has been recommended as a standard of care for individuals at risk of visual loss from intermediate AMD [8]. However, dietary habits can also influence plasma levels of L and Z [9].

The MP can be measured using several techniques including heterochromatic flicker photometry [10,11], fundus autofluorescence [12,13], and fundus reflectance [14]. MP can also be measured using Fluorescence lifetime imaging ophthalmoscopy (FLIO), as a strong correlation was identified between fluorescence lifetimes and MPOD levels [15].

FLIO is an emerging imaging technology, which enables in vivo measurements of emitted fluorescence of endogenous retinal fluorophores. FLIO has the potential to detect and evaluate variable metabolic and nutritional changes within the retina, as the fluorescence decay times are specific for fluorescent particles and their cellular and biochemical environment [16]. FLIO measurement in patients with AMD showed that the mean autofluorescence lifetimes of the retina in AMD was longer compared to age matched healthy control eyes [17].

The purpose of this study is to investigate the effects of L and Z supplementation on MPOD using FLIO and MPOD measurements in patients with AMD over a period of 6 months.

2. Materials & methods

The study was conducted at the University Hospital in Bern, Switzerland, in accordance with the Declaration of Helsinki ethical guidelines and local ethics committee approval was obtained. All patients provided written informed consent before study entry.

2.1. Subjects

Patients (eyes) of at least 50 years of age with the clinical diagnosis of nonexudative, early or intermediate AMD were included. The diagnosis was based on the presence of soft and/or reticular drusen, meeting the AMD level 2 or 3 according to the AREDS classification [18]. Ocular exclusion criteria included opacities of ocular media hindering detailed observation of the retina, such as severe cataract or corneal opacities, advanced stage AMD with choroidal neovascularization or geographic atrophy, late stage or uncontrolled glaucoma, any severe diseases of the vitreoretinal interface that would interfere with MP measurements, history of penetrating ocular trauma, and history of vitreoretinal surgery. Non-ocular exclusion criteria included the history of L and Z supplementation at any time, intake of other supplementation that could interfere with L and Z uptake, pathologies of the gastrointestinal system that would interfere with L and Z uptake, allergies against any substances used in this study, and bad compliance with the study protocol such as missing visits or non-adherence with study drug intake. Adherence was monitored by asking how many tablets were left in the blisters at each visit. Data of the medical history as well as macronutrient and micronutrient intake was collected with a questionnaire.

2.2. Intervention

After a baseline examination, we provided a nutritional supplement including a.o. 10 mg L and 2 mg Z, (Nutrof Total®, Théa PHARMA S.A., Schaffhausen, Switzerland), to all participants. The supplement was taken orally once per day over a course of 3 months. Patients were followed up for an additional 3 months after discontinuation of therapy.

2.3. Examination

All subjects underwent a monthly general ophthalmic evaluation including best-corrected visual acuity (BCVA), with the Early Treatment Diabetic Retinopathy Study (ETDRS) charts, visual contrast sensitivity using the Pelli Robson charts, slit lamp examination, non-contact tonometry, and fundoscopy. Optical coherence tomography (OCT) scans of the macula (Heidelberg Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) and color fundus photography (FF 450 plus; Carl Zeiss) were obtained. MPOD using dual wavelength retinal autofluorescence imaging (AFI) (mpHRA; Heidelberg Engineering, Heidelberg, Germany), heterochromatic flicker photometry (HFP) using the MPS II (Electron eye Technology, Cambridge UK), and FLIO images were obtained. Serum L and Z levels were measured at every visit.

2.4. Outcomes

MPOD (including HFP and AFI) and FLIO parameters were defined as the primary outcomes of this study. Serum L and Z concentration, BCVA, and contrast sensitivity were considered secondary outcomes.

2.5. Safety outcomes

Adverse events from L/Z substitutions treatment were monitored monthly. Patients were given a contact number to address at any time in any case of adverse events between these monthly visits.

2.6. Fluorescence lifetime imaging ophthalmoscope and imaging analysis

FLIO was used to obtain retinal fluorescence lifetime data, based on the Heidelberg Engineering Spectralis system. The principles and safety behind FLIO have been described previously [17,19].

Using a 473 nm pulsed diode laser at an 80 MHz repetition rate, retinal autofluorescence is excited within the central fundus. The emitted fluorescence photons are detected by two sensitive hybrid photon-counting detectors (HPM-100-40; Becker & Hickl, Berlin, Germany). Released fluorescence photons are detected in correspondence to their wavelength in two distinct spectral channels: a short spectral channel (SSC: 498–560 nm), and a long spectral channel (LSC: 560–720 nm). At least 1000 photons were registered per pixel for each channel. A high contrast confocal infrared image enabled each photon to be identified in its specific location within a 256 × 256 pixel frame.

