Bioavailability of lutein/zeaxanthin isomers and macular pigment optical density response to macular carotenoid supplementation: A randomized double blind placebo controlled study

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Abstract

**Purpose:** To examine the bioavailability of Lutein (L) and Zeaxanthin isomers (Zi) concentrations in serum and changes in MPOD over 12 weeks macular carotenoids supplementation in healthy young subjects.

**Methods:** In a randomized double blind placebo controlled study, twenty eight (N=28) healthy young male and female volunteers were randomized to receive one of three doses (6 mg L/1 mg Zi, 10 mg L/2 mg Zi or 20 mg L/4 mg Zi) for 12 weeks. Blood samples for serum L/Zi and macular pigment optical density (MPOD) were determined every two weeks over the 12 week study period. Serum lutein and zeaxanthin isomers concentration was determined by HPLC and MPOD by heterochromatic flicker photometry (HFP). The area under the curve (AUC) was calculated using the linear trapezoidal rule. Cmax  and t max  was determined over 12 weeks of supplementation.

**Results:** No significant difference in serum L/Zi concentrations of each dose group at baseline visit. Serum levels of L and Zi increased at 2 weeks, and peaked by 12 weeks. Median serum concentrations of 6 mg L, 10 mg L or 20 mg L groups from baseline to month 3 increased from 0.323 to 1.984 µg/dL (6-fold increase), from 0.353 to 2.234 µg/dL (7-fold increase), and from 0.372 to 3.163 (10-fold increase), respectively (all P<0.001). Median serum concentrations of 1 mg Zi, 2 mg Zi or 4 mg Zi groups from baseline to month 3 increased from 0.060 to 0.377 µg/dL (6-fold increase), from 0.096 to 0.350 µg/dL (4-fold increase), and from 0.117 to 0.391 (3.3 fold increase), respectively (all P<0.001). Area under curve (AUC) for serum lutein increased (p<0.01) and AUC for serum Zi increased (p<0.03) with increased dose of L/Zi over placebo. AUCL increased in 6 mg of L & 1 mg Zi by 6 fold, 8 fold in 10 mgL and 2 mg, and 12 fold in 20 mg L and 4 mg Zi over placebo, respectively. AUCZi increased in all three treatments over placebo by 3 fold, 4 fold and 5 fold, respectively. MPOD increased significantly from baseline to month 3 increased for all L/Zi treatments over placebo. No adverse events were observed with any dose of lutein.

**Conclusion:** Increasing doses of macular carotenoid supplementation significantly increased the serum AUC levels of lutein and zeaxanthin isomers, and doses up to 20 mg were safely administered. A long-term large clinical trial is necessary to investigate the safety and efficacy of macular carotenoids in health and disease.

Introduction

Lutein and zeaxanthin are 2 of the most abundant carotenoids present in the diet, and they are the pigments responsible for the bright colours of many fruits and vegetables. Lutein and zeaxanthin are isomers that differ by site of a single double bond [1,2]. Zeaxanthin exists as 3 stereoisomeric forms; (3R, 3'R)-zeaxanthin and (3R, 3'S)-zeaxanthin (also called meso-zeaxanthin) are the main forms present in the macula of the retina, while small amounts of (3S, 3'S)-zeaxanthin have also been detected [3,4]. Humans are unable to synthesize lutein and zeaxanthin isomers; thus, these nutrients are obtained from natural dietary sources or from supplementation. Circulating and tissue levels of xanthophylls increase with supplementation with lutein/zeaxanthin [5,6]. However, variability in their bioavailability has been reported [7-9], and has been related to factors such as the matrix of the formulation (e.g., presence of fat), the form in which they were administered (i.e., free versus esterified) and interactions with other nutrients [10,11]. Supplementation with lutein and zeaxanthin [i.e., (3R,3'R)-zeaxanthin and meso-zeaxanthin] is generally considered to be safe [12].

