

The Effect of Lutein and Zeaxanthin Supplementation on Metabolites of These Carotenoids in the Serum of Persons Aged 60 or Older

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PURPOSE. To investigate the effect of lutein supplementation at doses of 2.5, 5.0, and 10 mg/d for 6 months on distribution of these carotenoids and their metabolites in the serum of elderly human subjects, with and without age-related macular degeneration. To determine whether supplementation with lutein can interact with the serum levels of other dietary carotenoids, retinol, and α -tocopherol.

METHODS. Forty-five subjects received daily supplements of lutein (containing 5% zeaxanthin) for 6 months and were followed up for another 6 months after supplementation. Blood was collected at various intervals and lutein, zeaxanthin, and their metabolites in the sera were quantified by normal-phase high-performance liquid chromatography (HPLC)-UV/visible detection. Other dietary carotenoids, retinol, and α -tocopherol were identified and quantified on a C_{18} reversed phase HPLC column.

RESULTS. After 6 months of supplementation with 10 mg of lutein, the increases in the mean serum levels from baseline were: 210 to 1000 nM/L ($P < 0.0001$) for lutein and 56 to 95 nM/L ($P < 0.0001$) for zeaxanthin. Similarly, the mean concentrations (nM/L) of carotenoid metabolites increased from 49 to 98 ($P < 0.0001$) for 3-hydroxy- β , ϵ -caroten-3'-one (3'-oxolutein); 31 to 80 ($P < 0.0001$) for 3'-hydroxy- ϵ , ϵ -caroten-3-one; and 19 to 25 ($P < 0.0001$) for ϵ , ϵ -carotene-3,3'-dione. The serum levels of these carotenoids gradually decline within 6 months after supplementation.

CONCLUSIONS. The increase in the serum levels of lutein/zeaxanthin correlates with increases in the serum levels of their metabolites that have previously been identified in the ocular

tissues. Elderly human subjects with and without AMD can safely take supplements of lutein up to 10 mg/d for 6 months with no apparent toxicity or side effects. (*Invest Ophthalmol Vis Sci.* 2006;47:5234-5242) DOI:10.1167/iovs.06-0504

Age-related macular degeneration (AMD) is the leading cause of blindness among whites aged more than 60 years in the United States. The Eye Diseases Prevalence Research Group estimates that 1.8 million U.S. residents are legally blind, and another 7.3 million are at risk for vision loss from AMD.¹ In 1993, Bone et al.² established the complete identification and stereochemistry of the human macular pigment as (3*R*,3'*R*,6'*R*)-lutein, (3*R*,3'*R*)-zeaxanthin, and *meso*-zeaxanthin [(3*R*,3'*S*)-zeaxanthin]. It has been hypothesized that lutein and zeaxanthin protect the macula against photooxidative damage by functioning as antioxidants and/or optical filters.^{3,4} A comprehensive review of the biological mechanisms of the protective role of lutein and zeaxanthin in the eye has been published by Krinsky et al.⁵

In 1997, Khachik et al.⁶ provided preliminary evidence for the possible antioxidant role of lutein and zeaxanthin in the retina by identifying and quantifying lutein, zeaxanthin, and their oxidation products in human and monkey retinas. Lutein, zeaxanthin, their geometrical isomers, as well as their oxidative metabolites have also been identified and quantified in all ocular structures of the human eye, with the exception of vitreous, cornea, and sclera.⁷

The beneficial role of carotenoids and antioxidant vitamins in the prevention of neovascular AMD has been largely supported by the findings from several epidemiologic studies.^{8,9} In the meanwhile, two other epidemiologic studies did not find an association between serum carotenoid concentration and a reduced risk of AMD.^{10,11}

The first supplementation study of lutein in humans was conducted by Khachik et al.,¹² in which three healthy white men (nonsmokers) were given oral supplements containing 10 mg/d of lutein for 18 days.¹² The blood levels of lutein in all three subjects increased by four- to fivefold after 1 week of supplementation. The levels of the lutein oxidation products (metabolites) during this study also increased significantly. In a similarly designed study, one subject ingested 20 mg/d of lutein for 21 days, and the plasma carotenoid profile of the subject was monitored at various intervals up to 40 days.¹³ The blood levels of lutein increased by ninefold from 0.21 to 1.89 μ M/L within 3 weeks of treatment. At the end of the supplementation period, the levels of the lutein oxidative metabolites increased by two- to threefold.

Supplementation with 10 mg/d of zeaxanthin for 3 weeks in three subjects was also shown to result in a fourfold increase in plasma concentration of this carotenoid after 1 week.¹³ In addition, the plasma concentrations of the oxidation products of lutein and zeaxanthin (metabolites) was shown to increase significantly.

In 1997, Landrum et al.¹⁴ conducted a study involving two subjects who took supplements of lutein esters equivalent to

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Presented at the 14th International Symposium on Carotenoids, Edinburgh, Scotland, United Kingdom, July 2005.

Supported by DSM Nutritional Products, Parsippany, New Jersey (FK), and JIFSAN, University of Maryland-Food and Drug Administration (UM-FDA). EC is an NEI Principal Investigator.

Submitted for publication May 4, 2006; revised July 31 and August 30, 2006; accepted October 12, 2006.

Disclosure: F. Khachik, DSM Nutritional Products (F, P); F.F. de Moura, None; E.Y. Chew, None; L.W. Douglass, None; F.L. Ferris III, None; J. Kim, None; D.J.S. Thompson, None

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30 mg of free lutein per day for 140 days. In this study, serum lutein levels of the subjects increased by 10-fold within 20 days and plateaued at 1761 nM/L and remained at this level for the duration of the study.

These studies had certain design limitations because they were conducted in a small number of subjects and involved relatively short supplementation and postsupplementation phases. In addition, these studies were conducted in human subjects with no AMD and consequently could not provide the much-needed data with regard to the safety and serum response of patients with or without AMD who received various doses of lutein supplements. Therefore, we conducted a dose ranging study with lutein (containing 5% zeaxanthin) in 45 human subjects over the age of 60 without AMD, as well as those with the intermediate and advanced AMD as described by Rosenthal et al.¹⁵ in an earlier publication. Rosenthal et al. have described the serum carotenoid response of the subjects to supplementary lutein and have discussed the results of the extensive ophthalmic data obtained from this study. Here, we describe detailed serum analysis of lutein, zeaxanthin, and their metabolites in human subjects taking daily supplements of three doses of lutein (2.5, 5, and 10 mg) and focus on the metabolic aspects of this study.