Using SPCImage software version 4.6 (Becker & Hickl) fluorescence lifetime data was analyzed and a mean fluorescence decay curve was bi-exponentially approximated in each wavelength channel. The amplitude weighted mean fluorescence lifetime (tm)

was calculated utilizing the short and long lifetime components (T1 and T2) and their corresponding amplitudes α1 and α2. Using the "FLIO reader" software (ARTORG Center for Biomedical Engineering Research, University of Bern, Switzerland), the mean fluorescence lifetimes were further analyzed. To compute retinal autofluorescence lifetimes a standardized ETDRS grid with the following subfield diameters was used: 1 mm for the central area (C), 3 mm for the inner ring (IR), and 6 mm for the outer ring (OR).

2.7. Dual wavelength autofluorescence (AFI)

The MP was quantified in terms of MPOD and macular pigment optical volume (MPOV) [13]. Autofluorescence images of the macula were acquired with the modified confocal scanning laser ophthalmoscope (mpHRA; Heidelberg Engineering, Heidelberg, Germany). The principles of this imaging technique are based on Delori et al. [12] and the assessment has been previously described in detail. In summary, autofluorescence images of the macula at two distinct wavelengths (488 and 514 nm) were subtracted, and the resulting image was used as MPOD map. The central MPOD was obtained by measuring the optic density at 0.5°, 2° and 9° diameter subfield centered on the fovea. Quantitative data analysis were conducted using an inbuilt software provided by the manufacturer of the scanning laser ophthalmoscope and were presented as unitless density units.

2.8. Heterochromatic flicker photometry (MPS II)

The MP was quantified using the heterochromatic flicker photometry MPS II (Electron eye Technology, Cambridge UK) [10,11]. A characteristic absorption spectrum is found within the macula. The central stimulus had a one degree angular subtense and using an optical system, the objective viewing distance was fixed to infinity. The central stimulus is produced from white, green, and blue LEDs on a background luminance of 250 cd/m^2. The MPS II exhibits two light stimuli of distinctive wavelengths (blue 465 nm and green 530 nm). The light stimulus interchanges between a short wavelength of blue light, observed as a flicker, which is absorbed by the MP, while green light is not absorbed.

Patients were instructed to fixate at the central target and when observing a flicker, press the button. First, the threshold was determined by presenting five targets, followed by alterations of the radiance of blue versus the radiance of green until a constant target was perceived as having no flicker. This was marked as the lowest point on the graph. The MPOD measured was adjusted to account for the normal age-related yellowing of the lens and a final MPOD was reported in density units (du). The testing time for each eye was approximately 2 min.

2.9. Measurement of serum L and Z levels

Venous blood samples were taken at every visit. High performance liquid chromatography (HPLC) was utilized to quantify the combined serum concentration of L and Z. The principles of HPLC have been described elsewhere [20]. Analysis was performed at DSM Nutritional Products Ltd. R&D Human Nutrition and Care, Kaiseraugst, Switzerland.

2.10. Statistical analysis

The Shapiro–Wilk test was used to differentiate parametric from nonparametric data. The one-way analysis of covariance ANOVA test was used to compare normally distributed repeated clinical and imaging data and data was reported as mean ± standard deviation (SD), with mean difference (MD) and 95% confidence interval (CI). For nonparametric data, Friedman test with multiple comparisons was used, and data was reported as median and interquartile range (IQR). Pearson correlations of MPOD and FLIO were analyzed. P values < 0.05 were considered statistically significant. Descriptive statistics were provided additionally for all data.

3. Results

3.1. Baseline characteristics

Twenty-eight participants were recruited for the study (10 male, 18 female; 28 AMD). Of these, 7 were lost to follow up (2 did not come to baseline visit, 2 withdrew informed consent, 1 left the country, 1 converted to advanced AMD, 1 interrupted due to general health condition). Twenty-one participants completed the study and remained for analysis. The mean (±SD) age of the subjects was 73.5 (±9.9) years, ranging from 50 to 87 years. The food questionnaire showed that 13 patients had regular vitamin and/or mineral supplements intake, of these were: Calcium ($n = 5$), Magnesium ($n = 4$), Vitamin D ($n = 4$), Vitamin B ($n = 2$), and Vitamin C ($n = 2$). Overall macronutrient intake was evenly distributed over all participants: All patients reported at least 4–6 times per week consumption of green vegetables and fruits, as well as egg intake at least once weekly. Two patients did not eat any fish or meat at the time of the study. 12 patients used many different oil for cooking, while 7 used olive oil and 2 used rape oil only. No significant relationships between vitamin-, micro- and macronutrient intake with the L and Z concentration or other characteristics were found, and no tendency could be determined due to the small sample size. No interference between the aforementioned vitamin intake and the L and Z levels was expected since relevant substances were excluded. Other demographics and other baseline characteristics are presented in Table 1.

3.2. Effect of L and Z supplementation

Both, L and Z serum concentrations showed a significant increase in serum values (graphs show mean and 95% CI) at all three months of substitution, as illustrated in Fig. 1.

