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Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin – the LUXEA (LUtein Xanthophyll Eye Accumulation) study

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Abstract

The xanthophylls lutein (L) and zeaxanthin (Z) form the macular pigment with the highest density in the *macula lutea*. We investigated Macular Pigment Optical Density (MPOD) responses to supplementation with identically formulated (Actilease™) L or Z (OPTISHARP™) or L + Z over 6–12 months using doses of 10 or 20 mg/day. MPOD as well as blue light sensitivity in fovea and parafovea were measured monthly by heterochromatic flicker photometry. Average xanthophyll plasma concentrations, analysed monthly by HPLC, increased up to 27-fold. MPOD increased by 15% upon L or L + Z supplementation. Supplementation of Z alone produced similar pigment accumulation in fovea *and* parafovea, which confounded MPOD measurements. After correction for this, a 14% MPOD increase resulted for Z. Thus, during supplementation with xanthophylls, L is predominantly deposited in the fovea while Z deposition appears to cover a wider retinal area. This may be relevant to health and disease of the retina.

Keywords: Lutein; Zeaxanthin; Carotenoids; Supplementation; Heterochromatic Flicker Photometry; AMD; Macular pigment; Fovea; Parafovea; OPTISHARP™

The natural xanthophylls lutein (L)[1] and zeaxanthin (Z) are the main constituents of the yellow pigment that is deposited throughout the human retina and forms a visible yellow spot (*macula lutea*) centred on the fovea. The foveal location of the xanthophylls, their blue light absorption characteristics and their anti-oxidant properties have given rise to the hypothesis that they may offer optical and/or metabolic protection against blue light and reactive oxygen species and that intake of L and Z may contribute to risk reduction of retinal diseases such as age-related macular degeneration (AMD) [1].

Usually, L and Z are ingested in dark green vegetables or yellow to orange fruits. After intestinal absorption and subsequent transport in plasma within lipoproteins, a fraction of the xanthophylls is transferred to the retina. There, in the *macula lutea*, the xanthophylls are accumulated to the highest concentration found anywhere in the human body [2]. Numerous studies have examined whether MPOD can be

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[1] *Abbreviations used:* L, lutein; Z, zeaxanthin; MPOD, Macular Pigment Optical Density; HFP, heterochromatic flicker photometry; MP, macular pigment; GEE, Generalized estimating equations; AMD, Age-related macular degeneration.

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augmented by supplementation. Most of these have investigated the response to supplementation with L. Macular pigment optical density (MPOD) measurements in these studies have yielded a wide range of results. Pigment increases of around 40% [3–5] and more [6] were reported, but also modest changes of 15–23% [7–10], smaller [11] or no responses [12,13]. Supplementation with Z has received less attention and there is a paucity of published data. In one of the studies, Bone et al. [14] supplemented 30 mg/day of pure Z extracted from Flavobacteria for 4 months and reported statistically significant MPOD increases of about 10%.

A direct comparison of the effects of L and Z on MPOD by dietary studies is hampered by factors such as the excess L content in most fruits and vegetables, thereby complicating equivalent dosing. Furthermore, the bioavailability of the xanthophylls depends on their matrix embedment and possible ester linkage at xanthophyll hydroxyl groups. In the present study, such difficulties were overcome by administering comparable doses of non-esterified xanthophylls incorporated within the same formulation. We investigated the retinal accumulation of xanthophylls in a multiple dosing study by following MPOD responses to daily supplementation of identically formulated Z and L, administered to healthy volunteers either individually or in combination. Plasma concentrations of xanthophylls were measured and MPOD was monitored monthly by heterochromatic flicker photometry (HFP). The investigation was designed as a prospective, single-centred, randomized, double-blind, placebo-controlled, pilot supplementation study with chemically synthesised zeaxanthin and natural lutein.

Methods

Subjects

Throughout the entire study the tenets of the Declaration of Helsinki were followed. The research was approved by the institutional review board. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study and its extension.