MATERIALS AND METHODS

Selection of Human Subjects

Forty-five subjects, male and female, aged 60 years and older participated in the study. Subjects not diagnosed with AMD were recruited by advertisement posted in the Newsletter of the National Institutes of Health (NIH) and the patients with AMD were recruited from the Retina Clinic at the National Eye Institute (NEI). The procedures and methods complied with the tenets of the Declaration of Helsinki and were reviewed and approved by the Institutional Review Boards of the NEI and the University of Maryland, College Park. Written, informed consent was obtained from all participants.

Source and Formulation of Lutein Containing 5% Zeaxanthin

For the present study, commercially available lutein (Kemin Health, Des Moines, IA) that contains approximately 5% zeaxanthin was formulated into water-dispersible beadlets using a controlled release technology (ActiLease; DSM Nutritional Products, Basel, Switzerland). The beadlets typically contain collagen, disaccharides, ascorbyl palmitate, natural vitamin E (1%), and modified food starch.

Study Design

The study was designed as a double-blind, randomized clinical trial involving 45 subjects. An equal number of subjects were recruited in the following three disease categories: 15 with minimal or small drusen not diagnosed as AMD, 15 with large drusen in one or both eyes (intermediate AMD), and 15 with advanced AMD in one eye (geographic atrophic, retinal pigment epithelial detachment, neovascular AMD). Five subjects in each strata were randomized to receive one of the three doses of lutein, 2.5, 5, and 10 mg/d for 6 months. At the end of the supplementation period (month 6), the subjects were followed up for another 6 months. The lutein supplements consisted of 1 tablet that was taken once a day with a meal.

Blood samples were collected 1 week before supplementation (week -1) and the day just before supplementation. Because the qualitative and quantitative profiles of carotenoids, their metabolites, and vitamins A and E in the sera of each subject at the two time points were nearly identical, the data from week -1 were used as baseline measurements. In addition, blood samples were collected throughout the entire study at months 1, 3, 6 (end of supplementation), 9, and 12. The sera were immediately separated and stored at -80°C until the

samples were shipped on dry-ice to the University of Maryland (College Park) for extraction and analysis. These sera samples were used to determine the qualitative and quantitative distribution of lutein, zeaxanthin, and their metabolites, as well as that of other dietary carotenoids and vitamins A and E, in the study subjects.

Subjects were required to complete a medical history questionnaire and physical examination at baseline; the heights and weights of the subjects were also recorded. At all visits during which blood samples were collected, the subjects had a complete ophthalmic examination, visual acuity testing, a side-effects questionnaire, and a few questions modified from a Food Frequency Questionnaire to assess for dietary intake of lutein. However, the subjects were on a self-selected diet throughout the entire study.

Subjects were excluded from the study if they had ocular disease other than age-related macular degeneration or were taking any ocular medication. The subjects who had been taking lutein supplements for up to 3 months before the start of the study, those who presented abnormal liver function, and individuals with a history of lung cancer were excluded from the study.

Determination of Sample Size

The variance approximation for the estimation of sample size was obtained from lutein supplementation studies that have tested a similar hypothesis and used lutein as the dependent variable. Landrum et al.,¹⁴ observed an increase of approximately 0.7 to 0.9 $\mu\text{g/mL}$ (1.2-1.58 $\mu\text{M/L}$) in serum concentration of lutein after 140 days of supplementary doses equivalent to 30 mg. In another study, 21 normal volunteers had a mean baseline serum lutein concentration of $0.140 \pm 0.07 \mu\text{g/mL}$ ($0.245 \pm 0.120 \mu\text{M/L}$) which plateaued at a concentration of $0.275 \pm 0.1 \mu\text{g/mL}$ ($0.484 \pm 0.176 \mu\text{M/L}$) after 2.4 mg of supplementary lutein per day.¹⁶ In the presence of a significant interaction between dose and disease, nine pair-wise comparisons ($n = 5$), three in each disease-stage group, were made to assess the differences between the dose groups within the treatment groups. If the interaction were not significant, a total of three pair-wise comparisons ($n = 15$) were made to assess the differences between the dose groups across the disease-stage groups at a 5% level of significance. Depending on the significance of the interactions that reflect the number of pair-wise comparisons conducted, the power can range between 74% and ~100%.

Extraction and Analysis of Carotenoids and Vitamins A and E

Aliquots of human serum (ranging from 3 to 5 mL) were extracted and analyzed for carotenoids and vitamins A and E according to our previously published methods.^{17,18} The final extract was dissolved into 250 μL of the normal-phase HPLC injection solvent and half of it was used for normal-phase HPLC analysis. The other half was evaporated under nitrogen and reconstituted to the appropriate volume using the reversed-phase HPLC injection solvent for reversed-phase HPLC analysis.

Source and Purity of Carotenoid Standards

(3*R*,3'*R*,6'*R*)-lutein (85% pure) was isolated from a saponified extract of marigold flowers (Kemin Health) and was purified to greater than 98% by two consecutive crystallizations.¹⁹ (3*R*,3'*S*,6'*R*)-lutein (3'-epi-lutein) was synthesized from (3*R*,3'*R*,6'*R*)-lutein according to a published procedure.²⁰ 3-Hydroxy- β , ϵ -caroten-3'-one (3'-oxolutein) was synthesized according to the procedure developed by Lilaen-Jensen and Hertzberg.²¹ 3'-Hydroxy- ϵ , ϵ -caroten-3-one¹⁷ and ϵ , ϵ -carotene-3,3'-dione²² were prepared according to published procedures. (3*R*,3'*R*)-Zeaxanthin and all-*E*-lycopene were provided by DSM Nutritional Products. Geometrical isomers of lutein and zeaxanthin were prepared according to a published procedure²³ and 2,6-cycloycopene-1,5 diols I and II (cycloycopenes) was prepared by partial synthesis from lycopene.²⁴

Reproducibility and Accuracy of Extraction and HPLC Analysis

The accuracy of extraction was monitored on a regular basis by extraction and analysis of carotenoids in standardized Red Cross plasma. No internal standard in the extraction and analysis of various samples was used because of the possibility that the HPLC peak would interfere with the presence of unknown carotenoids. To monitor the reproducibility of the normal-phase HPLC analysis of carotenoids, we routinely analyzed a solution containing known concentrations of lutein, zeaxanthin, and 3'-epilutein to evaluate the consistency of retention times and peak areas. Similarly, for the C₁₈ reversed-phase HPLC a standardized mixture of lycopene, α -carotene, β -carotene, α -cryptoxanthin, and β -cryptoxanthin was analyzed. The accuracy of the plasma extraction and HPLC analyses of carotenoids in this study were consistent and measured in the range of 92% to 96%.