The mean (SD) L and Z levels increased significantly from 185.5 (76.7) ng/ml to 430.3 (265.0) ng/ml (MD 244.8 ng/ml; 95% CI: 81.26–419.9), and from 49.6 (18.5) ng/ml to 77.1 (37.4) ng/ml (MD 27.5 ng/ml; 95% CI: 5.3–52.0), respectively, as quantified in detail in Table 2.

3.3. Fluorescence lifetime and MPOD measurements

A significant negative correlation was found between FLIO and MPOD ($r2 = 0.57$, $p < 0.0001$). Median FLIO lifetimes showed a significant decrease from 277.3 ps (IQR 230.2–339.1) to 261.0 ps (IQR 231.4–334.4), after 3 months of L and Z supplementation in the SSC (rank sum difference 16, $p = 0.027$). No significant difference was detected in the LSC, with a decrease from 367.3 ps (IQR 314.0–398.6) to 366.5 ps (IQR 308.7–405.0), (rank sum difference 6, $p = 0.65$). An illustration of the lifetime measurements is presented in Fig. 2.

Significant shorter lifetimes were observed in the SSC after three months ($p < 0.05$, Friedman's multiple comparisons test). Changes in the LSC were not significant.

MPOD and MPOV determined by AFI revealed a significant increase in MP after 3 months of L and Z supplementation. Mean (SD) MPOD at 0.5° increased from 0.57 (0.25) to 0.62 (0.27) units (MD 0.06; 95% CI: 0.02–0.09). Mean (SD) MPOV increased from 307 (133) to 334 (135) units (MD 26.5; 95% CI: 4.1–48.9) at 5°, from

Table 1
Demographics of the study participants. Values are expressed in mean ± standard deviation (SD) or total numbers and percentage. (BMI: Body mass index).

Parameters	Mean ± SD
age [years]	73.5 ± 9.9
weight [kg]	79.4 ± 14.5
height [cm]	167.8 ± 9.0
BMI [kg/m^2]	28.3 ± 5.5
	Total n = 21 (%)
sex	
female	7 (33%)
smoker	
active	2 (10%)
former	8 (38%)
never	11 (52%)
lens	
phakic	12 (57%)
pseudophakic	9 (43%)

2309 (1144) to 2510 (1145) units (MD 202; 95% CI: 58–345) at 2°, and from 6422 (3733) to 7289 (3412) units (MD 1033; 95% CI: 288–1668) at 9°, as presented in Table 2 and Fig. 3. However, HFP/MPS II was not able to detect significant changes.

3.4. BCVA and clinical parameters

Mean (SD) BCVA was stable at 81.4 (6.9), 82.81 (7.0), and 81.3 (7.2) ETDRS letters at 1, 3, and 6 months, respectively. There was no significant increase or decrease at 3 and 6 months. Median (IQR) Pelli-Robson contrast sensitivity charts showed a slight increase after 3 months, however this was not statistically significant with 1.50 (1.35–1.65) to 1.50 (1.35–1.50), p = 0.09).

3.5. Safety outcomes

One patient was diagnosed with oesophageal carcinoma during follow up. The diagnosis was considered as clearly not related to the study intervention.

4. Discussion

MP is composed of the carotenoids L and Z, which accumulate in the macula and contribute to normal visual function. Oxidative stress, as in cigarette smoking, has been established a relevant modifiable risk factor in the pathophysiology of AMD, as reports of the AREDS study showed [21]. MP is thought to play a protective role in the delay or prevention of AMD by reducing oxidative stress factors, anti-inflammatory, and filtering blue light, while progressive disease is associated with low MP values [22]. Epidemiological studies that assessed the carotenoid levels have demonstrated an inverse correlation between the risk of advanced AMD and serum levels of L and Z and high dietary intakes [23,24]. L supplementation of 10 mg daily showed a significant increase of MPOD in a randomized trial with 112 patients with early AMD [25]. Also, a recent systematic review from 46 trials concluded that oral L and Z dosages over 5 mg/d for ≥3 months can increase MPOD

