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Clinical Trials

Supplementation with All Three Macular Carotenoids: Response, Stability, and Safety

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Purpose. This study was designed to investigate serum and macular response to, and safety of supplementation with, *meso*-zeaxanthin (MZ), lutein (L), and zeaxanthin (Z), the carotenoids that constitute macular pigment (MP).

Methods. Forty-four healthy subjects were recruited into this randomized, placebo-controlled, clinical trial. Subjects consumed one tablet per day containing 10.6 mg MZ, 5.9 mg L, and 1.2 mg Z (intervention, I group) or placebo (P group). The spatial profile of MP optical density (MPOD) was measured with customized heterochromatic flicker photometry (cHFP), and serum concentrations of L and Z were quantified by using high performance liquid chromatography (HPLC). Subjects were assessed at baseline and at 3 and 6 months. Clinical pathology analysis was performed at baseline and 6 months.

Results. Serum concentrations of L and Z increased significantly in the I group ($P = 0.001$ and 0.003, respectively) and remained stable in the P group ($P > 0.05$). There was a significant increase in central MPOD in the I group (0.25°: $P = 0.001$; 0.5°: $P = 0.001$), with no significant change in the P group ($P > 0.05$). Clinical pathology analysis confirmed that all variables remained within the normal reference range, with the exception of total cholesterol and low-density lipoprotein (LDL), which exhibited baseline values outside the accepted normal reference range before supplementation.

Conclusions. Subjects supplemented with MZ, L, and Z exhibited significant increases in serum concentrations of these carotenoids and a subsequent increase in central MPOD. Pathology analysis suggested no adverse clinical implications of consuming these carotenoids. (http://isrctn.org number, ISRCTN60816411.) (*Invest Ophthalmol Vis Sci.* 2011;52:9207–9217) DOI:10.1167/iovs.11-8025

The macula houses a yellow pigment, attributable to the carotenoids *meso*-zeaxanthin (MZ), lutein (L), and zeaxanthin (Z). Indeed, this pigment lends its name to the macula lutea (Latin for yellow), and has been more recently referred to as macular pigment (MP).[1] Interestingly, of the more than 700 carotenoids identified in nature, these three dietary carotenoids selectively accumulate at the macula,[1–3] indicating an exquisite degree of biological selectivity in this retinal tissue.

An average Western diet contains 1.3 to 3 mg/d of L and Z combined,[4] with substantially more L than Z (represented by an estimated ratio of ~7:1). It has been reported that approximately 78% of dietary L and Z is sourced from vegetables, with L found in highest concentrations in dark green, leafy vegetables.[5] It appears that humans ingest relatively low levels of MZ, although it should be noted that there has been no satisfactory published investigation of MZ concentrations in the foods of a typical diet. Interestingly, despite its absence or low concentrations in a normal diet, MZ accounts for about one third of total MP at the macula, consistent with the hypothesis that retinal MZ is produced primarily by isomerization of retinal L at the macula.[6]

Age-related macular degeneration (AMD) is a degenerative condition of the macula, and its late form is the most common cause of blind registration in the developed world.[7] It is now accepted that AMD is the result of (photo) oxidation-induced retinal injury. However, the anatomic (central retinal),[8] biochemical (antioxidant),[9] and optical (short wavelength–filtering)[10] properties of MP suggest that this pigment may confer protection against AMD (protective hypothesis).[11] Also, its optical (short wavelength–filtering) properties suggest that MP plays a role in visual performance and experience in the normal population (visual performance hypothesis).[12] The protective and visual performance hypotheses of MP have led to significant research in this area. However, questions asked by eye care professionals often relate to the response to (in blood and at the macula) and safety of supplementation with these carotenoids.

This study was designed to assess response and also the safety of consumption of the macular carotenoids MZ, L, and Z by analyzing blood samples for changes in renal and liver function, as well as lipid profile, hematologic profile, and markers of inflammation after 6 months of supplementation.

Methods

Study Design

The *meso*-zeaxanthin ocular supplementation trial in normals (MOST-N) is a double blind, randomized, placebo controlled, clinical trial registered with the International Standard Randomized Controlled Trial Register. All subjects signed an informed consent document, and the experimental

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measures conformed to the tenets of the Declaration of Helsinki. The study was reviewed and approved by the Research Ethics Committee, South East Region, Waterford Regional Hospital and by the Ethics Committee at Waterford Institute of Technology, Waterford, Ireland.

Forty-four healthy subjects were recruited into the study, which consisted of two groups: intervention (I, $n = 22$) and placebo (P, $n = 22$). The inclusion criteria were as follows: men and women between the ages of 18 and 61 years; absence of ocular disease by self-report; no clinical signs of retinal disease, according to expert assessment of fundus photographs; visual acuity of at least 20/60 in the study eye; not currently taking supplements containing MZ, L, and Z; and not pregnant.

Supplementation and Examination Procedures

The formulation for this study was manufactured by Industrial Organica SA (Monterrey, Mexico) by isomerizing L obtained from marigold extracts. A proportion of L (60%) was converted into MZ, and the small quantity of Z in the extract remained unchanged. The resulting composition was microencapsulated after dilution with rice starch. After consistency testing, it was confirmed that the capsules contained 10.6 mg MZ, 5.9 mg L, and 1.2 mg Z (confirmed by high-performance liquid chromatography [HPLC] analysis). The placebo consisted of rice starch and was microencapsulated to look identical with the carotenoid I capsule.

All subjects were instructed to take one capsule per day with a meal for 6 months. At baseline, demographic, lifestyle, and vision information was also collected from each subject, including name, contact information, age, sex, body mass index (BMI), smoking habits, lifestyle, medication, and vision history. Baseline dietary intakes of L and Z were quantified with a self-administered, semiquantitative food frequency questionnaire developed by the Scottish Collaborative Group at the University of Aberdeen (Scotland, UK), recently described by O'Connell et al.[13]

Best corrected visual acuity (BCVA) was measured at baseline with a computer-generated logMAR test chart (Test Chart 2000 Pro; Thompson Software Solutions, Hatfield, UK) at a viewing distance of 4 m, using the Sloan ETDRS letterset. BCVA was recorded by using a letter-scoring visual acuity rating, with 20/20 visual acuity assigned a value of 100. BCVA was scored relative to this value, with each letter correctly identified assigned a nominal value of one, for example, a BCVA of $20/20^{+1}$ equated to a score of 101 and $20/20^{-1}$ to 99. The eye with better visual acuity was chosen as the study eye; however, when both eyes had the same corrected acuity, the right eye was chosen as the study eye.