Identification of Carotenoids and Their Metabolites

A total of 12 dietary carotenoids, 13 of their *E/Z*-stereoisomers, and 4 major metabolites of (3*R*,3'*R*,6'*R*)-lutein and/or (3*R*,3'*R*)-zeaxanthin were identified and quantified. The four metabolites were 3-hydroxy- β , ϵ -caroten-3'-one (3'-oxolutein), 3'-hydroxy- ϵ , ϵ -caroten-3-one, ϵ , ϵ -carotene-3,3'-dione, and (3*R*,3'*S*,6'*R*)-lutein (3'-epilutein). Two metabolites of lycopene, 2,6-cyclolycopene-1,5-diols I and II (cyclolycopenes), were also measured in the sera.^{18,24}

Statistical Analysis

Data were analyzed using mixed model procedures (SAS, ver. 8.2; SAS Institute Inc., Cary, NC). More specifically, a two-way analysis of variance and covariance model for repeated measurements was fitted to determine the differences in the serum concentrations of lutein, zeaxanthin, and their metabolite among the three groups of subjects with no AMD, intermediate AMD, and advanced AMD. The model included the fixed effects of treatment (2.5 mg/d of lutein and 0.13 mg/d of zeaxanthin; 5.0 mg/d of lutein and 0.25 mg/d of zeaxanthin; or 10 mg/d of lutein and 0.5 mg/d of zeaxanthin), stage of disease (no AMD, intermediate AMD, or advanced AMD), and the time of repeated measures (baseline and months 1, 3, 6, 9, and 12). Goodness-of-fit statistics was used to select an appropriate variance-covariance structure for the repeated measures. After selecting the variance-covariance structure, nonsignificant higher sources of variation were removed one at a time from the initial full model until the model contained only the variables and covariates and their significant interactions. For all the analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Nomenclature

Lutein and zeaxanthin refer to (3*R*,3'*R*,6'*R*)-lutein and (3*R*,3'*R*)-zeaxanthin, respectively. 3'-Epilutein [(3*R*,3'*S*,6'*R*)-lutein], a presumed metabolite of dietary lutein and/or zeaxanthin, is absent in foods but present in human plasma and ocular tissues.^{6,17} Zeaxanthin has three configurational isomers: (3*R*,3'*R*)-, (3*R*,3'*S*,*meso*)-, and (3*S*,3'*S*)-zeaxanthin. *meso*-Zeaxanthin is absent in foods, human plasma and liver but present in nearly all ocular tissues.²⁵ The chemical structures of these carotenoids are shown in Figure 1. The chemical structures and systematic names of the metabolites of lutein and zeaxanthin and lycopene that have also been measured in the human sera in the present study are shown in Figure 2.^{18,24}

Dietary Intake of Lutein and Baseline Characteristics

Subjects with intermediate AMD had the highest dietary intake of lutein (3.1 ± 0.19 mg/d) in comparison with subjects with-

out AMD (2.2 ± 0.19 mg/d; $P = 0.0006$) as well as those with the advanced AMD (2.4 ± 0.19 mg/d; $P = 0.0109$). There were no significant differences in dietary intake of lutein between subjects without AMD and subjects with the advanced AMD ($P = 0.3701$). The dietary intake of lutein was also significantly different among subjects receiving different treatments. Subjects on 2.5 mg/d of lutein supplements showed a significantly lower dietary intake of lutein (1.9 ± 0.18 mg/d, $P = 0.0001$) in comparison with subjects on a 5-mg/d dose (3.0 ± 0.18 mg/d) and those on a 10-mg/d dose (2.9 ± 0.18 mg/d; $P = 0.0001$).

Details regarding demographics and baseline serum levels of the subjects including, body mass index (BMI), alcohol consumption, and smoking status have been provided in an earlier publication.¹⁵

Serum Lutein Concentration

The data were analyzed by two-way analysis of variance and covariance with repeated measures fitted with the autoregressive heterogeneous first-order model [ARH (1)]. The heterogeneous compound symmetry structure presented a better fit based on the goodness-of-fit statistics and was chosen as the variance-covariance structure. Only two covariates were significant in the final model, total serum cholesterol ($P = 0.0024$) and dietary intake of lutein ($P = 0.0039$). The mean serum concentrations of lutein were significantly different in subjects on different treatments ($P = 0.0086$) and at different weeks ($P < 0.0001$); however, these values were not significantly different ($P = 0.6017$) among the three disease strata. Because the interaction between treatment and the three disease strata were not significant ($P = 0.1866$), the mean serum concentrations (nM/L) of lutein in subjects receiving the three different doses of lutein regardless of the presence or absence of AMD were combined and are shown in Table 1.

After 1 month of receiving supplements, all subjects showed a significantly higher serum concentration of lutein in comparison with their baseline levels that were in the range of 210 to 280 nM/L. In addition, after 3 months of supplementation, subjects who received 10 mg/d of lutein continued to have a significantly higher mean serum concentration (920 ± 94 nM/L; 4.4-fold increase) in comparison with subjects who were on 5-mg/d (560 ± 94 nM/L; 2.7-fold increase) and 2.5-mg/d (550 ± 94 nM/L; 2-fold increase) doses of this carotenoid. As the mean serum levels of lutein in subjects increased with supplementation over time, the standard errors corresponding to these means also increased.

At the end of the supplementation period (month 6), the mean serum lutein level in subjects (1000 ± 111 nM/L) on the lutein dose of 10 mg/d was not significantly different from that of subjects who were on the 5.0-mg/d dose (720 ± 108 nM/L). However, the mean serum concentrations of lutein in subjects receiving the 10-mg/d dose were higher than those who were on the 2.5-mg/d dose (500 ± 110 nM/L). After 3 months of follow-up (month 9), the mean serum concentrations of lutein in all 45 subjects declined to approximately 300 nM/L and remained at this level for the remaining 3 months of the follow-up period. There were no significant differences in the lutein serum levels among all subjects at the 5% level of significance after 3 and 6 months of follow-up.