One hundred and twenty-six male volunteer subjects gave informed consent to be considered for recruitment to the study. Recruitment criteria included normal health and Caucasian race. Ethnicity was limited to Caucasians to decrease potential variability related to ethnic differences in macular pigmentation, which have been reported anecdotally. Ophthalmic exclusion criteria included inability to obtain measurements via HFP, current night blindness, an abnormal retina or turbid ocular media. Only males were considered in order to exclude variability induced by the monthly changes of hormone levels [15,16]. Subjects were aged 18–45 years with a body mass index (BMI) of between 18 and 28 kg/m^2. Volunteers agreed to refrain from taking carotenoid supplements other than those supplied during the study. No further dietary restrictions were imposed except that subjects on vegetarian or vegan diets were excluded from participation. Ninety-two subjects fulfilled the entry criteria and 23 subjects each were randomized to the following four supplementation groups (Table 1): L, Z, C (L + Z) and P (placebo). Subjects visited the study site monthly, where procedures included distribution and collection of supplements, collection of blood specimens and the measurement of MPOD.

Study supplements

Study supplements were provided in hard shell gelatine capsules containing identically formulated Actilease™ beadlets (DSM Nutritional Products Ltd.) of either synthetic zeaxanthin (OPTISHARP™) or non-esterified lutein extracted from marigold (*tagetes erecta*), both, or placebo. The synthetic zeaxanthin was lutein-free, while the lutein from marigold contained about 7.5% zeaxanthin. The analysis of the capsules by HPLC resulted in xanthophyll doses administered as shown in Table 1. The Z beadlets contained 81% all-*trans* zeaxanthin and 19% *cis* isomers with 13-*cis* being the major *cis* isomer. The L beadlets contained 92% all-*trans* lutein and 8% *cis* isomers. Subjects had to take their assigned capsules together with breakfast on a daily basis and were instructed not to fundamentally change their breakfast and general dietary habits during the course of the study.

Study extension

After 6 months, when the study had been expected to conclude, two new psychophysical tests became available: one to measure visual performance and another to determine MPOD over a wider (±8°) eccentricity than in the employed HFP technique. In order to evaluate the effect of xanthophyll supplementation on these parameters, the study was extended for 6 months. The results of this study, conducted in a subgroup of the LUXEA subjects, have been recently published [17,18]. Presentation and discussion of results in the present manuscript, however, will only deal with results obtained using the original HFP technique.

Twenty subjects from the original study joined the extension, and 10 additional subjects were recruited as a new placebo group. All subjects gave informed consent for participation in the extension. After discontinuation of supplementation for 2–4 weeks, the supplementation regimes were started as shown in Table 1. Together with the original study, this created two groups supplemented with xanthophylls: (a) subjects supplemented

Table 1
Design of study: subjects, doses and abbreviation of supplementation groups

Supplementation phase	Supplementation groups				
	Lutein	Zeaxanthin	Combination	Placebo	
1st six months	L[a]	Z[a]	C[a]	P[a]	
Daily dose of L, mg	10.7	0	10.2	0	
Daily dose of Z, mg	0.8	12.6	11.9	0	
Subjects at beginning (V0)	23	23	23	23	
Subjects at end (V6)	18	16	19	20	
	Dose doubled	Dose doubled	Dose unchanged	Switched to C	New P group
2nd six months[b]	LL[a]	ZZ[a]	CC[a]	PC[a]	PP[a]
Subjects at beginning (V7)	3	6	5	6	10
Subjects at end (V13)	3	5	5	5	10

[a] One and two letter abbreviations of supplementation groups.
[b] The groups supplemented for the 2nd six months are subgroups of the respective population supplemented for the 1st six months.

Table 2
Baseline characteristics (mean ± standard deviation)

Supplementation group	N	Age (years)	BMI (kg/m^2)	MPOD (OD)	Plasma concentrations	
					Lutein (μmol/L)	Zeaxanthin (μmol/L)
L	23	26.96 ± 5.64	24.40 ± 2.37	0.44 ± 0.11	0.16 ± 0.07	0.05 ± 0.02
Z	23	26.26 ± 4.67	24.13 ± 2.11	0.37 ± 0.10	0.13 ± 0.08	0.04 ± 0.03
C	23	26.13 ± 5.00	24.21 ± 1.93	0.42 ± 0.13	0.17 ± 0.07	0.06 ± 0.03
P	23	24.39 ± 3.69	23.43 ± 2.12	0.40 ± 0.09	0.13 ± 0.04	0.04 ± 0.03

There were no statistically significant differences (ANOVA) between groups at baseline (V0).

for 6 months ($n = 58$) and (b), a cohort of (a) that was supplemented for a total of 12 months ($n = 13$); as well as two separate placebo populations, one for the 1st half year ($n = 20$) and one for the 2nd half year ($n = 10$). See Table 1 for details of the design of the study and Table 2 for baseline characteristics of the participating subjects.