Contrast sensitivity was measured with a computer-generated letter-contrast test similar in design to a Pelli-Robson chart.[14]

Retinotopic ocular sensitivity was assessed by microperimetry (MP1; Nidek, Gamagori, Japan). The central 6° of fixation were examined and are reported as macular mean sensitivity (MMS) within 2°, 4°, and 6° of the macula. Fundus photography was also performed at each study visit, and the photographs were assessed by a vitreoretinal specialist to confirm the absence of significant retinal pathology. MPOD, including its spatial profile (i.e., 0.25°, 0.5°, 1°, and 1.75°), was measured at each study (i.e., visit baseline, three and six months [V1, V2, and V3, respectively]) using a customized heterochromatic flicker photometry (cHFP) method previously described.[15]

A blood sample was collected at V1, V2, and V3, respectively for serum carotenoid analysis of L and Z, by a method previously described by our group.[15] Additional blood samples were collected at V1 and V3 for clinical pathology analysis.

Clinical Pathology Analysis

Clinical pathology analysis was performed on all subjects at V1 before supplementation and at V3 (i.e., after 6 months) to test for any change in renal and liver function, lipid profile, hematologic profile, and markers of inflammation after supplementation with MZ, L, and Z. To achieve this, nonfasting blood samples were collected at both visits by standard venipuncture techniques. The blood was collected in three plastic collection tubes as follows: Tube 1 (serum) contained an added clot activator and gel layer, tube 2 (glucose) contained sodium fluoride, and tube 3 (hematology) contained the anticoagulant dipotassium ethylene diamine tetra-acetic acid (K_2EDTA). All collection tubes were labeled with the subject's number, visit number, and date and were inverted a minimum of eight times to ensure appropriate mixing of the blood with each additive in the tubes.

The serum tube was centrifuged within 2 hours of collection, and a 1-mL sample was aliquotted into a clean, labeled, plastic tube that was then transported with the other two tubes to Biomnis Ireland (Dublin, Ireland; Irish National Accreditation Board certified), for independent analysis. All pathology variables tested are outlined in Table 1. Analysis at Biomnis Laboratories was conducted using one of two integrated diagnostic immunoassay systems (Abbott Architect ci8200; Abbott Labs, Abbott Park, IL, or Advia 120; Siemens Healthcare Diagnostics, Deerfield, IL), as appropriate. The reference ranges for this study were obtained from the insert kits for the instrumentation used by Biomnis Laboratories. The only exceptions were the lipids (high-density lipoproteins [HDL], LDL, total cholesterol, and triglycerides) for which the reference ranges were obtained from the European Guidelines on Cardiovascular Disease Prevention,[16] and glucose, for which the reference range came from the World Health Organization.[17]

Statistical Analysis

Means ± SDs are presented in the text and tables (SPSS ver. 17; SPSS, Chicago, IL, was used for analysis; SigmaPlot, ver. 8.0, SyStat Software, San Jose, CA, for graphic presentations). Between group differences in age, BMI, baseline serum carotenoid concentration, and baseline MPOD levels were investigated by using independent-samples *t*-tests. The between-group difference with respect to sex and smoking habits were investigated by using the standard χ^2 test. Pearson correlation coefficient analyses were conducted to investigate bivariate relationships. Repeated-measures analysis of variance was conducted to investigate changes in serum concentrations of L and Z and MPOD (including its spatial profile) across the three study visits, by a general linear model approach. Differences between two time points within subjects were assessed with paired-samples *t*-tests. We used the 5% level of significance throughout our analysis.

Results

Baseline

The demographic, lifestyle, dietary intake of L and Z (mg/d), serum concentrations of L and Z (μmol/L), and MPOD data at baseline for the I and P groups ($n = 44$) are presented in Table 2. There was no statistically significant difference between groups in terms of any of these variables at baseline ($P > 0.05$, for all). Statistically significant relationships between variables at baseline are presented in Table 3 and Figure 1.

Compliance with Study Visits

Of the 44 subjects recruited into the study, 18 from the I group and 17 from the P group attended and completed all study visits (i.e., V1, V2, and V3). Four subjects were lost to follow-up (personal reasons [e.g., death in family]), and the remainder did not attend V2.

Retinal Findings

No significant change was observed in retinal sensitivity at 6 months for any of the microperimetry tests performed (i.e., MMS 2°, MMS 4°, MMS 6°, $P > 0.05$, for all tests). There was no noticeable change in retinal findings at 6 months (confirmed by a vitreoretinal specialist).

Table 1. Clinical Pathology Variables Assessed at Baseline (V1) and after a 6-Months (V3) Supplementation with Meso-zeaxanthin, Lutein, and Zeaxanthin in Both the Intervention and Placebo Groups