Serum Zeaxanthin Concentration

The lutein supplements in the present study contained 5% zeaxanthin. The mean serum concentrations of zeaxanthin in all 45 subjects were only significantly different by month ($P < 0.0001$). The mean concentrations of zeaxanthin in the serum of all 45 subjects, regardless of the absence or severity of AMD or dose, were not significantly different. Therefore, these values for all 45 subjects at various time points were combined

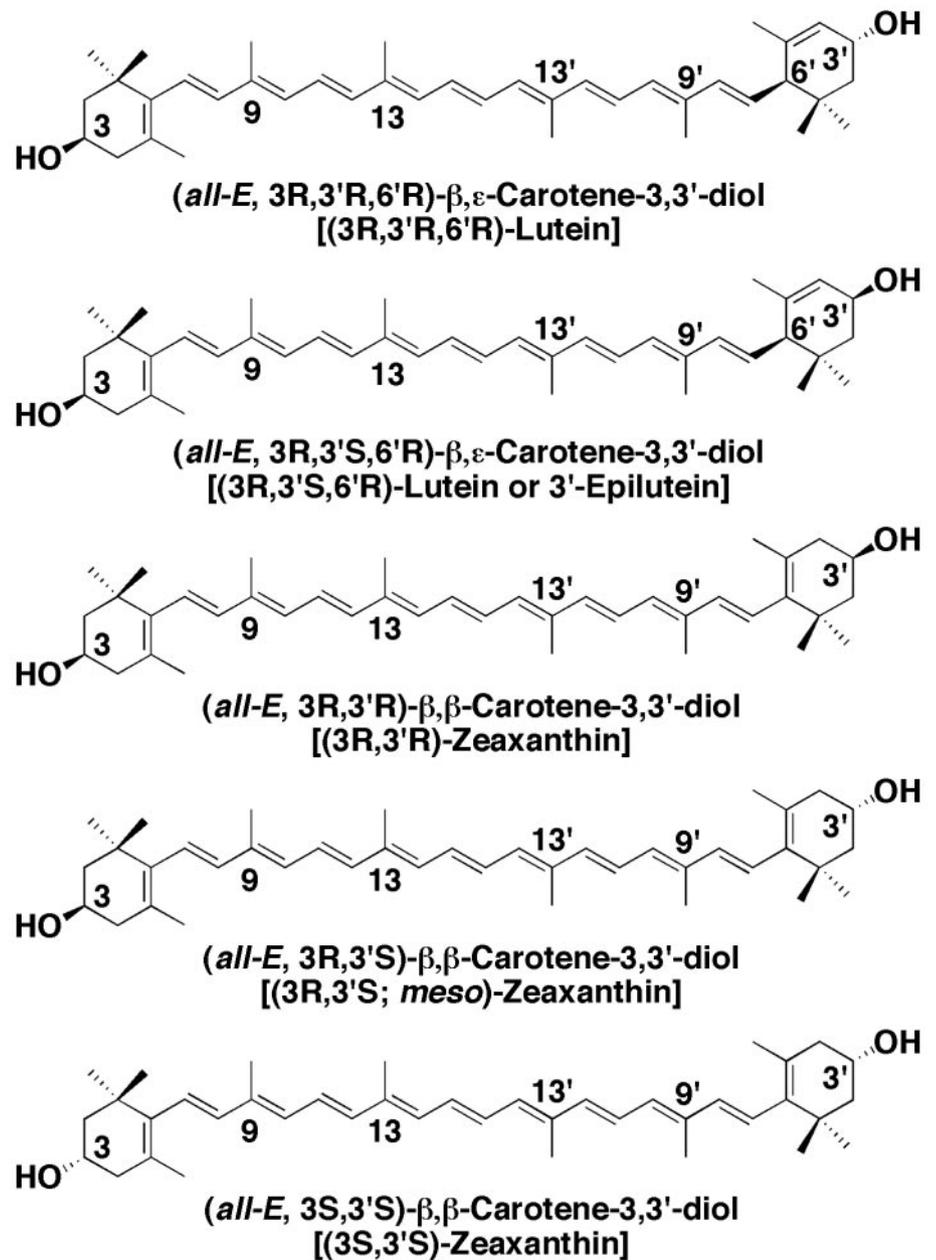


FIGURE 1. Chemical structures of dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin and their nondietary stereoisomers, (3R,3'S,6'R)-lutein (3'-epilutein), (3R,3'S; *meso*)-zeaxanthin, and (3S,3'S)-zeaxanthin. The correct systematic names of carotenoids are followed by their common names (shown in brackets).

(Table 1). The mean serum zeaxanthin concentrations in subjects increased significantly after 1 month of supplementation. The highest mean serum concentration of zeaxanthin (95 ± 9 nM/L) was achieved at the end of the supplementation period (month 6), resulting in nearly a twofold increase in the baseline levels (57 ± 6 nM/L) of this carotenoid. At the 9-month follow-up, the mean concentration of zeaxanthin in the serum of all subjects gradually declined to 71 ± 7 nM/L and remained approximately at this level for the remaining 3 months of follow-up. At the end of the follow-up period (month 12), the mean concentration of zeaxanthin (69 ± 7 nM/L) in the serum of all subjects was significantly different from baseline (57 ± 6 nM/L).

Serum Concentrations of the Metabolites of Lutein and Zeaxanthin

The oxidative metabolites of lutein and zeaxanthin that were identified and quantified in the serum of the subjects were:

3-hydroxy-β,ε-caroten-3'-one (3'-oxolutein), 3'-hydroxy-ε,ε-caroten-3-one, and ε,ε-carotene-3,3'-dione. In addition, the serum concentrations of 3'-epilutein in all subjects were similarly measured and will be discussed later. With the exception of 3'-epilutein, the mean serum concentrations of the other three oxidative metabolites of lutein and zeaxanthin as well as retinol and α-tocopherol are shown in Table 1. As indicated earlier, the mean serum lutein concentrations of the subjects were dependent on the three doses of supplementary lutein but were independent of the presence or absence of AMD. In contrast, the mean serum concentrations of zeaxanthin, the three oxidative metabolites, and fat-soluble vitamins (retinol and α-tocopherol) in all 45 subjects, did not reveal any significant correlation with dose, presence, or absence of AMD. Therefore, the mean serum concentrations of these compounds for all 45 subjects at various time points were combined. The mean serum levels of 3'-oxolutein, 3'-hydroxy-ε,ε-caroten-3-one, and ε,ε-carotene-3,3'-dione, were only