Epidemiological data indicate that the average intake of lutein and zeaxanthin from dietary sources is in the range of 1 to 2 mg/day (approximately 0.01 to 0.03 mg/kg body weight/day), corresponding serum concentrations of approximately 0.4 µmol/L have been measured [10,13,14]. Supplementation with lutein/zeaxanthin has been shown to increase levels in the blood and tissues where these
xanthophylls are selectively deposited (such as the macula lutea of the retina) [15,16]. However, considerable inter-individual variability in serum concentrations and macular pigment density has been reported following supplementation with lutein/zeaxanthin [17]. Some of the factors that may contribute to this variation include those that affect the absorption of xanthophylls, such as the matrix of the formulation, the form in which they were administered (i.e., free versus esterified). Lutein occurs as a single stereoisomer [(3R,3'R,6'R)-β,ε-carotene-3,3'-diol] while zeaxanthin occurs as a mixture of stereoisomers, with the 2 most prominent forms in the macula of the retina being, (3R,3'R)-β,β-carotene-3,3'-diol (referred to as zeaxanthin) and (3R,3'S)-β,β-carotene-3,3'-diol (referred to as mesozeaxanthin). The physical and chemical properties of lutein and zeaxanthin isomers are summarized in Figure 1. Most of the studies are single dose studies [7,18,19] and a multiple-dose pharmokinetics (PK) study [20] reported in the literature. The present study was designed to compare, in human subjects, the bioavailability of lutein and zeaxanthin isomers when ingested at different doses compared with placebo and to study the changes in MPOD by macular carotenoid dose over three months supplementation (Figure 2).

### Subjects and methods

Twenty eight (28) volunteers participated in this study recruited from the University of Georgia population in accordance with the IRB guidelines. This study was reviewed and approved by the University of Georgia Institutional Review Board. Informed consent was obtained for each subject, and the study adhered to the tenets of the Declaration of Helsinki. This study is registered at ISRCTN#54990825. Subjects were randomly assigned to one of four groups: Placebo (Group I, safflower oil, N=5), 6mg L/1mg Zi (Group II, n=7), 10mg L/2mg Zi (Group III, n=8), or 20mg L/4mg Zi (Group IV, n=8). Identical looking capsules containing only safflower oil was used as a placebo. Lutemax 2020 (L/Zi) at different doses (6mg L/1mg Zi; 10mg L/2mg Zi; 20mg L/4mg Zi) and placebos supplied by OmniActive Health Technologies Ltd., Mumbai, India. Subjects instructed to take one capsule per day with a meal for 12 weeks but otherwise to follow their normal diet. Compliance was ensured with weekly phone calls and subjects were requested to return bottles to count left over pills in the bottle.

Subjects’ anthropometric measurements, health habits and medical history recorded during their screening visit. Normal healthy subjects and no history of smoking included in the study. Subjects with chronic conditions excluded such as prescriptions or surgical treatments. Pregnancy and lactating women and subjects with a BMI higher than 27 and took supplements containing any of the carotenoids excluded. Subjects were instructed to keep up their current diet and not to change their diet during the study period. In consideration of MPOD testing, all subjects had uncorrected or contact lens-corrected visual acuity of 20/20 or better in the test (right) eye, and had no current or earlier history of ocular pathology.

Subjects were instructed to visit the laboratory every 2 weeks for blood draws and vision testing. Fasting blood draw samples were collected to assess serum L/Zi and Macular pigment measurement was assessed for each subject.

### Serum analysis

Serum concentrations of lutein and zeaxanthin isomers were obtained by HPLC according to a method described in detail [21]. Samples were taken at baseline and every 2 weeks over the 12-week study period.

Detection wavelengths were λ = 447 nm (lutein) and 450 nm (zeaxanthin isomers).

### Measurement of macular pigment optical density (MPOD)

MPOD in the central retina was assessed with a non-invasive, perceptual task called customized heterochromatic flicker photometry.

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**Table 1. Serum Analysis**

| Serum Analysis | P Value |
|---------------|---------|
| AUC<sub>L</sub> | P<0.0081 |
| AUC<sub>Zi</sub> | P<0.0259 |

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**Figure 1. Molecular Structures of Lutein and Zeaxanthin Isomers.**

**Figure 2. A and 2B Lutein and zeaxanthin isomers bioavailability measured by AUC (µg/mL) over 12 weeks of supplementation.**
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A densitometer (Macular Metrics Corp., Rehoboth, MA) described by Wooten et al. [23] was used for this purpose. The densitometer, detailed measurement procedures, and the principle of HFP have been fully described in earlier publications [23]. Measurements were taken at baseline and every 2 weeks over the 12 week study period. We obtained spatial profiles of MPOD at each visit, with measures at 10', 20', 30', 1.75 degrees, and 2.75 degrees of retinal eccentricity.