Pathology Variable	Function of Test	Reference Range (Unit)	V1 I*	V3 I	*P* Value I	V1 P†	V3 P	*P* Value P
Sodium	Renal profile	135–145 (mmol/L)	139.42 ± 1.68	139.26 ± 2.08	0.51	139.26 ± 2.05	139.26 ± 1.69	1.00
Potassium	Renal profile	3.3–5.3 (mmol/L)	4.16 ± 0.36	4.55 ± 0.40	**0.01**	4.26 ± 0.30	4.43 ± 0.24	**0.04**
Chloride	Renal profile	98–107 (mmol/L)	104.05 ± 2.55	98.89 ± 21.35	0.32	104.05 ± 1.72	103.11 ± 1.97	0.15
Urea	Renal profile	2.5–7.7 (mmol/L)	4.72 ± 1.16	5.03 ± 1.11	0.23	5.31 ± 1.40	5.37 ± 1.53	0.76
Creatinine	Renal profile	40–90 (μmol/L)	75.11 ± 14.13	76.84 ± 11.70	0.42	77.00 ± 14.36	74.68 ± 14.97	0.15
Total protein	Liver profile	64–83 (g/L)	72.63 ± 3.53	71.05 ± 3.12	0.10	71.63 ± 3.58	70.05 ± 4.97	0.12
Albumin	Liver profile	37–52 (g/L)	44.47 ± 1.84	44.58 ± 2.67	0.82	43.53 ± 1.98	44.21 ± 3.78	0.30
Globulins	Liver profile	21–36 (g/L)	28.16 ± 3.29	26.47 ± 2.95	0.11	28.11 ± 3.63	26.37 ± 4.11	**0.07**
Total bilirubin	Liver profile	3.4–21.0 (μmol/L)	8.73 ± 4.94	8.21 ± 3.85	0.59	8.05 ± 2.62	8.77 ± 2.99	0.29
Alanine aminotransferase	Liver profile	0–55 IU/L	24.32 ± 18.18	19.42 ± 7.62	0.18	22.47 ± 14.11	23.16 ± 14.72	0.63
Aspartate aminotransferase	Liver profile	5–36 IU/L	20.37 ± 4.68	19.05 ± 4.59	0.16	22.16 ± 8.25	21.89 ± 10.13	0.81
Alkaline phosphate	Liver profile	40–150 IU/L	78.84 ± 27.32	74.63 ± 17.65	0.41	79.00 ± 62.93	79.95 ± 76.25	0.80
Gamma glutamyl transpeptidase	Liver profile	9–36 IU/L	33.84 ± 40.39	25.05 ± 17.25	0.29	25.16 ± 12.33	23.89 ± 11.55	0.42
Cholesterol total	Lipid profile	<5.0 (mmol/L)	5.21 ± 0.92	5.24 ± 0.91	0.79	5.26 ± 0.93	4.92 ± 0.86	**0.02**
Triglycerides	Lipid profile	0.60–1.70 (mmol/L)	1.38 ± 0.75	1.66 ± 0.93	0.13	1.10 ± 0.44	1.09 ± 0.68	0.93
HDL	Lipid profile	1.00–1.55 (mmol/L)	1.46 ± 0.33	1.49 ± 0.31	0.63	1.54 ± 0.32	1.51 ± 0.32	0.46
Direct LDL	Lipid profile	<3.0 (mmol/L)	3.03 ± 0.75	3.25 ± 0.80	**0.01**	3.13 ± 0.84	2.98 ± 0.80	0.23
Calcium	Bone profile	2.10–2.60 (mmol/L)	2.38 ± 0.07	2.35 ± 0.10	0.33	2.36 ± 0.09	2.36 ± 0.12	0.80
Phosphate	Bone profile	0.80–1.56 (mmol/L)	1.16 ± 0.16	1.14 ± 0.15	0.63	1.10 ± 0.21	1.09 ± 0.13	0.82
Magnesium	Bone profile	0.65–1.10 (mmol/L)	1.00 ± 0.07	0.95 ± 0.09	**0.01**	0.98 ± 0.06	0.92 ± 0.06	**0.01**
Uric acid	Bone profile	155–394 (μmol/L)	263.47 ± 94.34	273.47 ± 85.91	0.19	274.68 ± 88.78	271.74 ± 85.68	0.76
Glucose	Bone profile	3.1–6.1 (mmol/L)	5.31 ± 2.10	5.77 ± 2.94	0.11	5.03 ± 0.41	4.94 ± 0.47	0.50
High sensitive reactive protein	Inflammation marker	<5.0 (mg/L)	4.00 ± 7.36	3.31 ± 4.88	0.57	1.49 ± 1.25	4.18 ± 13.40	0.40
Full blood count								
White cells	Hematology	3.88–10.49 (10e9/L)	7.07 ± 2.00	6.79 ± 1.49	0.24	5.97 ± 1.24	6.92 ± 2.34	0.10
Red cells	Hematology	3.73–5.02 (10e12/L)	4.53 ± 0.43	4.58 ± 0.40	0.35	4.64 ± 0.36	4.58 ± 0.36	0.30
Hemoglobin	Hematology	11.3–15.2 (g/dL)	14.23 ± 1.35	13.91 ± 1.37	**0.03**	14.46 ± 1.46	13.85 ± 1.28	**0.01**
Hematocrit	Hematology	0.323–0.462 (L/L)	0.40 ± 0.04	0.41 ± 0.04	**0.01**	0.40 ± 0.40	0.41 ± 0.03	0.38
MCV‡	Hematology	83.1–99.1 (fL)	87.93 ± 4.33	90.41 ± 4.54	**0.01**	87.06 ± 3.02	89.42 ± 3.22	**0.01**
MCH§	Hematology	28.3–33.9 (pg)	31.42 ± 1.51	30.38 ± 1.54	**0.01**	31.15 ± 1.58	30.28 ± 1.34	**0.01**
MCHC‖	Hematology	32.1–36.6 (g/dL)	35.75 ± 0.98	33.62 ± 0.93	**0.01**	35.78 ± 1.36	33.88 ± 1.09	**0.01**
Platlets	Hematology	164–382 (10e9/L)	295.47	287.00	0.24	313.28	299.00	**0.08**
Differential White Cell Count								
Neutrophils	Hematology	1.91–7.16 (10e9/L)	4.39 ± 1.57	4.05 ± 1.01	0.15	3.44 ± 0.72	4.18 ± 2.02	0.16
Lymphocytes	Hematology	1.01–3.13 (10e9/L)	1.85 ± 0.67	1.86 ± 0.57	0.92	1.72 ± 0.65	1.87 ± 0.73	**0.04**
Monocytes	Hematology	0.19–0.68 (10e9/L)	0.42 ± 0.10	0.39 ± 0.80	0.23	0.36 ± 0.08	0.40 ± 0.14	0.21
Eosinophils	Hematology	0.05–0.51 (10e9/L)	0.25 ± 0.20	0.27 ± 0.15	0.62	0.24 ± 0.17	0.23 ± 0.12	0.79
Basophils	Hematology	0.02–0.15 (10e9/L)	0.07 ± 0.03	0.07 ± 0.02	0.71	0.10 ± 0.07	0.07 ± 0.04	0.10
Large unstained cells	Hematology	0.00–0.30 (10e9/L)	0.14 ± 0.03	0.13 ± 0.03	0.81	0.12 ± 0.04	0.16 ± 0.06	**0.01**

Paired-samples *t*-tests were performed on all variables between baseline and 6 months. Bold *P* values indicate significant differences. Data are expressed as the mean ± SD for all variables tested.

* Intervention group.

† Placebo group.

‡ Mean corpuscular volume.

§ Mean corpuscular hemoglobin.

‖ Mean corpuscular hemoglobin concentration.

Table 2. Baseline Characteristics of the Intervention and Placebo Group

Characteristic	Intervention (n = 22)	Placebo (n = 22)
Age, y	43 ± 13	45 ± 12
18–30, *n*	5	4
31–40, *n*	3	3
41–50, *n*	6	6
51–60, *n*	6	9
61, *n*	2	0
BMI*	27.2 ± 6.1	26.8 ± 5
BCVA†	116 ± 7.8	116 ± 7.9
Log letter contrast sensitivity	1.61 ± 0.17	1.60 ± 0.25
Microperimetry		
MMS2°‡	13.43 ± 2.0	13.09 ± 2.3
MMS4°	13.05 ± 1.8	12.63 ± 1.7
MMS6°	11.05 ± 1.9	10.69 ± 1.8
Dietary lutein, mg/d	1.33 ± 0.76	1.19 ± 0.74
Dietary zeaxanthin, mg/d	0.19 ± 0.07	0.21 ± 0.16
Serum lutein	0.40 ± 0.12	0.40 ± 0.17
Serum zeaxanthin	0.18 ± 0.07	0.20 ± 0.08
MPOD		
0.25°	0.45 ± 0.21	0.45 ± 0.19
0.5°	0.37 ± 0.18	0.38 ± 0.19
1°	0.26 ± 0.13	0.23 ± 0.12
1.75°	0.13 ± 0.08	0.09 ± 0.09
Sex, *n*		
Male	8	9
Female	14	13
Smoking habits, *n*§		
Current	5	4
Past	8	4
Never	9	14

Data are presented as the mean ± SD, unless otherwise noted.