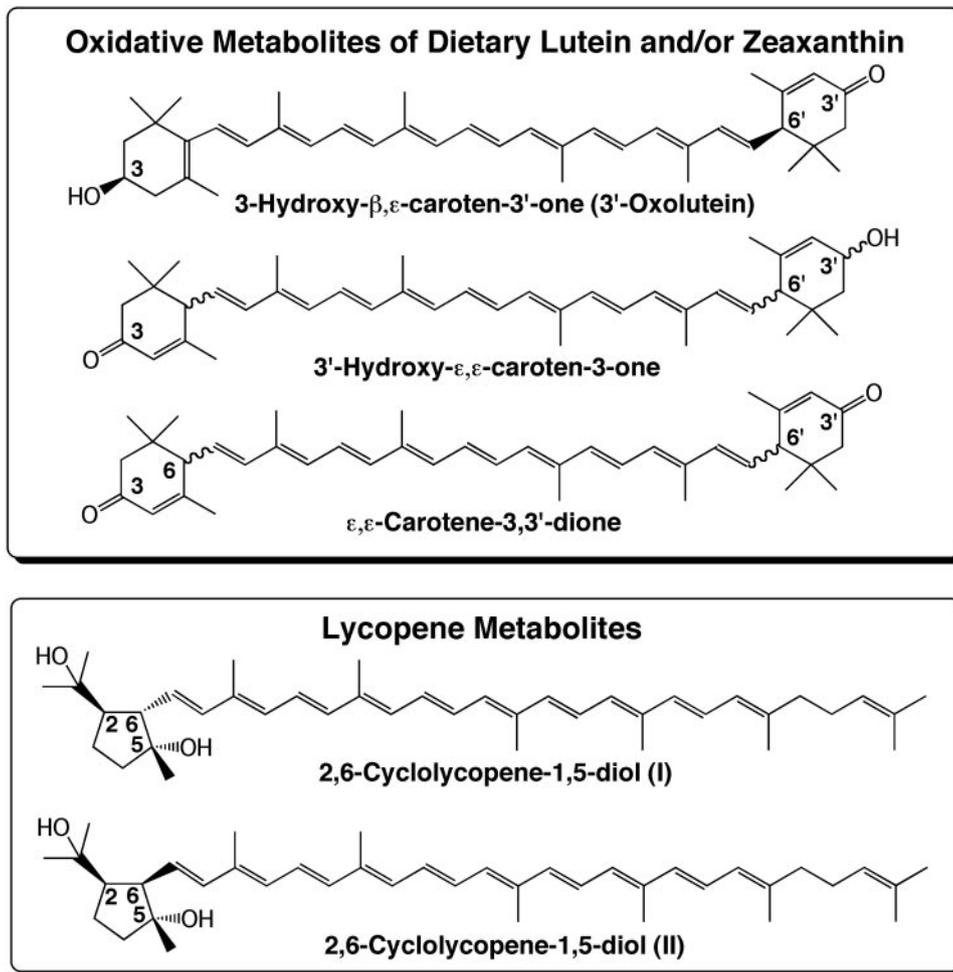


FIGURE 2. The chemical structures of the oxidative metabolites of dietary (3*R*,3'*R*,6'*R*)-lutein and/or (3*R*,3'*R*)-zeaxanthin and the metabolites of lycopene: 2,6-cyclolycopene-1,5-diol I and II. Only the relative, not the absolute, configurations of lycopene metabolites at C-2, C-5, and C-6 are known.

significantly different by month ($P < 0.0001$). After 1 month of supplementation with lutein, the mean serum level of 3'-oxolutein in all subjects increased from a baseline value of 49 ± 4 to 78 ± 6.0 nM/L, whereas the mean serum concentration of 3'-hydroxy- ϵ,ϵ -caroten-3-one in all subjects during the same time period increased from 31 ± 3 to 59 ± 5 nM/L. The highest mean serum levels for 3'-oxolutein (98 ± 7 nM/L) and 3'-hydroxy- ϵ,ϵ -caroten-3-one (80 ± 7 nM/L) in the serum of all 45 subjects were achieved after 6 months of supplementary lutein. Six months after supplementation (month 12), the mean serum concentrations of 3'-oxolutein (62 ± 4 nM/L) and 3'-hydroxy- ϵ,ϵ -caroten-3-one (38 ± 3 nM/L) were still significantly higher than their baseline values. The mean serum concentrations of 3'-oxolutein for all 45 subjects were also significantly different in relation to the covariates such as total serum cholesterol ($P = 0.0003$) and dietary intake of lutein ($P = 0.0469$). In addition, the mean serum concentration of 3'-hydroxy- ϵ,ϵ -caroten-3-one in all subjects at various time points was significantly different ($P = 0.0230$) when examined by the effect of their corresponding blood cholesterol levels.

The mean serum concentration of ϵ,ϵ -carotene-3,3'-dione in all subjects from a baseline value of 19 ± 1 nM/L increased to 28 ± 2 and 38 ± 3 nM/L after 1 month and 6 months of supplementation, respectively. The mean serum concentrations of ϵ,ϵ -carotene-3,3'-dione in all subjects were shown to be significantly different ($P = 0.0388$) by the effect of dietary intake of lutein.

The mean serum concentrations of 3'-epilutein were not significantly different by dose but were found to be significantly different among subjects with no AMD and those with

varying severity of AMD. According to our proposed pathways, 3'-epilutein can be either formed from stereospecific double-bond isomerization of zeaxanthin and/or stereospecific reduction of 3'-oxolutein (a metabolite of dietary lutein).^{6,12} The mean serum levels of 3'-epilutein for subjects with no AMD, intermediate AMD, and advanced AMD at various time points are shown in Table 2 and Figure 3. For comparison, the mean serum levels of zeaxanthin are also tabulated. After 1 month of supplementary lutein, subjects without AMD had a significantly higher ($P = 0.0017$) mean serum level of 3'-epilutein (35 ± 4 nM/L) than did those with intermediate AMD (21 ± 5 nM/L). The mean serum levels of 3'-epilutein in subjects with advanced AMD at various time points during the supplementation period were lower than those of subjects without AMD; however, the results were not significantly different ($P = 0.1243$). The mean serum concentrations of 3'-epilutein in all 45 subjects were also significantly different by month ($P = 0.0235$) as well as total serum cholesterol ($P < 0.0001$), gender ($P = 0.0148$), and dietary intake of lutein (0.0066).