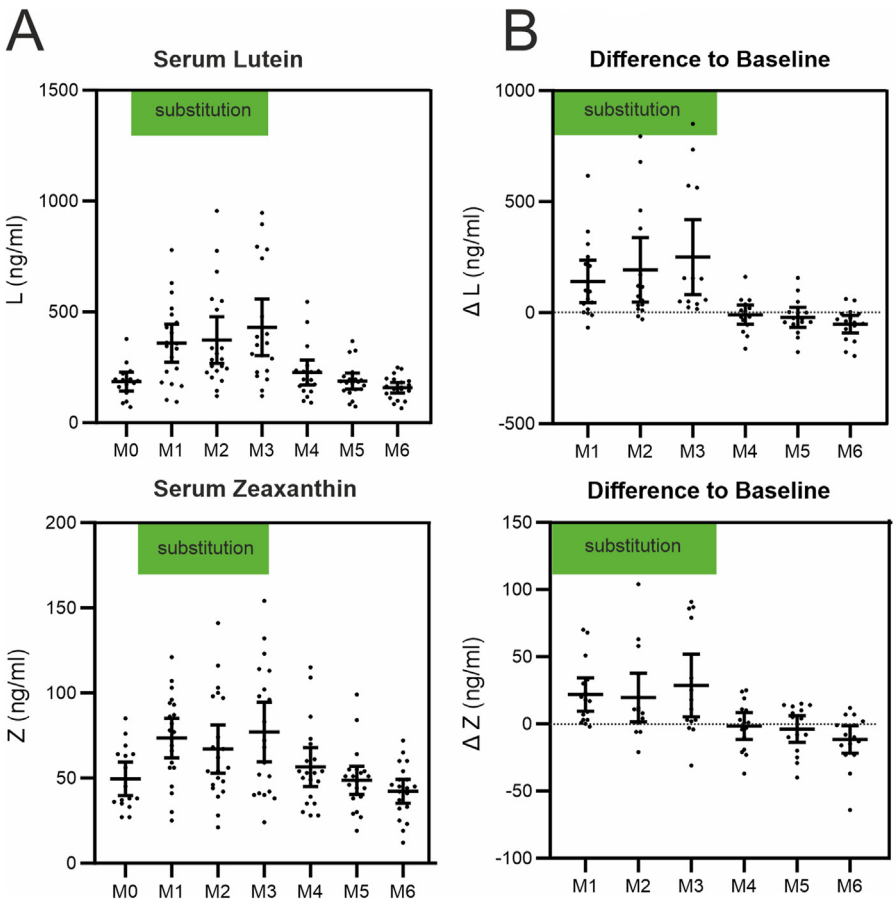

Fig. 1. A) Serum level of lutein (L) and zeaxanthin (Z) was administered from baseline (M0) for three months (M1, M2 and M3) in the unit of ng/ml. B) Difference from baseline (delta Z) in monthly follow up (M1-M6) (Graphs show mean and 95% CI).

Table 2
Clinical and imaging data over time. Macular pigment optical density values are without units (†) and at 0.5, 2, 9° (°) of eccentricity. Nonparametric data is indicated with an asterisk (*). Abbreviations: Fluorescence lifetime imaging ophthalmoscopy (FLIO), Short spectral channel (SSC), macular pigment optical density (MPOD), macular pigment optical volume (MPOV), dual wavelength autofluorescence imaging (AFI), macular pigment screener II (MPSII), heterochromatic flicker photometry (HFP), best corrected visual acuity (BCVA, in ETDRS letters), standard deviation (SD), interquartile range (IQR).

	Baseline	3rd month			6th month		
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	Mean difference (95% CI), or rank sum difference	P	Mean ± SD or Median (IQR)	Mean difference (95% CI), or rank sum difference	P
Lutein [ng/ml]	185.5 ± 76.7	430.3 ± 265.0	244.8 (81.26–419.9)	0.01	158.3 ± 50.8	−27 (−38.06–6.1)	0.19
Zeaxanthin [ng/ml]	49.6 ± 18.5	77.1 ± 37.4	27.5 (5.3–52.0)	0.02	42.3 ± 15.3	−7.3 (−21.6–1.3)	0.07
FLIO, SSC [ps]*	277.3 (230.2–339.1)	261.0 (231.4–334.4)	16	0.027	277.8 (247.4–350.1)	−4.0	>0.99
FLIO, LSC [ps]*	367.3 (314.0–398.6)	366.5 (308.7–405.0)	6	0.65	367.3 (307.2–400.8)	3.0	0.65
MPOD 0.5°, AFI†	0.57 ± 0.25	0.62 ± 0.27	0.06 (0.02–0.09)	0.0075	0.60 ± 0.27	0.03 (0.0–0.1)	0.06
MPOV 0.5°, AFI†	307 ± 133	334 ± 135	26.5 (4.1–48.9)	0.04	331 ± 144	23.3 (3.7–42.9)	0.04
MPOV 2°, AFI†	2309 ± 1144	2510 ± 1145	202 (58–345)	0.02	2483 ± 1201	175 (-113–462)	0.2
MPOV 9°, AFI†	6422 ± 3733	7289 ± 3412	1033 (288–1668)	0.02	7179 ± 3510	757 (21–1413)	0.04
MPS II, HFP†	0.44 ± 0.28	0.47 ± 0.28	0.034 (−0.11–0.18)	0.8	0.51 ± 0.27	0.073 (−0.06–0.18)	0.35
BCVA [ETDRS letters]	81.38 ± 6.9	82.81 ± 7.0	1.43 (−3.9–1.1)	0.3	81.29 ± 7.2	0.01 (−2.4–2.6)	0.9
Pelli-Robson†*	1.50 (1.35–1.50)	1.50 (1.35–1.65)	13	0.09	1.50 (1.35–1.65)	3.50	>0.99