Monitoring of supplementation effects

At each monthly visit, blood was collected in the morning after an overnight fast and before intake of the next dose. Plasma was analyzed by HPLC for concentrations of L and Z according to published procedures [19–24].

MPOD was determined monthly by heterochromatic flicker photometry (HFP) [25,26] using a portable instrument, which has been described in detail elsewhere and has been validated against motion photometry in normal subjects [27]. HFP luminance matches were made between near-monochromatic lights that are absorbed (blue, peak 465 nm) or not absorbed (green, peak 530 nm) by the macular pigment (MP). Adjustments to set flicker to a minimum were made by varying the luminance of the blue light, one match in a centrally fixated circular foveal test-field with a diameter of 1°, and one match in a concentric annular parafoveal test-field with inner and outer radii of 5° and 6°, respectively resulting in a mean eccentricity of ±5.5°. The instrument independently recorded, in arbitrary units, the foveal (Lum_{fovea}) and the parafoveal ($Lum_{parafovea}$) luminance of blue light required to attain a minimum flicker match (the null point) [27]. MPOD was computed from $\log(Lum_{fovea}/Lum_{parafovea})$. The method is based on the assumption that MP is mainly present at the fovea and that there is negligible MP at the reference location of ±5.5° eccentricity. In this idealized situation, the amount of blue light required to achieve a minimum flicker match at the fovea is greater than that needed at the reference location, and the difference is attributable to MP. During each measurement session, at least 4 matches per viewing condition (i.e., foveal vs. parafoveal) were made. As it was easier to obtain matches with the parafoveal annulus, these matches were made first and the right eye was always tested before the left eye.

Statistical analyses

General

All statistical evaluations were done with SAS (release 8.02) and S-PLUS (release 6.2). Baseline values were compared by analysis of variance.

GEE (Generalized estimating equations)

The statistical analysis of the MPOD data was complicated by a number of factors, including subject drop out, especially at the 6 month extension point. This gave rise to a considerable number of missing data. Furthermore, the extension of the study caused different individual lengths of supplementation. And finally, all measurements were done in both eyes. To account for these factors and the related data structure, statistical analysis was based on the *Generalized Estimating Equations (GEE)* method [28]. This is an approach used to evaluate the longitudinal follow-up of correlated response variables, measured in correlated entities (here: both eyes of a subject). The GEE method allows an unequal number of observations per subject, caused, for example, by missing values or different lengths of follow-up. GEE as applied in this evaluation generates an average integrated intensity that describes the overall MPOD or luminance responses to supplementation by taking into account the responses of individual eyes as well as the identity and individual duration of supplementation. GEE analysis was performed using the GENMOD procedure available in SAS 8.02. The mathematical details of this method are published elsewhere [29].

Results

Baseline characteristics

Table 2 shows the demographic baseline characteristics as well as baseline values for MPOD and plasma concentrations of L and Z of the participating subjects. Differences between the supplementation groups were not statistically significant (ANOVA).

Plasma response

Supplementation with 10 mg of either L or Z generated comparable plasma concentrations for L and Z (Figs. 1A and B, and Table 3). Combined supplementation with L + Z, each at a dose of 10 mg, resulted in plasma concentrations that were lower than that seen with L or Z alone. Total xanthophyll (L + Z) concentrations of the three supplementation regimes were statistically not different from each other (data not shown).