Serum Concentrations of Retinol and α -Tocopherol

The mean serum concentrations of α -tocopherol were found to be significantly different ($P = 0.0001$) at baseline and each scheduled month visit (Table 1). However, the changes in the mean serum concentrations of α -tocopherol at various time points were too small to be of any biological or clinical significance.

TABLE 1. Mean Concentrations of (3R,3'R,6'R)-Lutein, (3R,3'R)-Zeaxanthin, and Their Oxidative Metabolites and Fat-Soluble Vitamins in the Serum of Subjects with Three Doses of Lutein Supplementation throughout the Study

Follow-up Time Point	Lutein			Oxidative Metabolites				Fat-Soluble Vitamins	
	Treatment 1 (2.5 mg) (n = 15)	Treatment 2 (5 mg) (n = 15)	Treatment 3 (10 mg) (n = 15)	3-Hydroxy-β,ε-caroten-3'-one (n = 45)	3'-Hydroxy-ε,ε-caroten-3-one (n = 45)	ε,ε-Carotene-3,3'-dione (n = 45)	Retinol (n = 45)	α-Tocopherol (n = 45)	
Baseline	280 ± 34 _x ^c	210 ± 34 _x ^b	210 ± 34 _x ^c	49 ± 4 ^a	31 ± 3 ^a	19 ± 1 ^c	1.4 ± 0.1 ^a	14 ± 1 ^a	
1	450 ± 86 _x ^{a,b}	490 ± 86 _x ^a	810 ± 86 _x ^a	78 ± 6 ^c	59 ± 5 ^c	28 ± 2 ^c	1.3 ± 0.1 ^a	15 ± 1 ^{a,b,c}	
3	550 ± 94 _x ^a	560 ± 94 _x ^a	920 ± 94 _x ^a	89 ± 7 ^{c,d}	72 ± 6 ^d	32 ± 3 ^b	1.3 ± 0.1 ^a	17 ± 1 ^{c,d}	
6	500 ± 110 _x ^{a,b,c}	720 ± 108 _x ^a	1000 ± 111 _x ^a	98 ± 7 ^d	80 ± 7 ^d	38 ± 3 ^a	1.3 ± 0.1 ^a	16 ± 1 ^{b,c,d}	
9	300 ± 47 _x ^{b,c}	290 ± 46 _x ^{b,y}	330 ± 48 _x ^b	60 ± 3 ^b	41 ± 4 ^b	25 ± 2 ^d	1.6 ± 0.1 ^a	18 ± 1 ^d	
12	290 ± 33 _x ^{b,c}	250 ± 32 _x ^b	260 ± 33 _x ^b	62 ± 4 ^b	38 ± 3 ^b	25 ± 2 ^d	1.4 ± 0.1 ^a	18 ± 1 ^d	

Data are the mean ± SEM (nmol/L) serum concentrations of lutein, zeaxanthin, carotenoid oxidative metabolites; mean ± SEM serum concentrations of fat soluble vitamins are expressed in μM/L. The results revealed no significant correlation with dose or presence or absence of AMD. Within a column, mean results followed by identical superscript letters (a, b, c, d, e) are not significantly different by month, as determined by repeated-measures analysis of variance with means comparison by least significant difference (LSD) at the 5% level of significance. Within a row, mean results followed by identical subscript letters (x, y) are not significantly different by treatment, as determined by repeated-measures analysis of variance with means comparison by LSD at the 5% level of significance.

Serum Concentrations of Other Carotenoids

Other major dietary carotenoids that were also identified and quantified in the serum of the subjects, were α-carotene; β-carotene; phytofluene; phytoene; lycopene and its metabolites 2,6-cyclolycopene-1,5-diols I and II (cyclolycopenes); α-cryptoxanthin; β-cryptoxanthin; and anhydrolutein. The analysis of the mean serum concentrations of these carotenoids in all subjects found no interaction with the mean serum concentrations of the supplementary lutein at the three dose levels at various time points throughout the study.

DISCUSSION

This study is the first lutein supplementation trial to involve a relatively large number of participants: 45 elderly persons with and without AMD. After 1 month of supplementation, the mean lutein concentration in the serum of all 45 subjects was significantly higher in comparison with baseline. At the end of month 1, the mean lutein serum concentration of the subjects who received 10 mg/d of lutein increased by nearly fourfold and was significantly higher than the mean serum lutein levels of subjects receiving the 2.5- or 5-mg/d doses. In the present study, after 3 months of receiving supplements, subjects receiving lutein at doses of 2.5 or 5.0 mg/d showed 2-fold and 2.7-fold increases in their mean lutein serum concentrations, respectively. However, there was no significant difference between the mean lutein serum concentrations of these two treatment groups after 3 months. Therefore, the results from the present study (Table 1) and the published data to date strongly suggest that serum concentration of lutein in human subjects is dependent on the supplemental dose of this carotenoid.

Because of the presence of zeaxanthin in lutein supplements, the subjects receiving 2.5, 5, or 10 mg/d of lutein also took daily supplements if 0.13, 0.25, or 0.5 mg of zeaxanthin, respectively. These zeaxanthin doses, although they appear to be low, are either comparable or several times higher than the highest dietary intake of this carotenoid.²⁶ In the present study, the serum concentrations of zeaxanthin in all subjects increased with supplementation, and there were no significant differences among the subjects with respect to either their disease stage or dose of this carotenoid (Table 1). Although the subjects received much lower doses of zeaxanthin than lutein, the mean serum concentration of zeaxanthin increased by 1.7-fold at the end of the supplementation period (month 6) in comparison with the mean serum baseline level. The results of the present study clearly demonstrate that the presence or absence of AMD has no impact on the mean serum concentrations of lutein or zeaxanthin in subjects who have been taking these carotenoid supplements. These findings are in agreement with a recent lutein supplementation study conducted by Koh et al.²⁷ that involved human subjects with and without AMD whose serum lutein levels were not affected by the presence or absence of disease.

The serum levels of other dietary carotenoids and fat-soluble vitamins (retinol, α-tocopherol) in all 45 subjects were also monitored throughout the entire study, to evaluate possible interaction with the three dose levels of supplementary lutein. The carotenoids quantitated were: α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lycopene and its metabolites 2,6-cyclolycopene-1,5-diols I and II (cyclolycopenes), phytofluene, and phytoene. In the present study, lutein supplementation at the three dose levels of 2.5, 5, and 10 mg/d for 6 months did not result in any significant interaction with the serum levels of other dietary carotenoids. However, due to the small sample size this finding is not conclusive.