* Defined as body weight in kilograms divided by height in square meters (kg/m^2).

† Recorded using a letter-scoring visual acuity rating, with 20/20 visual acuity assigned a value of 100. BCVA was scored relative to this value, with each letter that was correctly identified assigned a nominal value of 1. For example, a BCVA of 20/20^{-1} equated to a score of 101, and 20/20^{-1} to 99.

‡ Defined by the mean retinotopic ocular sensitivity within 2°, 4°, and 6° of the macula.

§ Smoking habits: Never smokers had smoked less than 100 cigarettes in their lifetime. Past smokers had smoked at least 100 cigarettes in their lifetime, but had not smoked for at least 1 year before the investigation. Current smokers had smoked at least 100 cigarettes in their lifetime and had at least one cigarette in the year before the investigation. Independent-samples *t*-tests showed no significant difference between groups; differences between smoking and the sex of the subject were analyzed using χ^2 analysis.

Lutein and Zeaxanthin Response in Serum

There was a statistically significant increase in serum concentrations of L and Z (μmol/L) from baseline at 3 (L: $P = 0.001$; Z: $P = 0.001$) and 6 (L: $P = 0.001$; Z: $P = 0.001$) months in the I group. There was no significant change from baseline in serum concentrations of L or Z in the P group over this period ($P > 0.05$, for both). These findings are consistent with repeated-measures analysis of variance, which showed a statistically significant time–study arm interaction effect ($P = 0.001$ for L and $P = 0.003$ for Z; Figs. 2A, 2B).

MPOD Response

There was a statistically significant increase in MPOD at 0.25° retinal eccentricity from baseline at 3 and 6 months in the I group ($P = 0.001$, for both). There was no significant change from baseline in MPOD at 0.25° retinal eccentricity in the P group at either time point ($P > 0.05$, for both). Repeated-measures analysis did not show a statistically significant time–study arm interaction effect ($P > 0.05$; Fig. 2C).

There was a statistically significant increase in MPOD at 0.5° retinal eccentricity from baseline at 3 and 6 months in the I group ($P = 0.001$ and 0.01, respectively). No significant change was observed at this eccentricity in the P group at either time point ($P > 0.05$, for both). Repeated-measures analysis showed a significant time–study arm interaction effect ($P = 0.016$; Fig. 2D).

There was no statistically significant increase at 1° and 1.75° retinal eccentricity from baseline at 3 or 6 months in either the I or P group ($P > 0.05$, for all).

Clinical Pathology Analysis

We report a statistically significant variation from baseline to 6 months (in both positive and negative directions) in 8 of the 25 variables assessed in the I group and 9 of the 25 variables assessed in the P group after supplementation with the macular carotenoids (Table 1). However, all variables remained within the normal reference range, with the exception of total cholesterol and LDL, which had a baseline value outside the accepted normal reference range in both the I and P groups before supplementation (i.e., at baseline) with the macular carotenoids (HDL, LDL, and total cholesterol reference ranges were taken from the European Guidelines on Cardiovascular Disease Prevention).

Discussion

The MOST-N study was designed to measure serum and macular response to a dietary supplement containing all three macular carotenoids (MZ, L, and Z) in the normal population (Irish Republic), as part of a randomized, double-blind, placebo-controlled clinical trial, and to assess the safety of consumption of these carotenoids by performing clinical pathology analysis.

To date there have been many published studies in the scientific literature that have reported on the effect of macular carotenoid supplementation on serum concentrations of these carotenoids, with the majority of the studies reporting significant increases in serum concentrations of L and Z after supplementation with these carotenoids (Table 4), and recent studies have reported and confirmed significant serum MZ response after supplementation with this carotenoid. Consistent with these previous studies, we report statistically significant increases in serum concentrations of L and Z in the I group, whereas, as expected, the P group remained stable over the study

Table 3. Significant Relationships between Baseline Variables for the Entire Study Group before Intervention

Dependent Variable	Independent Variable	Pearson Coefficient (r)	Significance (P)
MPOD 0.5°	BMI	−0.322	0.035
MPOD 1°	BMI	−0.355	0.019
MPOD 1.75°	BMI	−0.322	0.035
Serum lutein	BMI	−0.516	0.001
Serum zeaxanthin	BMI	−0.524	0.001
MMS 2°	Age	−0.409	0.007
MPOD 0.25°	Serum zeaxanthin	0.373	0.016
MMS 2°	MPOD 0.25°	0.304	0.050
MPOD 1°	Serum zeaxanthin	0.343	0.028
Serum Lutein	Age	0.318	0.040
Total Cholesterol	Age	0.439	0.004
BCVA	Serum lutein	0.318	0.040
Serum lutein	Diet lutein	0.374	0.017

$n = 44$.