Plasma concentration time courses for the 2nd six months of supplementation (data not shown) resulted in qualitatively similar concentration–time profiles, although higher plateau concentrations were attained after doubling the doses for L and Z (Table 3). Average L or Z plasma concentration increased from the beginning of the 2nd six months by a factor of 4 (L) or 5 (Z) for subjects on the doubled doses of L or Z. In subjects receiving the combination, plasma concentrations increased by a factor of approximately 2 (L) or 4 (Z). During supplementation with L a slight rise in Z concentration is evident and reflects the natural content (about 7.5%) of Z in the marigold-based lutein beadlets (Fig. 1B).

Macular pigment response

Changes in MPOD following supplementation were evaluated using the GEE technique (Table 4). In comparison to placebo, supplementation with L or C caused significant increases in MPOD of 14.5% and 15.1% respectively ($p = 0.04$). In contrast, the effect of supplementation with Z appeared to be much smaller with only a 2.7% ($p > 0.1$)

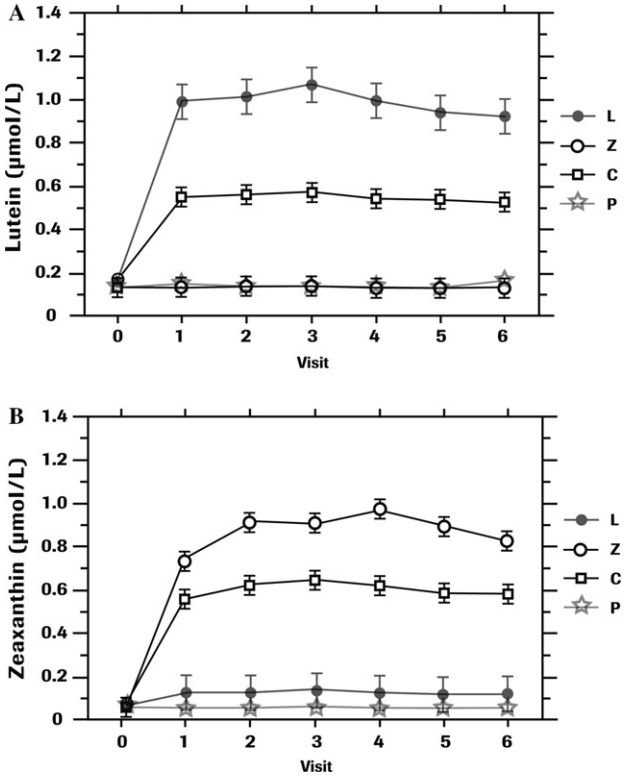

Fig. 1. Time course of lutein (A) and zeaxanthin (B) plasma concentrations (μmol/L, average ± standard error, see also Table 3). Measured in subjects supplemented for 6 months with lutein (L, 10 mg/day), zeaxanthin (Z, 10 mg/day), their combination (C, L + Z, 10 mg/day each) or placebo (P). The time between visits is one month.

increase in apparent MPOD (but see below). In addition to MPOD, the foveal *and* parafoveal luminance values (465 nm) required to achieve a minimum flicker match (null point) were recorded independently during supplementation (Table 4). In subjects taking the combination (L + Z), a mean luminance increase of 16.9% ($p = 0.04$) was required to attain a minimum flicker match at the fovea and a 4.8% increase was needed at the parafovea, compared with placebo. In subjects who received L alone there was a similar but slightly smaller increment at the fovea (13.1%) but no increase at the parafoveal location. In subjects who received Z alone, a mean luminance increase of 4.6% was recorded at the fovea but unlike the other groups, a comparable increment (3.2%) was recorded at the reference location. Analysis of one subject supplemented with Z for six months (Fig. 2) and six subjects supplemented with Z for one year (Fig. 3) reveal a corresponding trend at both retinal locations (Figs. 3A and B). The resulting MPOD measurements for the six subjects are shown in Fig. 3C, documenting no apparent change over time. Computation of MPOD based on comparison of the actual foveal value with the parafoveal value at baseline resulted in a +14% difference in the Z-alone group, compared with placebo. In contrast to the supplementation groups, luminance values did not change in the placebo group (Fig. 4).