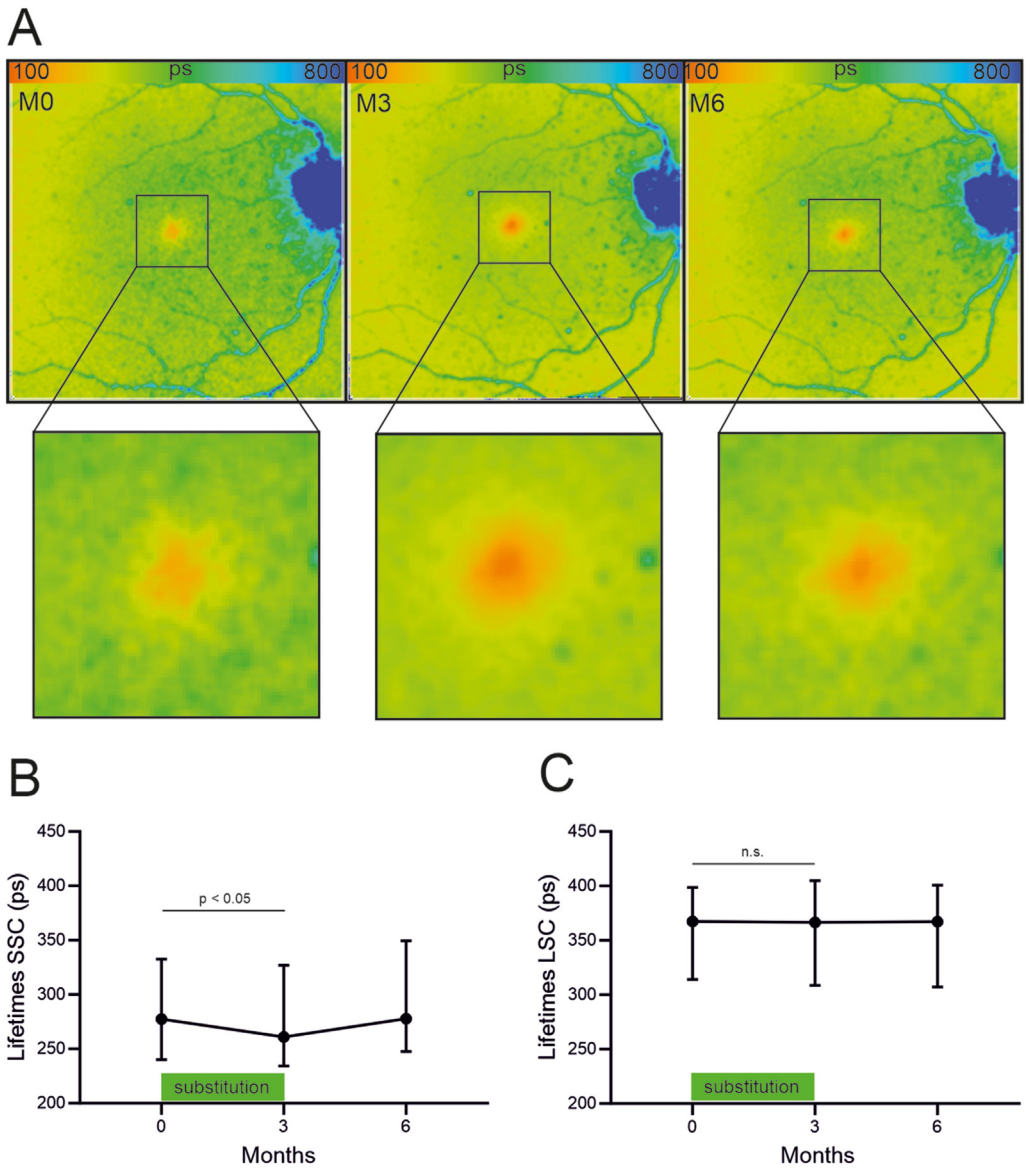

Fig. 2. The FLIO images show the macular and foveal (enlarged images) lifetimes of a right eye at month 0, 3, and 6 (M0, M3, M6), respectively. The graphs illustrate the lifetimes of the short spectral channel (B, SSC, left graph), and long spectral channel (C, LSC, right graph) at baseline, 3 and 6 months, values are presented as the median ± interquartile range.