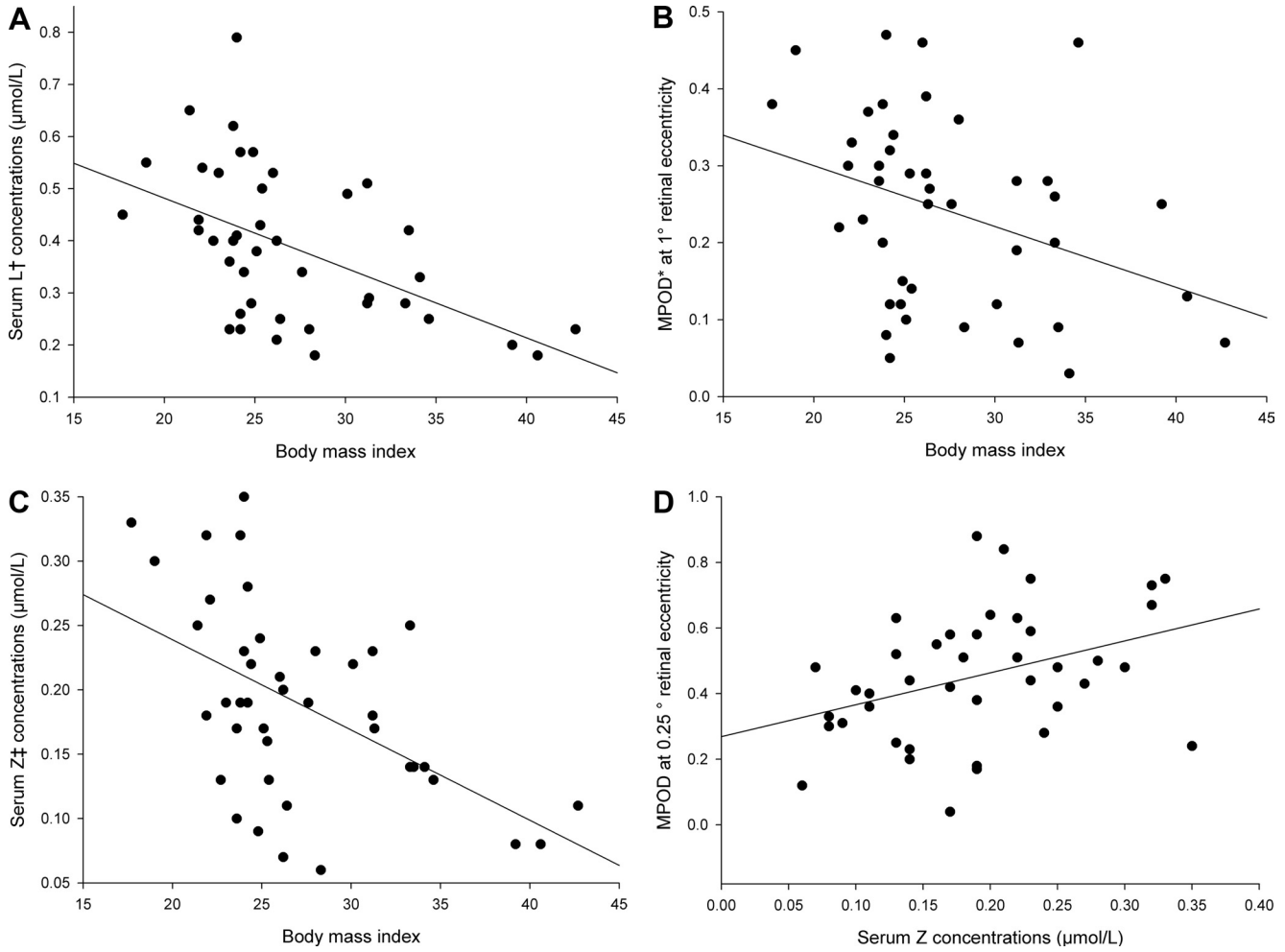

FIGURE 1. Statistically significant relationships between baseline variables ($n = 44$). *MPOD, macular pigment optical density; †L, Lutein; ‡Z, Zeaxanthin.

period. MZ was not quantified separately as part of the present study; however, MZ response is detected as part of the Z peak in the HPLC assay used herein. Indeed, we report a 1.5-fold increase in serum concentrations of L (baseline, 0.39 ± 0.15 μmol/L; final, 0.50 ± 0.22 μmol/L), and a 1.6-fold increase in serum concentrations of Z (baseline, 0.21 ± 0.03 μmol/L; final, 0.72 ± 0.11 μmol/L), which are somewhat poorer responses than other studies of supplementation with similar amounts of these carotenoids.[18,31] Possible reasons for this lower than normal response are discussed below, after our discussion on MPOD.

We report significant increases in MPOD at 0.25° and 0.5° retinal eccentricity, at 3 and 6 months in the I group; whereas, as expected, MPOD remained stable in the P group. This result is consistent with those in previous studies that also measured central MPOD and supplemented with similar amounts of the macular carotenoids at 3 months; however, at 6 months we report a slightly lower than normal MPOD response at 0.25° (Table 5).

Given that the supplement used in the present study had higher amounts of MZ (10.6 mg) than L (5.9 mg) or Z (1.2 mg), we feel it important to make direct comparison with other studies that also supplemented with MZ. To date, there have been only two published studies that have reported on MPOD response after supplementation with this carotenoid in humans. Bone et al.[25] performed a study on 10 subjects supplemented with a soy bean oil–based supplement containing 14.9 mg MZ, 5.5 mg L, and 1.4 mg Z and reported an average increase of ~0.07 (~17%) ODU at 0.75° retinal eccentricity over a 120-day period. A pilot study by our group, where 10 subjects (5 with and 5 without AMD) were assessed over an 8-week study period after supplementation with 7.3 mg MZ, 3.7 mg L, and 0.8 mg Z and showed an average increase of ~0.16 (56%) ODU in MPOD at 0.25° retinal eccentricity.

Also, it is interesting to note that only central MPOD, as discussed above, increased significantly in the I group, which is most likely because we used an MZ-dominant supplement. Given the known anatomic (central retina),[8] biochemical (antioxidant),[9] and optical (short-wavelength filtering)[10] properties of MP, there is a consensus that this pigment may confer protection against AMD, making the above findings with respect to central MP augmentation important for patients with, or at risk of developing, AMD.

The differing serum carotenoid and MP responses reported between studies (again, see Tables 4 and 5) may be due to several factors, such as dose of carotenoids consumed per day, type of carotenoids in the supplement (e.g., free versus ester), matrix in which carotenoids are consumed (e.g., oil versus microencapsulated), whether the supplement was consumed alone or in the presence of other antioxidants, and noncompliance with the study supplement regimen.