The primary study endpoint was the 12 week area under the curve for plasma lutein (AUC). Secondary endpoints included the maximum concentration ($C_{\text{max}}$), the time at which the maximum concentration was observed for plasma lutein ($T_{\text{max}}$) and $C_{\text{max}}$, and $T_{\text{max}}$ was also calculated for zeaxanthin. The area under the curve was calculated using the linear trapezoidal rule. In order to meet the assumption of normality, statistics on AUC and $C_{\text{max}}$ were based on log transformed values for individual subjects.

**Statistical analysis**

Descriptive statistics (mean and standard deviation) were reported. One-way and repeated-measures analysis of variance, curve fitting, and correlational analyses were conducted. All statistical analyses performed with SAS (NC). Tukey-Kramer adjusted $P$ values were used to find where the post hoc differences occurred within statistically significant interaction or main effects, with significance set at $P<0.05$. Multiple models comparing group differences analyzed (raw values at all time points, raw values adjusted for baseline, and change scores). Statistical significance determined at $P=0.05$ level. Interaction and main effects were considered statistically significant at $P<0.05$ and trends at $P<0.1$.

**Results**

**Baseline characteristics**

Table 1 provides baseline characteristics of the study. No significant difference was found in any of the groups.

**Lutein bioavailability**

The mean serum plasma and AUC$_{\text{L}}$ concentrations were significantly higher ($P<0.001$) compared to placebo. Highly significant difference in AUC$_{\text{L}}$ was observed between Group I (placebo) and Group IV (20 mg L, $P<0.001$) followed by group III (10 mg L, $P<0.019$) and a trend of significant difference in AUC$_{\text{L}}$ between Group I (placebo) and Group II (6 mg L, $P<0.1$) was observed. There was a significant difference between Group II (6 mg L and 20 mg) and Group IV over 12 week period ($P<0.03$) (Table 2).

Between treatment analysis of covariance, significant differences were observed between Group I and II (placebo vs. 6 mg L, $P<0.0494$), Group I and Group III (placebo vs. 10 mg L, $P<0.034$) and Group I and Group IV (placebo vs. 20 mg L, $P<0.0002$). Significant difference in $C_{\text{max}}$ was observed between Group I and Group IV ($P<0.015$) and Group III and Group IV (10 mg L vs. 20 mg, $P<0.023$) Table 3). The time to reach maximum concentration ($T_{\text{max}}$) for lutein was not significantly different for treatments.

**Zeaxanthin isomers bioavailability**

Highly significant difference in AUC$_{\text{Zi}}$ was observed between placebo and Group IV (4 mg Zi, $P<0.005$) followed by group III (2 mg Zi, $P<0.02$) and a trend of significant difference in AUC$_{\text{Zi}}$ between Group I and Group II (placebo and 6 mg L, $P<0.1$) observed. There was a trend of significant difference between Group I and IV (6 mg L and 20 mgL) over 12 week period ($P<0.07$).

Between treatment analysis of covariance, significant difference between Group I and II (placebo vs. 1 mg Zi, $P<0.0541$), Group I and Group III (placebo vs. 2 mg Zi, $P<0.0114$) and Group I and Group IV (placebo vs. 4 mg Zi, $P<0.0005$) was observed. Significant difference in Cmax was observed between Group I and Group IV ($P<0.0363$, Table 3) and no significance in other groups was observed. The time to reach maximum concentration ($T_{\text{max}}$) for Zi was not significantly different for treatments.

**Change in MPOD**

MPOD responses were detected at 4 weeks in Group II and Group III (NS) at the standard, 30' retinal locus followed by a significant change in MPOD in Group II at 12 weeks. A non-significant change in MPOD was observed in Group III and IV at week 6. Significant change in MPOD was observed in Group III and IV at week 8 to week 12 ($P<0.001$). No significant difference between Group III and IV were observed.