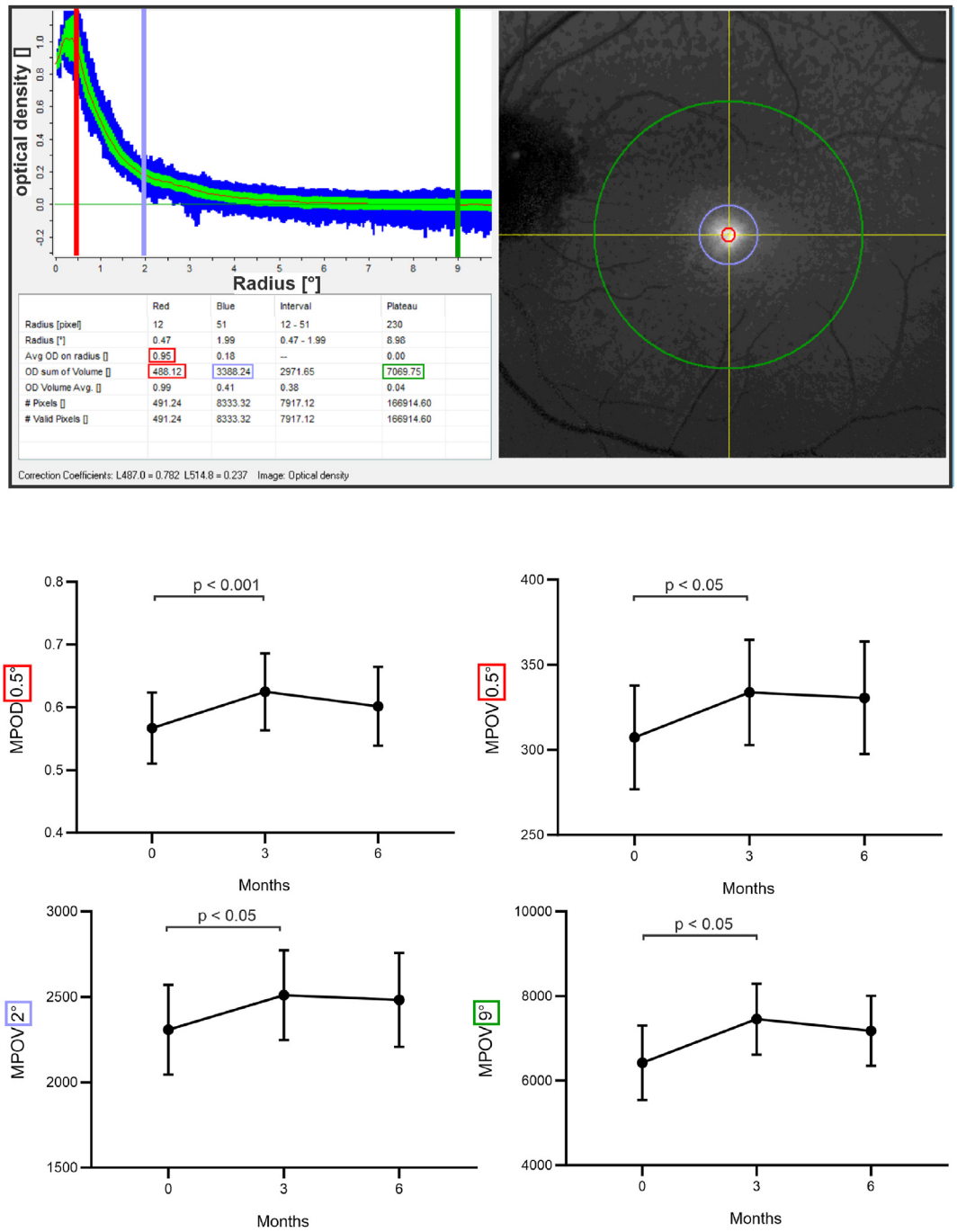

Fig. 3. Macular pigment optical density and optical volume (MPOD, MPOV) at their corresponding eccentricities at months 0, 3, 6, respectively. MP values are without units.

concentrations significantly [26]. However, the pathophysiology leading to AMD is not fully elucidated, with genetics playing an increasingly important role. Advances in multimodal imaging have provided further insight in the pathophysiology of AMD.

To date, there has been no clear gold standard technique to measure MPOD [13]. The subjective psychophysical heterochromatic flicker photometry test is the most common non-invasive method for measuring human MP levels, involving a color intensity matching of a light beam directed at the foveal and parafoveal area. However, this technique requires a cooperative, alert subject with a decent visual acuity and is rather time consuming. Furthermore, in the presence of a significant macular pathology or in the presence of low MP densities, it exhibits a high intrasubject variability. Therefore, in the population group at risk for AMD, the usefulness of this technique for evaluating MP levels may be limited. In contrast, AFI evades limitations emerging subjective testing. By illuminating the macula using autofluorescence technique and image subtraction, a more detailed and objective illustration and quantification of MP is possible [12,27]. However, MPOD has been described at various eccentricities, which might cause difficulties to compare MP among studies. MPOV as an integral over all MPOD values over a certain eccentricity has therefore been introduced as a robust and maybe more precise endpoint parameter [13]. FLIO on the other hand is a imaging technique with the ability to identify subtle alterations and pathophysiological processes within the retina, providing

supplementary data in comparison to other retinal imaging techniques [15,19,28–31].

