Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 458 (2007) 128–135
ABB
www.elsevier.com/locate/yabbi

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

Wolfgang Schalch [a,*], William Cohn [a], Felix M. Barker [b], Wolfgang Köpcke [c], John Mellerio [d], Alan C. Bird [e], Anthony G. Robson [e], Fred F. Fitzke [f], Frederik J.G.M. van Kuijk [g]

[a] *DSM Nutritional Products Ltd., NRD-CH, Building 203/855, Wurmisweg 576, CH-4303, Kaiseraugst, Switzerland*
[b] *Pennsylvania College of Optometry, Philadelphia, USA*
[c] *University of Münster, Münster, Germany*
[d] *St Thomas' Hospital, London, UK*
[e] *Moorfields Eye Hospital, London, UK*
[f] *Institute of Ophthalmology, London, UK*
[g] *University of Texas, Galveston, TX, USA*

Received 14 July 2006, and in revised form 19 September 2006
Available online 7 November 2006

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

* Corresponding author. Fax: +41 61 687 1847.
E-mail address: wolfgang.schalch@dsm.com (W. Schalch).

[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.

0003-9861/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2006.09.032

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.