Discussion

The results of the present multiple-dosing study indicate that L and Z supplementation caused markedly increased

Table 4
Changes of MPOD and luminance in response to supplementation

Supplementation	MPOD		Luminance at null point (465 nm)			
			Fovea		Parafovea	
	% change	p	% change	p	% change	p
Lutein	14.5	0.04	13.1	>0.1	−0.3	>0.1
Combination	15.1	0.04	16.9	0.04	4.8	>0.1
Zeaxanthin	2.7	>0.1	4.6	>0.1	3.2	>0.1
Zeaxanthin[a]	14.0					

Expressed as % change in relation to placebo values over the 12 months of monitoring (p-values estimated by GEE). Mean values for MPOD, foveal and parafoveal luminances in subjects on placebo (100%) were 0.371, 1.264, and 0.517, respectively.

[a] Corrected for parafoveal luminance increases (Fig. 3D).

Table 3
Xanthophyll doses given, attained average plateau[a] plasma concentrations, and factors of increases[b]

Supplementation group	Xanthophyll[c] dose (μmol)	Plasma concentrations			
		Lutein		Zeaxanthin	
		Fold increase	Plateau[a] (μmol/L)	Fold increase	Plateau[a] (μmol/L)
1st six months					
L	20.2[(a)]	6.9 ± 3.7	0.99 ± 0.39	2.9 ± 2.1	0.10 ± 0.04
Z	22.1	1.1 ± 0.4	0.13 ± 0.06	27.2 ± 15.7	0.85 ± 0.32
C	38.8	3.7 ± 1.4	0.55 ± 0.12	13.5 ± 7.9	0.61 ± 0.16
P	0	1.1 ± 0.5	0.14 ± 0.04	1.3 ± 0.7	0.04 ± 0.02
2nd six months					
LL	40.4[(b)]	4.0 ± 2.6	1.35 ± 0.87	2.6 ± 1.1	0.14 ± 0.04
ZZ	44.3	1.2 ± 0.4	0.17 ± 0.07	5.2 ± 1.6	1.09 ± 0.41
CC	38.8	2.1 ± 0.9	0.53 ± 0.24	3.8 ± 1.6	0.52 ± 0.30
PC	38.8	2.0 ± 1.1	0.32 ± 0.25	5.3 ± 5.4	0.29 ± 0.32
PP	0	1.0 ± 0.2	0.13 ± 0.04	1.3 ± 0.6	0.04 ± 0.02

For a description of supplementation groups, including number of subjects, see Table 1. Baseline concentrations are shown in Table 2.

[a] During visits 2–6 (1st six months) and 9–13 (2nd six months) respectively.

[b] The ratio of concentration at plateau divided by the concentration at baseline (V0, 1st six months) and visit 7 (V7, 2nd six months) respectively.

[c] L + Z. (a) Including 1.4 μmol Z. (b) Including 2.8 μmol Z.