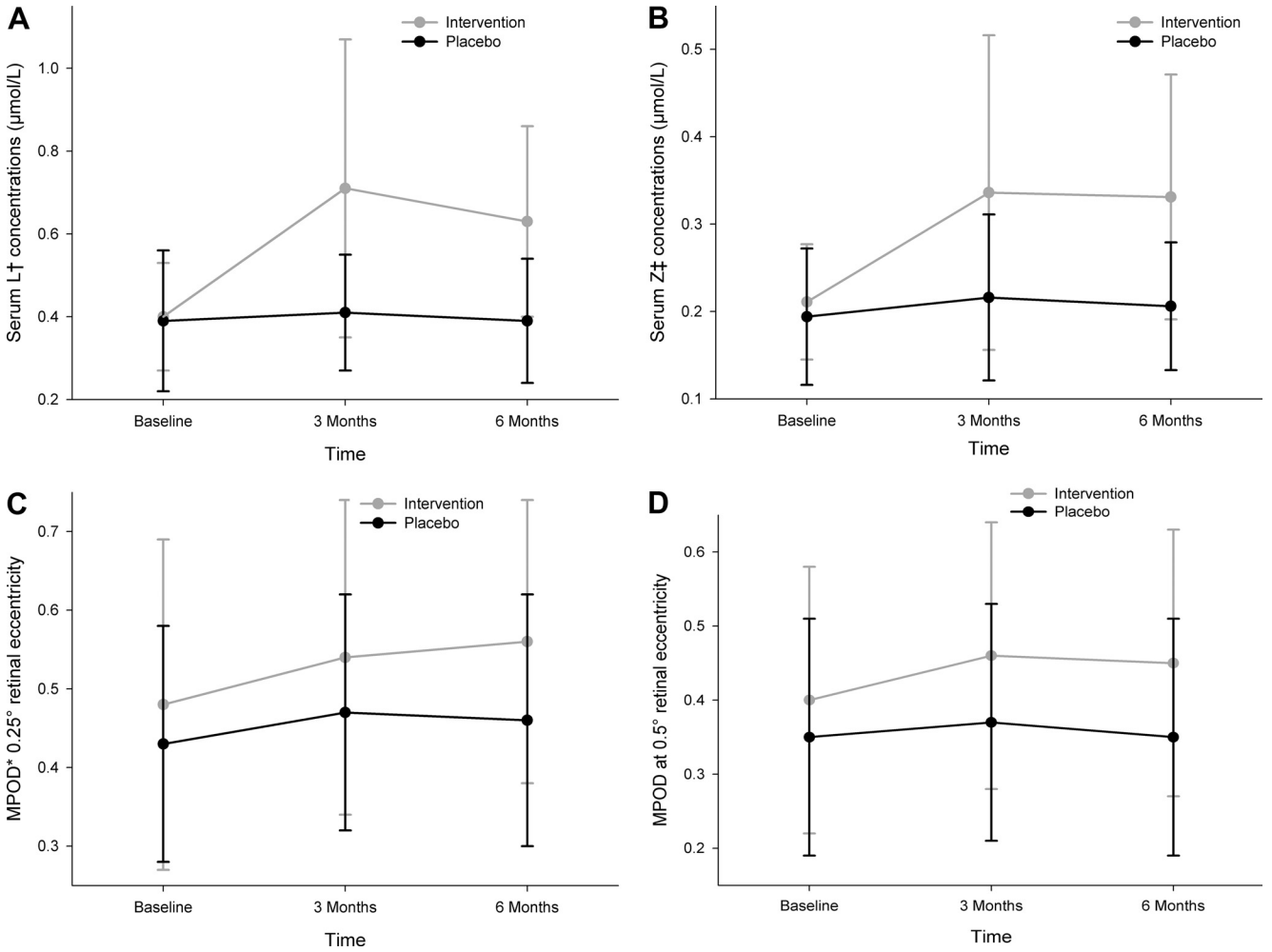

FIGURE 2. Change in (**A, B**) serum L and Z concentrations and (**C, D**) central MPOD for the intervention and placebo group. *MPOD, Macular pigment optical density; †L, Lutein; ‡Z, Zeaxanthin; The data are the mean ± SD for subjects who attended each study visit (n = 18, I group; n = 17, P group).

An in-depth analysis of our study data with respect to nonresponse in serum and MPOD confirms the following. We found that there was one nonresponder in serum for L and Z (subject 28), which was not due to a lack of compliance with the supplement regimen (confirmed by tablet counting). Surprisingly, however, this subject showed a significant response in central MPOD. This finding is difficult to explain, but may indicate that this subject exhibited a rapid uptake of the carotenoids into the retina (suggesting a need of the macula to absorb these carotenoids). This finding is also provocative, given that this subject had a confirmed family history of AMD and was a current cigarette smoker. These two risk factors have been suggested to prevent the formation of MZ at the central macula from retinal L (although the exact mechanism remains unclear). One explanation rests on the possibility that this subject cannot generate MZ from retinal L (hence, the lack of central baseline MP in this subject; MPOD at 0.25° = 0.18 and at 0.5° = 0.11), but could respond to a supplement containing MZ. Indeed, this notion is consistent with our previous pilot study reporting on MZ.[15] It is also possible that this subject initially consumed the macular carotenoid supplement, containing MZ, which had a positive effect on his MP; however, given that serum levels provide information on recent carotenoid intake, it is possible that this subject did not comply with taking the supplement by 3 or 6 months, explaining the apparent nonresponse in this subject's serum levels.

With respect to MPOD nonresponse, we found that only two subjects (subjects 1 and 15) demonstrated little, or no, response in MP (although both these subjects demonstrated significant response in serum concentrations of these carotenoids). We suggest that a simple explanation for this nonresponse in these two subjects rests on their high baseline MPOD values of 0.73 and 0.51, respectively (i.e., we suggest that they were already at their saturation points of MP). Other interesting findings with respect to MPOD response can be seen in the MPOD spatial profiles of subjects in this study. In brief, we identified three subjects with central dips in their baseline MP spatial profiles (see Kirby et al.[40] and Connolly et al.,[15] for discussion on central dips in MP spatial profiles), which were normalized after supplementation of MZ, L, and Z. This, again, is consistent with the hypothesis that these subjects are unable to generate MZ from L at the macula, but do respond to a supplement containing MZ. Moreover, and consistent with our suggestion that family history of AMD and smoking cigarettes may inhibit MZ generation from L at the macula, the subjects in the present study who exhibited baseline central dips in their MP spatial profiles had either a positive family history of AMD or a history of smoking cigarettes, but, importantly, did respond to the MZ supplement resulting in a "normal" MP profile after this carotenoid intervention.

TABLE 4. Serum Carotenoid Response after Supplementation with the Macular Carotenoids