**Discussion**

In several conducted studies where lutein preparations were repeatedly administered at doses ranging from 4 to 20 mg/day for up to 20 weeks, plasma concentrations of lutein increased by 3- to 8-fold compared to controls or baseline, with levels back to baseline by 3 to 4 weeks following cessation of treatment [9,24-26]). This is our first attempt of this type.
The pharmacokinetics of lutein in humans was assessed in two studies utilising [14C] and [13C] labelled lutein from spinach and kale, respectively [29,30]. The 14C-lutein concentrations reached its peak (Cmax) were determined based on the concentrations of L and Zi from see the changes of AUC L/Zi over 12 weeks. Maximum concentrations individual data sets(Table 3). Ocular tissues, particularly the retina, are being taken up by the tissues. Hence we saw significance in change levels of L and Zi are consistent with the dose. At week 12 the higher dose appears to plateau. These results suggest the macular carotenoids are being taken up by the tissues. Hence we saw significance in change in MPOD at 12 weeks in all doses. These results suggest the presence of a striking treatment effect where relative to placebo, greater Lutein dose increased over placebo. AUCL increased 6 folds higher in Group II over placebo (Group I), 8 folds higher Group III and 12 folds higher in Group III over placebo (all P<0.01). AUC_L increased by 3, 4 and 5 folds in Group II, III and IV over Group I (all P<0.05). The difference is due to differential spatial accumulation of lutein relative to zeaxanthin may be relevant to retinal health.

Conversely, no significant differences in serum lutein levels were reported following supplementation with free lutein (6.0 mg) or esterified lutein (5.5 mg of free lutein) for 9 days in a cross-over study with 10 healthy males [11]. In this study, both formulations were provided as crystalline suspensions in oil in soft gel capsules and administered with a test meal. In another cross-over study, subjects administered a single dose of a formulation containing unesterified lutein or lutein diesters (0.5 and 0.67 µmol lutein/kg body weight in 10 and 8 subjects, respectively), along with a test meal [38]. Supplementation with lutein diesters produced a significantly higher maximum serum concentration of lutein and a higher mean AUC (by 61.6%), compared to supplementation with free lutein. It should be noted, though, that different formulations were used for the test articles, with free lutein administered as a crystalline oil suspension in soft gel capsules, whereas esterified lutein was administered as a powder in hard gel capsules. As such, the interpretation of these findings is unclear as they may have been confounded by differences in formulation dissolution. In the current study, median serum concentrations of free lutein, 10 mg L or 20 mg L groups from baseline to month 3 increased from 0.323 to 1.984 µg/dL (6-fold increase), from 0.353 to 2.234 µg/dL (7-fold increase), and from 0.372 to 3.163 (10-fold increase), respectively (all P<0.001). Median serum concentrations of free L/Zi, 2 mg Zi or 4 mg Zi groups from baseline to month 3 increased from 0.060 to 0.377 µg/ dL (6-fold increase), from 0.096 to 0.330 µg/dL (4-fold increase), and from 0.117 to 0.391 (3.3 fold increase), respectively (all P<0.001).

The bioavailability of esterified versus free zeaxanthin has also been evaluated in 1 study where a single dose of esterified or free 3R,3’R-zeaxanthin (5mg) was administered to 12 healthy volunteers in a cross-over study design [39]. Both test articles were suspended in sunflower oil and mixed with a yogurt which was consumed along with a standardized breakfast. Supplementation with 3R,3’R-zeaxanthin
palmitate (esterified) produced approximately 2-fold higher AUC values compared to supplementation with free 3R, 3’R-zeaxanthin (p<0.05). Supplementation with L/Zi (free) had higher AUC values and very quick response and MPOD also detectable at 4 weeks but significance observed at 8 to 12 weeks.

The role of lutein and zeaxanthin in eye health has been further supported by some epidemiological studies reporting an inverse relationship between lutein/zeaxanthin intake and eye disease, particularly AMD and cataracts [16,40-44]. Several controlled intervention studies have also indicated that macular pigment density or dietary supplementation with lutein improves parameters of visual function, such as visual acuity [45,46], glare recovery, and contrast sensitivity [26,45,47-49]. A number of clinical studies have evaluated the pharmacokinetic properties of lutein and zeaxanthin. Overall, an increased intake of lutein and zeaxanthin, either through natural dietary sources or supplementation, produces corresponding increases in levels of these carotenoids in systemic circulation.

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Conflict of interest

VF is an employee of OmniActive Health Technologies.

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