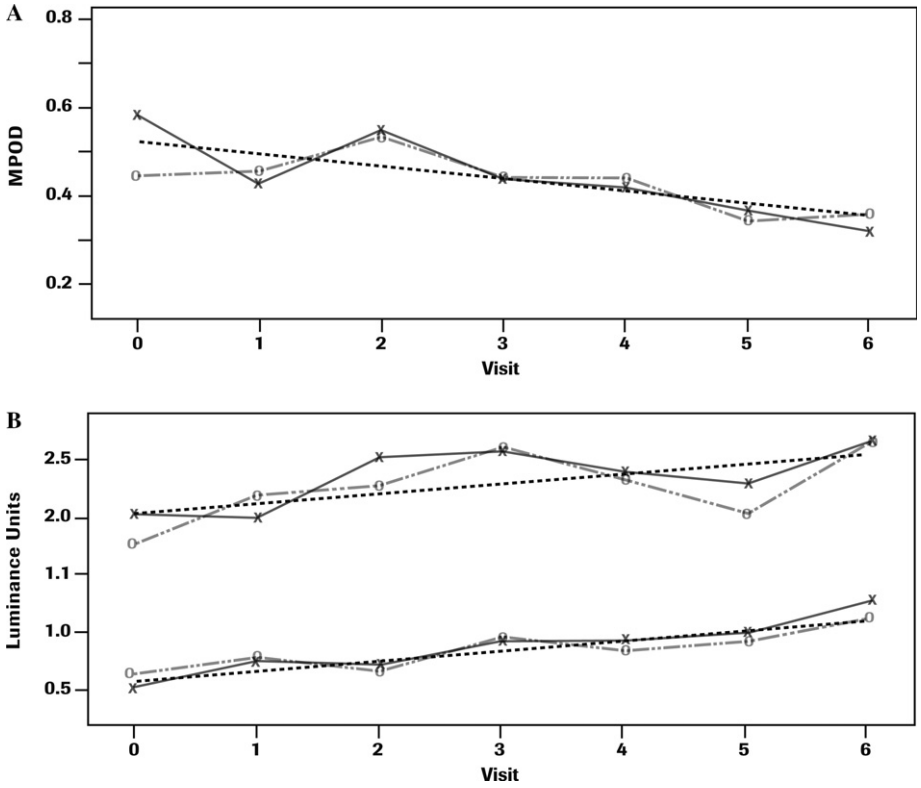

Fig. 2. Time course of macular pigment density parameters for one subject supplemented with zeaxanthin (10 mg/day) for six months. (A) MPOD; (B) corresponding changes in the luminance of the 465 nm stimulus component required to achieve minimum flicker in the fovea (upper lines) and the parafovea (lower lines). Open circles: left eye; crosses: right eye; dashed line: linear regression of averages between eyes.

xanthophyll plasma plateau concentrations and that supplementation resulted in enhanced MPOD. There are two important and novel elements of this study: firstly that the supplementation with pure Z, both alone and in a 1:1 combination with L was evaluated, and secondly that during supplementation changes at the parafoveal and foveal locations were recorded and analyzed separately.

Plasma response

In agreement with previous findings ([23,24]), apparent steady state xanthophyll concentrations in plasma were reached within one month of supplementation and the magnitude of this effect documents a substantial systemic exposure to these xanthophylls, an important theoretical prerequisite for xanthophylls being incorporated into the retina. As shown in Fig. 1, administering the xanthophylls together appeared to lower the relative bioavailability of both. This may be due to the high chemical similarity of L and Z. It can be conceived that their uptake into plasma may be limited by competition for the same absorption mediator, leading to non-linear plasma responses as described previously [23,24].

Macular pigment response

There was close correspondence in MPOD between eyes (evident in Fig. 2) and high inter-ocular correlation ($r > 0.9$, data not shown). However, intersession variability was substantial (see Figs. 3A and 4 (upper line)). This may be related to difficulties performing the psychophysical task or other factors inherent in the HFP method. The minimum flicker task was generally considered more difficult at the fovea compared with the parafoveal location. This is also suggested by the relatively low variability of the parafoveal measurements (Figs. 3B and 4 lower line). Measurement error at the foveal location may partly relate to the rapidly changing distribution of MP over this 1° area [30] and may additionally be compounded by small fixation errors. The choice of a 1° test-field in the current study was guided by several factors. Small punctate stimuli [31] would better define a profile in its steepest region but microsaccades may increase matching errors as field size is reduced [32]. Peak optical density may be underestimated if isoluminance is determined for the edge of a circular stimulus [33] or if there is visual integration across the whole of a stimulus field. The latter may occur in colour matching studies and results in values equivalent to those made for an annulus at 70–80% of the central circular field radius [34]. In flicker studies the equivalent match has been estimated to occur at 51% of the field radius [33]. These effects may be larger when the MP profile has a sharp cusp or when large circular test-fields are employed, resulting in possible underestimation of peak MPOD in some subjects, but are less likely to influence longitudinal intra-subject comparisons. The 1° field in the current study was considered optimal, in keep-