Study	*n*	L (mg/day)	Z (mg/day)	MZ (mg/day)	Duration (wk)	Baseline L (μmol/L)	Final L (μmol/L)	Rise (%) L	Baseline Z (μmol/L)	Final Z (μmol/L)	Rise (%) Z	Baseline MZ (μmol/L)	Final MZ (μmol/L)	Rise (%) MZ
Berendschot et al.[18]	8	10	0	0	12	0.18 ± 0.08	0.9 ± 0.18	400	—	—	—	—	—	—
Johnson et al.[19]	7	19.7	1	0	15	0.37 ± 0.05	0.67 ± 0.11	81	0.06 ± 0.01	0.07 ± 0.01	17	—	—	—
Hughes et al.[20]	21	15	0	0	4	0.37	1.753	374	—	—	—	—	—	—
Bone et al.[21]	21	2.4	0	0	24	0.245 ± 0.12	0.484 ± 0.176	98	—	—	—	—	—	—
	2	30	1.5	0	20	0.158	2.06	1204	—	—	—	—	—	—
	2	0	30	0	12	—	—	—	0.09	0.52	478	—	—	—
Koh et al.[22]	6	10	0	0	19	0.27 ± 0.1	1.95 ± 1.06	622	—	—	—	—	—	—
Zhao et al.[23]	8	12	0	0	8	0.17	0.874	514	—	—	—	—	—	—
Schalch et al.[24]	18	10.7	0.8	0	24	0.16 ± 0.07	1.104	590	0.05 ± 0.02	0.145	190	—	—	—
	16	0	12.6	0	24	0.13 ± 0.04	0.303	133	0.04 ± 0.03	1.09	2625	—	—	—
	19	10.2	11.9	0	24	0.17 ± 0.07	0.63	270	0.06 ± 0.03	0.81	1250	—	—	—
Bone et al.[25]	10	5.5	1.4	14.9	17	0.31 ± 0.13	0.38 ± 0.12	23	0.097 ± 0.05	0.26 ± 0.07	168*	0	94.5	—
Wenzel et al.[26]	3	30	2.7	0	17	—	—	~1500	—	—	~278	—	—	—
Thurnham et al.[27]	19	10.8	1.2	8	3	0.28 ± 0.13	0.88 ± 0.33	221	0.05 ± 0.02	0.37 ± 0.15	640	0	0.21 ± 0.13	—
Johnson et al.[28]	11	12	0.5	0	16	0.28 ± 0.04	0.60	114	—	—	—	—	—	—
					16	0.32 ± 0.04	0.81	153	—	—	—	—		
Bone et al.[29]	24	20	0	0	20	0.199	1.62	714	—	—	—	—	—	—
	14	20	0	0	20	0.289	1.35	367	—	—	—	—	—	—
	22	10	0	0	20	0.301	1.01	235	—	—	—	—	—	—
	17	5	0	0	20	0.289	0.743	157	—	—	—	—	—	—
Connolly et al.[15]	5	3.7	0.8	7.3	8	0.31 ± 0.086	0.386	25	0.17 ± 0.78	0.19	12	0.02 ± 0.01	0.066	230
Nolan et al.[30]	61	12	1	0	48	0.57	1.40	146	0.36	0.39	8	—	—	
												—	—	
									—	—	—			
Koh et al.[22]†	7	10	0	0	19	0.32 ± 0.22	1.89 ± 0.29	491	—	—	—	—	—	—
Khachik et al.[31]†	15	2.5	0.13	0	24	0.28 ± 0.03	0.5 ± 0.11	79	0.057 ± 0.01	0.095 ± 0.01	67	—	—	—
	15	5	0.25	0	24	0.21 ± 0.03	0.72 ± 0.11	243	0.057 ± 0.01	0.095 ± 0.01	67	—		
	15	10	0.5	0	24	0.21 ± 0.03	1 ± 0.11	376	0.057 ± 0.01	0.095 ± 0.01	67	—		
Trieschmann et al.[32]†	97	12	1	0	36	0.158	0.44	178	—	—	—	—	—	—
Huang et al.[33]†	20	10	2	0	24	0.316	0.877	177	0.08	0.19	138	—	—	—
	20	10	2	0	24	0.369	0.650	76	0.08	0.15	88	—		
Connolly et al.[15]†	5	3.7	0.8	7.3	8	0.29 ± 0.13	0.336	17	0.093 ± 0.036	0.15	61	0.02 ± 0.01	0.052	160

* Includes MZ supplementation.

† Supplementation studies with AMD subjects.

TABLE 5. Studies Reporting on MPOD Response to Supplementation with the Macular Carotenoids

Study	*n*	Age (y)	L (mg/d)	Z (mg/d)	MZ (mg/d)	Duration (wk)	Tec	Retinal ECC	PF	MP Rise	Sig.
Normal Subjects: Dietary Modification											
Hammond et al.[34]	10	30–65	11.2	0.6	0	15	HFP	0.5°	5.5°	~0.05	$P < 0.05$
	2	30–65	0.4	0.3	0	15	HFP	0.5°	5.5°	~0.05	—
	1	30–65	10.8	0.3	0	15	HFP	0.5°	5.5°	~0.05	$P < 0.05$
Johnson et al.[19]	7	33–54	11.2	0.57	0	15	HFP	0.5°	5.5°	~0.07	$P < 0.05$
Normal Subjects: Supplement Modification											
Landrum et al.[35]	2	42–51	30	0	0	20	HFP	0.75°	8°	~0.20	—
Berendschot et al.[18]	8	18–50	10	0	0	12	SLO	0.75°	14°	~0.05	$P = 0.022$
	8	18–50	10	0	0	12	SA	0.75°	—	~0.04	$P < 0.001$
Aleman et al.[36]	8	11–59	20	0	0	24	HFP	0.17°	5–7°	0.07	p, 0.04
	8	11–59	20	0	0	24	HFP	0.5°	5–7°	0.07	—
	8	11–59	20	0	0	24	HFP	1°	5–7°	0.08	—
	8	11–59	20	0	0	24	HFP	2°	5–7°	0.04	—
Bone et al.[21]	2	19–59	30	1.5	0	20	HFP	0.75°	8°	~0.20	—
	1	53	0	30	0	17	HFP	0.75°	8°	~0.07	—
	21	19–59	2.4	0	0	17	HFP	0.75°	8°	~0.04	—
	12	19–60	20	0	0	17	HFP	0.75°	8°	~0.06	$P < 0.05$
	2	26–27	5	0	0	17	HFP	0.75°	8°	~0.03	—
Koh et al.[22]	6	64–81	20	0	0	20	HFP	0.5°	6°	0.07	$P > 0.05$
Bernstein et al.[37]	8	<61	20	0	0	16	HFP	0.75°	8°	0.04	—
	8	<61	20	0	0	16	RRS	-	-	76RC	—
Bone et al.[25]	10	21–58	5.5	1.4	15	17	HFP	0.75°	8°	~0.07	$P < 0.05$
Wenzel et al.[26]	3	24–52	30	2.7	0	17	HFP	0.33°	7°	0.07	$P < 0.001$
	3	24–52	30	2.7	0	17	HFP	0.5°	7°	0.07	$P < 0.002$
	3	24–52	30	2.7	0	17	HFP	1°	7°	0.046	$p < 0.002$
	3	24–52	30	2.7	0	17	HFP	2°	7°	0	—
Schalch et al.[24]	23	18–45	10.7	0.8	0	17	HFP	0.5°	5.5°	0.06	$P = 0.04$
	23	18–45	0	12.6	0	17	HFP	0.5°	5.5°	0.01	$P > 0.1$
	23	18–45	10.2	11.9	0	17	HFP	0.5°	5.5°	0.06	p, 0.04
Johnson et al.[28]	11	60–80	12	0.5	0	16	HFP	1.5°	7°	—	$P < 0.05$
	11	60–80	12	0.5	0	16	HFP	3°	7°	—	$P < 0.01$
Stringham et al.[38]	40	17–41	10	2	0	24	HFP	0.25°	10°	0.19	—
	40	17–41	10	2	0	24	HFP	0.5°	10°	0.16	—
	40	17–41	10	2	0	24	HFP	1°	10°	0.1	—
	40	17–41	10	2	0	24	HFP	3°	10°	0.07	—
	40	17–41	10	2	0	24	HFP	7°	10°	0.03	—
Connolly et al.[15]	5	30–85	3.7	0.8	7.3	8	HFP	0.25°	7°	0.16	$P < 0.05$
	5	30–85	3.7	0.8	7.3	8	HFP	0.5°	7°	0.16	$P < 0.05$
Nolan et al.[30]	61	18–41	12	1	0	52	HFP	0.25	7°	0.12	p, 0.001
	62	18–42	12	1	0	52	HFP	0.5	7°	0.11	p, 0.001
AMD Subjects											
Koh et al.[22]	7	64–81	20	0	0	20	HFP	1°	6°	0.07	$P > 0.05$
Trieschmann et al.[32]	108	51–87	12	1	0	24	AF	1°	6°	0.10	$P < 0.001$
Richer et al.[39]	76	—	10	0	0	52	HFP	1°	7°	0.25	$P < 0.05$
Connolly et al.[15]	5	30–85	3.7	0.8	7.3	8	HFP	0.25°	7°	0.16	$P < 0.05$
	5	30–85	3.7	0.8	7.3	8	HFP	0.5°	7°	0.16	$P < 0.05$