The current study was designed to investigate the effects of L and Z supplementation on MP in patients with age-related macular degeneration, using these different imaging methods.

Our findings confirm that the supplementation of L and Z leads to significantly higher serum levels of L and Z, as well as higher MPOD and MPOV. This study also demonstrates that FLIO is able to detect changes in MPOD and MPOV after L and Z supplementation. Our findings are consistent with prior studies in which we found significantly prolonged lifetimes within the macular region in patients with AMD, even in the early stages of this disease, compared to healthy subjects [28]. The prolongation of retinal fluorescence lifetimes has been seen in previous measurements of progressive age as well as other retinal pathologies, which can be interpreted by a progressive remodeling of the retina and buildup of lipofuscin [28,32]. Raised lipofuscin levels confined in the retinal pigment epithelium may precede or co-occur at early stages of pathology in AMD. In support of this idea, previous studies have reported on a significantly reduced formation of lipofuscin when the antioxidant substances L and Z had been supplemented. Their protective photo-oxidative and chain-breaking properties could be of importance for the prevention of the accumulation of lipofuscin. This may be a relevant feature in the pathogenesis of AMD, suggesting the prospect that this common cause of impaired vision could be prevented or delayed by the supplementation of antioxidants, and that the cellular microenvironment might be monitored using the FLIO technique.

Although FLIO measurements can identify fluorescence lifetime data from retinal fluorophores, as well as their interface with the embedding matrix, controversy over the source of the computed lifetime signal still exists, requiring additional explanation on the participating fluorophores. To date, melanin, lipofuscin, collagen, elastin, flavin adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide (NADH) have been proposed to contribute towards the detected fluorescence lifetime signal [17].

4.1. Limitation

The current study has a relatively small number of participants; however, this was conducted as a pilot study to preliminarily examine the effect of L and Z supplementation on FLIO and MP. Another limitation is, that the effects of L and Z on changes in fluorescence lifetimes and MPOD were not identified separately. To evaluate the effects of each component, additional studies using L and Z supplements alone and a combined L and Z supplement would be desirable. Furthermore, seven subjects were lost to follow-up during the 6-month study period. Also, it should be acknowledged that the fluorescence of the crystalline lens may impact the fluorescence lifetime dynamics of the retina [33].

5. Conclusion

FLIO can detect metabolic alterations within the macula, following supplementation with L and Z in patients with early and intermediate AMD over time. A key finding was a shortening of foveal lifetime patterns identified in AMD eyes during the substitution period. We further confirmed an inverse correlation between FLIO and MPOD measurements demonstrated in an earlier study [15].

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Author contributions

Damian Jaggi: Conceptualization, Investigation, Writing – original draft.

Yasmin Solberg: Conceptualization, Investigation, Writing – original draft.

Joel Lincke: Data curation, Formal analysis, Writing – review & editing.

Oussama Habra: Formal analysis, Writing – review & editing.

Adrian Wyss: Investigation, Data curation.

Sebastian Wolf: Project administration, Supervision.

Martin S. Zinkernagel: Conceptualization, Project administration, Supervision.

Declaration of competing interest

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S. Wolf: Roche, Chengdu Kanghong Biotechnology, Zeiss, Heidelberg Engineering (all consulting and speakers honoraria, not related to the here presented work), Bayer, Novartis (both Reading Center Fees, not related to the current work).

References

[1] Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. Br J Ophthalmol 1999;83(7):867–77.
[2] Wu J, Cho E, Willett WC, Sastry SM, Schaumberg DA. Intakes of lutein, zeaxanthin, and other carotenoids and age-related macular degeneration during 2 decades of prospective follow-up. JAMA Ophthalmol. 2015;133(12):1415–24.
[3] Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res 1997;65(1):57–62.
[4] Dagnelie G, Zorge IS, McDonald TM. Lutein improves visual function in some patients with retinal degeneration: a pilot study via the Internet. Optometry 2000;71(3):147–64.
[5] Hammond Jr BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, et al. Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 1997;38(9):1795–801.
[6] Ma L, Yan SF, Huang YM, Lu XR, Qian F, Pang HL, et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. Ophthalmology 2012;119(11):2290–7.
[7] Weigert G, Kaya S, Pemp B, Sacu S, Lasta M, Werkmeister RM, et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. Invest Ophthalmol Vis Sci 2011;52(11):8174–8.
[8] Age-Related Eye Disease Study 2 Research G, Chew EY, Clemons TE, Sangiovanni JP, Danis RP, Ferris 3rd FL, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. JAMA Ophthalmol 2014;132(2):142–9.
[9] Handelman GJ, Nightingale ZD, Lichtenstein AH, Schaefer EJ, Blumberg JB. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. Am J Clin Nutr 1999;70(2):247–51.
[10] Bone RA, Landrum JT. Heterochromatic flicker photometry. Arch Biochem Biophys 2004;430(2):137–42.
[11] Wooten BR, Hammond Jr BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. Invest Ophthalmol Vis Sci 1999;40(11):2481–9.
[12] Delori FC. Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. Arch Biochem Biophys 2004;430(2):156–62.