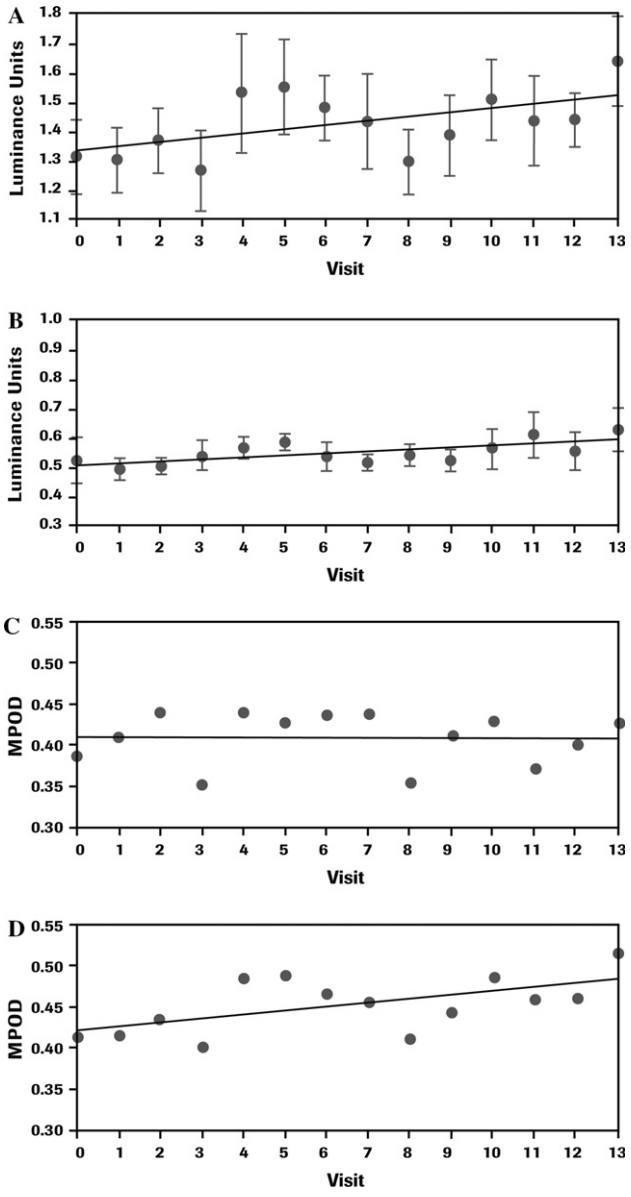

Fig. 3. Time courses of mean luminance (465 nm, arbitrary units, average ± standard error) values (average of both eyes, 6 subjects) supplemented with zeaxanthin for one year. (A) Fovea; (B) Parafovea; (C) MPOD, computed from foveal luminance values relative to parafoveal luminance values (see methods for details); (D) MPOD, recomputed at the fovea relative to baseline (V0) parafoveal values.

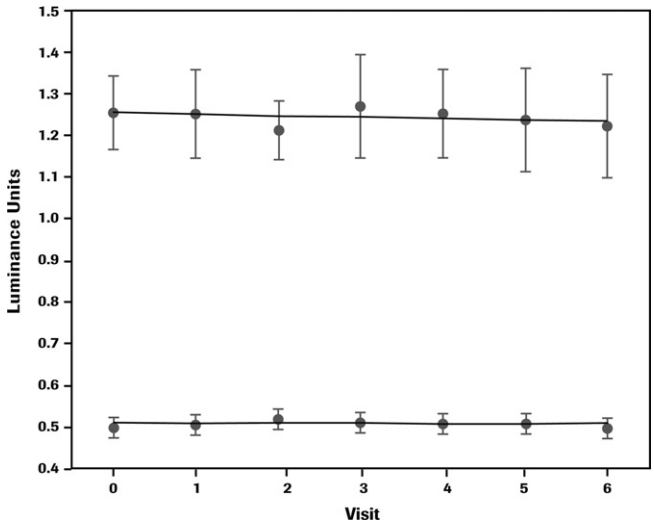

Fig. 4. Time courses of mean luminance (465 nm) values (arbitrary units, average ± standard error, right eye) in subjects on placebo ($N = 20$) for the 1st six months: fovea (upper line) and parafovea (lower line). The situation in placebo subjects of the 2nd six months is equivalent (data not shown).

ing with numerous other psychophysical studies of MP that have utilized identical or similar central field sizes (e.g. [30,35–39]).