Retinal ECC, retinal eccentricity; PF, parafovea stimulus; ODU, optical density units; AF, autofluorescence; SLO, scanning laser ophthalmoscope; SA, spectral analysis; AMD, age-related macular degeneration; RRS, resonance Raman spectroscopy; —, data unavailable.

The most novel aspect of the present study concerns the efforts made to investigate safety of consumption of the macular carotenoids by performing clinical pathology analysis to assess renal and liver function, lipid profile, hematologic profile, and markers of inflammation in subjects at baseline (V1) and after 6 months (V3). It is important to point out that, although clinical pathology analysis demonstrated significant statistical variation from baseline to 6 months (in both positive and negative directions) in 8 of the 25 variables assessed in the I group and 9 of the 25 variables assessed in the P group, all variables remained within the normal reference range given, with the exception of total cholesterol and LDL in the I group ($P = 0.01$), which had a baseline value outside the accepted normal reference ranges before carotenoid supplementation commenced. Adverse events were also monitored during the study period; each subject was questioned at each visit regarding any adverse effects arising from consuming the supplements. There were no adverse events recorded or reported by any subject taking part in the study after supplementation with all three macular carotenoids.

Of note, there are currently no published clinical trials performed in human subjects that have assessed the safety of supplemental macular carotenoids by conducting comprehensive clinical pathology analysis, such as that performed in the present study. However, several human intervention studies have been conducted involving supplementation with high doses of L for extended periods of time, with no adverse effects reported (assessment limited by self report).[41–43] Indeed, doses of 20 mg/d for up to 6 months were not associated with any side effects.[36,44]

Even doses of 30 mg/d for 5 months[35] or 40 mg/d over 2 months were not associated with any adverse effects.[45] The only side effect reported as a result of L supplementation in humans has been carotenodermia, which is a harmless and reversible cutaneous hyperpigmentation of the skin.[41,46,47] Carotenodermia is itself not known to be associated with any specific adverse effects on human health and results only from excessive intake of L.[48] The majority of studies assessing safety of supplemental Z involving humans have also been observational in design and did not include appropriate clinical pathology safety testing. Of note, none of these studies reported any adverse effects or ocular toxicity after supplementation with this carotenoid.[19,21,49–55] However, there has been one (unpublished) pharmacokinetic study in five men and five women that was designed to assess the safety of Z consumption.[41] In this study conducted by Hoffmann-La Roche (now DSM Nutritional Products Europe, Ltd., Basel, Switzerland), the men and women were given capsules containing either 1 or 10 mg per day of Z for 42 days. Clinical chemistry measures and adverse events were recorded. Several clinical laboratory results fell outside the normal ranges, but there was only one adverse event where the possibility of an association with dosing was deemed even remotely plausible. The conclusion from this study was that all the adverse events were rated as mild to moderate in severity and were unlikely to be related to the supplement.[56]

In the animal model, there have been two investigations into the possibility of toxicological and/or mutagenic effects of MZ. A toxicity study performed by Chang in 2006, investigated the effect of administering 2, 20, and 200 mg/kg/d of MZ for 13 weeks consecutively.[57] In their study, Chang[57] reported that MZ was tolerated well, and the no-observed-adverse-effect level (NOAEL) of MZ in rats is >200 mg/kg/d when administered orally for 13 consecutive days. The potential for mutagenic activity has also been tested using the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2uvrA in both the presence and absence of microsomal enzymes prepared from polychlorinated biphenyl–induced rat liver. This report also found no mutagenic effect with various doses of MZ.[58]

Kruger et al.[59] published a review on the safety of consumption of a crystalline L product (FloraGLO; Kemin Health, Europe, Linda-a-Veha, Portugal) and concluded that crystalline L is safe and a generally recognized as safe (GRAS) source of L, corroborated also by animal toxicology studies, and therefore suitable for human consumption. This is also, consistent with a recent publication in Wistar rats that demonstrated no toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weight gains, feed consumption, and organ weight after oral gavage administration of a lutein-zeaxanthin concentrate to rats during 13 weeks at levels up to 400 mg/kg/d.[60] A published report by the International Programme on Chemical Safety by the World Health Organization, Geneva, summarizes some clinical, toxicologic, and mutagenicity tests that have been performed on animals with Z.[61] This report presented findings from a 13-week study on mice and rats receiving oral doses of Z, who received 250, 500, 1000 mg/kg per day of Z for 13 weeks. No treatment-related effects were observed throughout the study. In addition, hematology, blood chemistry, and urine analysis measurements showed no evidence of toxicity. The NOAEL for this study was 1000 mg/kg per day of Z (i.e., the highest dose tested).[62] Also, ocular toxicity studies have been performed on monkeys that also reported no evidence of treatment-related changes.[63,64]

In conclusion, we have shown that subjects supplemented with all three macular carotenoids, including MZ, demonstrate a statistically significant increase in serum concentrations of L and Z, and central MPOD, over a 6-month study period. Moreover, clinical pathology analysis after supplemental MZ, L, and Z is not suggestive of associated toxicity.

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