[13] Green-Gomez M, Bernstein PS, Curcio CA, Moran R, Roche W, Nolan JM. Standardizing the assessment of macular pigment using a dual-wavelength autofluorescence technique. Transl Vis Sci Technol 2019;8(6):41.
[14] Berendschot TT, van Norren D. Objective determination of the macular pigment optical density using fundus reflectance spectroscopy. Arch Biochem Biophys 2004;430(2):149–55.
[15] Sauer L, Andersen KM, Li B, Gensure RH, Hammer M, Bernstein PS. Fluorescence lifetime imaging ophthalmoscopy (FLIO) of macular pigment. Invest Ophthalmol Vis Sci 2018;59(7):3094–103.
[16] Schweitzer D, Kolb A, Hammer M, Anders R. [Time-correlated measurement of autofluorescence. A method to detect metabolic changes in the fundus]. Ophthalmologe: Zeitschrift der Deutschen Ophthalmologischen Gesellschaft 2002;99(10):774–9.
[17] Dysli C, Wolf S, Berezin MY, Sauer L, Hammer M, Zinkernagel MS. Fluorescence lifetime imaging ophthalmoscopy. Prog Retin Eye Res 2017;60:120–43.
[18] The age-related eye disease study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the age-related eye disease study report number 6. Am J Ophthalmol 2001;132(5):668–81.
[19] Dysli C, Quellec G, Abegg M, Menke MN, Wolf-Schnurrbusch U, Kowal J, et al. Quantitative analysis of fluorescence lifetime measurements of the macula using the fluorescence lifetime imaging ophthalmoscope in healthy subjects. Invest Ophthalmol Vis Sci 2014;55(4):2106–13.
[20] Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. Invest Ophthalmol Vis Sci 2013;54(3):1781–8.
[21] Clemons TE, Milton RC, Klein R, Seddon JM, Ferris 3rd FL. Risk factors for the incidence of advanced age-related macular degeneration in the age-related eye disease study (AREDS) AREDS report no. 19. Ophthalmology 2005;112(4):533–9.
[22] Kijlstra A, Tian Y, Kelly ER, Berendschot TT. Lutein: more than just a filter for blue light. Prog Retin Eye Res 2012;31(4):303–15.
[23] Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. Invest Ophthalmol Vis Sci 2001;42(1):235–40.
[24] Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. Invest Ophthalmol Vis Sci 2003;44(6):2461–5.
[25] Huang YM, Dou HL, Huang FF, Xu XR, Zou ZY, Lin XM. Effect of supplemental lutein and zeaxanthin on serum, macular pigmentation, and visual performance in patients with early age-related macular degeneration. BioMed Res Int 2015;2015:564738.
[26] Wilson LM, Tharmarajah S, Jia Y, Semba RD, Schaumberg DA, Robinson KA. The effect of lutein/zeaxanthin intake on human macular pigment optical density: a systematic review and meta-analysis. Adv Nutr 2021;12(6):2244–54.
[27] Johnson EJ, Avendano EE, Mohn ES, Raman G. The association between macular pigment optical density and visual function outcomes: a systematic review and meta-analysis. Eye (London, England) 2021;35(6):1620–8.
[28] Dysli C, Fink R, Wolf S, Zinkernagel MS. Fluorescence lifetimes of drusen in age-related macular degeneration. Invest Ophthalmol Vis Sci 2017;58(11):4856–62.
[29] Jaggi D, Solberg Y, Dysli C, Ebneter A, Wolf S, Zinkernagel MS. Fluorescence lifetime imaging ophthalmoscopy: findings after surgical reattachment of macula-off rhegmatogenous retinal detachment. Retina 2020;40(10):1929–37.
[30] Lincke JB, Dysli C, Jaggi D, Solberg Y, Wolf S, Zinkernagel MS. Longitudinal foveal fluorescence lifetime characteristics in geographic atrophy using fluorescence lifetime imaging ophthalmoscopy (FLIO). Retina 2021;41(11):2391–8.
[31] Sauer L, Schweitzer D, Ramm L, Augsten R, Hammer M, Peters S. Impact of macular pigment on fundus autofluorescence lifetimes. Invest Ophthalmol Vis Sci 2015;56(8):4668–79.
[32] Solberg Y, Dysli C, Wolf S, Zinkernagel MS. Fluorescence lifetime patterns in macular Telangiectasia Type 2. Retina 2020;40(1):99–108.
[33] Dysli C, Dysli M, Lincke J, Jaggi D, Wolf S, Zinkernagel MS. Imaging artifacts in fluorescence lifetime imaging ophthalmoscopy (FLIO). Retina 2021;41(11):2378–90.