TABLE 2. Mean Concentrations of (3R,3'R)-Zeaxanthin and 3'-Epilutein in the Serum of the Subjects with or without AMD Who Took (3R,3'R,6'R)-Lutein Supplements throughout the Study

Months	(3R,3'R)-Zeaxanthin* (n = 45)	3'-Epilutein [(3R,3'S,6'R)-Lutein]†		
		No AMD (n = 15)	Intermediate AMD (n = 15)	Advanced AMD (n = 15)
Baseline	57 ± 6 ^a	27 ± 4 _x ^a	16 ± 5 _x ^a	24 ± 4 _x ^{a,b}
1	80 ± 8 ^{c,d}	35 ± 4 _y ^{a,b}	21 ± 5 _x ^{a,b}	31 ± 4 _{x,y} ^{b,c,d}
3	86 ± 8 ^{d,e}	34 ± 4 _x ^a	25 ± 5 _x ^b	25 ± 4 _x ^{a,b,c}
6	95 ± 9 ^e	44 ± 4 _y ^b	19 ± 5 _x ^{a,b}	37 ± 5 _y ^d
9	71 ± 7 ^{b,c}	34 ± 4 _y ^a	19 ± 5 _x ^{a,b}	34 ± 5 _y ^{c,d}
12	69 ± 7 ^b	36 ± 4 _y ^{a,b}	17 ± 5 _x ^{a,b}	23 ± 5 _x ^{a,b}

Data are the mean ± SEM (nmol/L). Superscript letters are explained in the footnote to Table 1. Within a row, mean results followed by identical subscript letters (x, y) are not significantly different by disease stage at the 5% level of significance.

* Mean ± SEM serum concentrations of (3R,3'R)-zeaxanthin in subjects with and without AMD were not significantly different by dose or presence or absence of AMD, and as a result these values for all 45 subjects at various time points were combined.

† Mean ± SEM serum concentrations of 3'-epilutein were not significantly different by the three doses of supplemental lutein.

There was no significant interaction between lutein and retinol in subjects who received lutein supplements at the three dose levels ($n = 15$ for each dose level). This was also shown to be the case for α -tocopherol. Of note, the mean serum level of α -tocopherol in all 45 subjects was significantly higher ($P < 0.0001$) after 3 months of supplementary lutein, regardless of the dose, in comparison with baseline, and these levels remained elevated throughout the follow-up period. The low level of α -tocopherol (1%) that was present in the lutein supplements could have caused this increase. It must be noted that the dietary intake of α -tocopherol was not assessed in subjects and, as a result, the elevated mean serum levels of α -tocopherol may be due to a greater dietary intake of this vitamin.

Metabolites of Lutein and Zeaxanthin in the Serum of the Subjects

In 1992, we reported on characterization and quantification of several carotenoids in human plasma that were not of dietary origin and based on their chemical structures, we proposed

that these compounds result from metabolism of dietary lutein and zeaxanthin.¹⁷ These carotenoids which were also identified in the serum of all 45 subjects who received supplementary lutein and low doses of zeaxanthin in the present study, were 3-hydroxy- β , ϵ -caroten-3'-one (3'-oxolutein), 3'-hydroxy- ϵ , ϵ -caroten-3-one, ϵ , ϵ -carotene-3,3'-dione, and (3R,3'S,6'R)-lutein (3'-epilutein). In addition to human serum, the same carotenoid metabolites have been identified in the extracts of human breast milk, as well as major organs and tissues.^{18,28} In 1995, we conducted two separate human studies of lutein and zeaxanthin supplementation that provided preliminary evidence for the in vivo conversion of these dietary carotenoids to their aforementioned metabolites.^{12,13} Subsequent to these findings, the same metabolites of lutein and zeaxanthin were also identified in all ocular tissues of humans and in the retina of a monkey.^{6,7} On the basis of all our findings to date, we have proposed the most likely pathways that lead to the formation of lutein and zeaxanthin metabolites in humans.^{6,12,13,25,28,29} As summarized in Figure 4, these transformations involve a series of

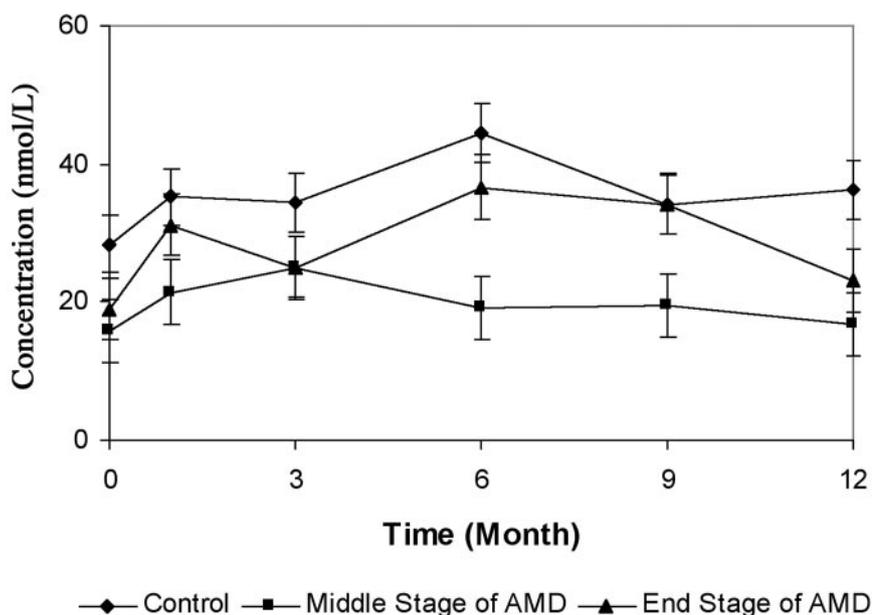


FIGURE 3. Mean ± SEM serum concentration (nmol/L) time curve of 3'-epilutein [(3R,3'S,6'R)-lutein] in subjects without AMD ($n = 15$), with intermediate AMD ($n = 15$), and with advanced AMD ($n = 15$) who received one of three doses of supplementary lutein for 6 months.