In view of the close chemical similarity of L and Z, their identical formulation and substantial MPOD increase of 15% observed after L and L + Z supplementation, the relatively small 3% increase in MPOD during supplementation with Z alone was unexpected (Table 4 and Fig. 3C). A possible explanation is that during supplementation MP may increase at both the foveal and the reference retinal locations resulting in underestimation of foveal MP. This is suggested by Figs. 2B and 3(A and B) that show almost parallel changes in sensitivity to blue light at the foveal and parafoveal locations. If foveal MPOD is recomputed relative to pre-supplementation parafoveal measurements, the increase equates to 14% (Fig. 3D). This value is in the same order of magnitude as the MPOD increase measured following supplementation with L or C (Table 4).

Supplementation with L alone resulted in a statistically significant 15% change of MPOD at the fovea. In this group the stimulus luminance required for the parafoveal flicker match was stable (Table 4) suggesting that deposition of L in this group had mainly occurred in the fovea, consistent with reports in the literature. It is somewhat difficult to reconcile the more widespread deposition of Z with previous reports of L and Z distribution patterns published by Bone and Landrum [40], who reported Z being the dominant xanthophyll in the fovea. However, Bone and Landrum investigated presumably un-supplemented subjects and the situation during supplementation may be different. One possibility is that the normal retinal distribution of xanthophylls could influence the pattern of MP deposition during supplementation. The relatively higher levels of Z already present at the fovea may limit the amount of Z being additionally accumulated, thus favouring deposition of L in this area. Similarly, the relatively higher levels of L naturally present in the parafovea may limit the uptake of L, possibly resulting in enhanced deposition of Z within this area.

With regard to plasma concentrations, all of the three supplementation regimes, L, Z and L + Z were similarly effective in increasing the plasma concentration of total xanthophylls. If attaining a certain plasma concentration of the xanthophylls were a prerequisite for MPOD augmentation, it could be supposed that L, Z and L + Z would be potentially effective for this purpose. However, the results of this study showed that a balanced mixture of L *and* Z, as administered via the L + Z combination, resulted in the

greatest (15.1%, Table 4) statistically significant increase in MPOD. This increase occurred in spite of a decrease in blue light sensitivity in the parafovea. This suggests that, possibly because of the presence of Z (see above), supplementation with L *and* Z produces a wider MP distribution over the posterior pole of the retina than supplementation with L alone.

Supporting observations from independent studies

Parafoveal pigment deposition during supplementation has been described by Neuringer et al. [41] and Johnson et al. [42], who carried out HPLC analyses of dissected retinas of monkeys supplemented with L or Z. Their results indicated a marked MP accumulation in concentric annuli well outside of the central MP peak. By using HFP with a reference location at ±7°, Snodderly et al. [43] monitored MPOD in humans supplemented with L and DHA (docosahexaenoic acid) and found evidence of statistically significant ($p < 0.05$) parafoveal MP accumulation at an eccentricity of ±5°, which is close to the parafoveal reference location of ±5.5° used in our study. In six LUXEA subjects who were supplemented with the L + Z combination for six months (PC group), Rodriguez-Carmona et al. [17] recorded MPOD profiles across ±8° eccentricity. Their results revealed that at ±5.5° eccentricity the amount of blue light transmitted by the supplemented subjects was still 12% less than in the placebo group and that this difference decreased to 3% four months after supplementation was discontinued, suggesting that the 12% difference was related to supplementation with the L + Z combination. Furthermore, working with the same instrument as used in our study, Berendschot et al. [44] recently reported age-related parafoveal pigment increases.

Potential relevance of parafoveal MP deposition

Parafoveal pigment deposition during supplementation of xanthophylls, as suggested by this study, may have implications for the study of xanthophylls and AMD. Given that the presence of MP has been associated with risk reduction of AMD [4] and that AMD lesion patterns usually extend beyond the centre of the macula, the possibility of increasing paracentral Z deposition may prove significant when considering the possible protective influence of xanthophyll supplementation. It is tempting to speculate that Z may not only influence the development of central retinal disease, such as AMD, but may also influence more peripheral diseases. Furthermore, it has been shown that in healthy subjects, parafoveal MP may play a role in improving visual performance under mesopic conditions, as suggested recently by Kvansakul et al. [18].

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