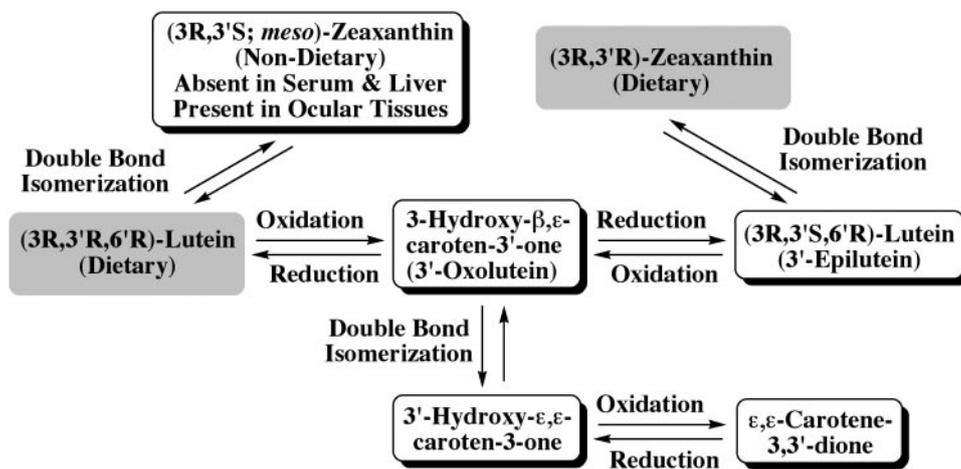


FIGURE 4. Proposed metabolic pathways for transformation of dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin into their metabolites in humans. With the exception of (3R,3'S; meso)-zeaxanthin, which is absent in human liver and serum but present in ocular tissues, all other carotenoids have been detected in serum, major organs, and ocular tissues. The chemical structures are shown in Figures 1 and 2.

oxidation–reduction and double-bond isomerization reactions of dietary lutein and zeaxanthin. Undoubtedly, in the absence of human supplementation studies with stable isotopes of lutein and zeaxanthin, these studies could not unequivocally establish the true metabolic pathways of these carotenoids. However, the present study that was conducted in a larger number of subjects, particularly those with AMD, could provide valuable insight into the possible metabolic consequence of supplementation with the three doses of lutein and zeaxanthin. As shown in Table 1, supplementation with lutein containing low levels of zeaxanthin resulted in a significant increase in the mean serum concentrations of 3'-oxolutein (3-hydroxy-β,ε-caroten-3'-one), 3'-hydroxy-ε,ε-caroten-3-one, and ε,ε-carotene-3,3'-dione in all 45 subjects after 1 month. 3'-Oxolutein is the key metabolite that can most likely serve as a precursor to the other two metabolites, 3'-hydroxy-ε,ε-caroten-3-one and ε,ε-carotene-3,3'-dione (Fig 4). 3'-Oxolutein itself can be directly formed from lutein by allylic oxidation and perhaps to a lesser extent from zeaxanthin, via 3'-epilutein. We conducted extensive statistical analyses using Pearson correlations to correlate separately the mean serum concentrations of lutein and zeaxanthin in the subjects with the mean serum levels of 3'-oxolutein and 3'-epilutein. Although the data suggest that the mean serum concentration of 3'-oxolutein in all subjects correlates well with the mean serum levels of lutein and zeaxanthin, the extent to which this metabolite may be formed from lutein and/or zeaxanthin remains inconclusive. It is interesting to note that the mean serum concentration of 3'-epilutein and 3'-oxolutein in the subjects were not dependent on the dose of supplementary lutein. One explanation for this observation may be that once 3'-epilutein and 3'-oxolutein are formed, these metabolites mostly revert to their parent dietary lutein and zeaxanthin. This notion is consistent with the expected mechanism of action of antioxidants. It should be noted that the mean serum concentration of 3'-epilutein in subjects without AMD was significantly higher than in those with intermediate AMD, but not when compared with the level in subjects with advanced AMD. In supplementation studies with lutein and zeaxanthin, Khachik et al.¹² did not find any significant increase in the plasma concentration of 3'-epilutein in healthy humans who received supplementary lutein but found a significant increase in the plasma levels of this metabolite when the subjects received supplementary zeaxanthin.

The metabolic pathways shown in Figure 4 also depict the conversion of dietary lutein to the nondietary (3R,3'S; meso)-zeaxanthin, which was not detected in the serum of the sub-

jects who took lutein supplements. However, as we have recently shown, this carotenoid is absent in human serum and liver but present in human ocular tissues.²⁵ In a study of rhesus monkeys that received supplementary lutein and zeaxanthin, Johnson et al.³⁰ provided additional support for metabolic transformation of lutein to meso-zeaxanthin. They demonstrated that meso-zeaxanthin that was absent in the retinas of xanthophyll-free and zeaxanthin-fed monkeys, was present in the retinas of xanthophyll-free monkeys after supplementary lutein.

Despite the fact that the metabolites of lutein and zeaxanthin have been isolated and characterized in nearly all human ocular tissues such as ciliary body, retinal pigment epithelium (RPE), iris, lens, macula, and peripheral retina, their role and biological functions, if any, remain unclear.⁷ The presence of lutein and zeaxanthin metabolites in human ocular tissues may be attributed to photoinduced and/or enzymatic metabolic transformation of these carotenoids by a process unique to the eye or, alternatively, these metabolites may be simply transported to and accumulated in ocular tissues via the circulatory system.

CONCLUSION

Although supplementary lutein increased the mean serum level of this carotenoid in all subjects in a dose-dependent manner, there were no differences in the serum lutein response of the subjects with and without AMD. The exception to the findings was the mean serum level of 3'-epilutein which was significantly higher in subjects without AMD than in those with intermediate AMD, but was not higher in those with advanced AMD. Supplementary lutein containing low levels of zeaxanthin (5%) resulted in an increase in the serum concentrations of the metabolites of these carotenoids. The mean serum concentrations of lutein, zeaxanthin, and 3'-epilutein in human subjects gradually return to baseline levels within 6 months after supplementation. In contrast, 6 months after supplementation, the mean serum concentrations of 3'-oxolutein, 3'-hydroxy-ε,ε-caroten-3-one, and ε,ε-carotene-3,3'-dione remained significantly higher than baseline. No toxicity or side effects were associated with supplementation with lutein up to a dose of 10 mg/d, based on results of liver function tests and visual function examinations.

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