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Ocular Effects of Prenatal Carotenoid Supplementation in the Mother and Her Child: The Lutein and Zeaxanthin in Pregnancy (L-ZIP) Randomized Trial - Report Number 2

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Purpose: Lutein (L) and zeaxanthin (Z) are xanthophyll carotenoids that have been promoted to enhance maternal health and infant visual and neurodevelopment. In this study, we determined the effects of prenatal L and Z supplementation on systemic and ocular carotenoid status in the mother and her newborn infant (NCT03750968). This report focuses on the ocular effects of prenatal carotenoid supplementation.

Design: A prospective randomized clinical trial with 47 subjects randomly assigned by 1:1 allocation to receive standard-of-care prenatal vitamins along with 10 mg L and 2 mg Z softgel (Carotenoid Group) or standard-of-care prenatal vitamins with a placebo softgel (Control Group) starting in the first trimester.

Subjects: We enrolled low-risk pregnancy subjects aged ≥18 years from the obstetrics and gynecology clinic of the University of Utah Hospital.

Methods: Maternal macular, skin, and serum carotenoid concentrations were measured using autofluorescence imaging, resonance Raman spectroscopy, and high-performance liquid chromatography, respectively. Infants' ocular carotenoids and retinal architecture were measured by blue light reflectance imaging and spectral-domain OCT, respectively.

Main Outcome Measures: Changes in maternal and infant macular pigment, skin, and serum carotenoid status over the study period. Differences in infants' retinal maturity indicators between the 2 study groups.

Results: Following supplementation, there was a statistically significant increase in maternal macular pigment optical volume ($P < 0.001$) in the Carotenoid Group relative to the Control Group at all study time points, and there was no detectable maternal ocular carotenoid depletion. Infant skin and serum carotenoids increased significantly in the Carotenoid Group compared with the Control Group. As exploratory endpoints, infants in the Carotenoid Group had a 20% increase in macular pigment optical density ($P = 0.242$) and more mature foveal parameters compared with those in the Control Group.

Conclusion: Prenatal carotenoid supplementation significantly increased maternal and infant systemic carotenoids and caused a pattern of increased infant ocular carotenoid status, which may benefit both mothers and their infants' ocular development and function. This study provides important data to design and power a future multicenter study of prenatal carotenoid supplementation in higher-risk pregnancies.

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The human macula, the functional center of the retina, mediates distinct spatial vision, including facial recognition, reading, driving, and color vision. It is metabolically active and highly susceptible to photo-oxidative damage, especially by short-wavelength visible (blue) light. The xanthophyll carotenoids, particularly lutein (L), zeaxanthin (Z), and *meso*-Z, collectively called the macular pigment (MP),[1–4] are selectively concentrated at the fovea of the human eye where they protect the macula from the deleterious effects of blue light by acting as light filters[5,6] and scavengers of free radicals.[7,8] Functionally, the MP has been shown to improve visual performance (i.e., contrast sensitivity [CS] and glare disability),[9,10] mitigate the onset and progression of age-related macular degeneration,[11–13] and enhance cognition.[14–16] These carotenoids cannot be synthesized de novo in the human body, so they must be obtained solely through dietary sources such as green leafy vegetables, orange/yellow

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fruits and vegetables, eggs, and dairy products, or by supplementation.[17,18]

The accumulation of xanthophyll carotenoids in the developing baby's retinal and neural tissues begins even before birth,[19–21] with detectable amounts present at full-term and gradually increasing until ≥7 years of age.[22] These findings suggest that carotenoids may have a protective and physiologic role in infant visual and central nervous system development. Also, carotenoid deposition in utero mediated by transplacental transfer during the third trimester (T3) of pregnancy[23] could put mothers at risk for systemic and ocular carotenoid depletion.

Given the metabolic and physiologic changes associated with pregnancy, it is important that mothers obtain adequate nutrition to support this crucial developmental phase. Hence, prenatal micronutrient supplementation is standard-of-care worldwide during pregnancy to support the health of mothers and their babies;[24] however, many prenatal micronutrient recommendations are not yet supported by comprehensive clinical research. Although MP accumulates in infants' retina and neural tissues before birth, its incorporation in prenatal formulations has not been studied in a randomized clinical trial, and there is limited information on how commonly maternal ocular and systemic carotenoid depletion occurs during pregnancy.[22,25,26] In 2014, Abbott Nutrition introduced a prenatal vitamin formulation including 6 mg of L (*Similac Prenatal Vitamins*, distributed by Abbott Nutrition) with the stated intention of improving maternal and infant health. However, the product was subsequently withdrawn from the market because of poor sales that may have been related to the paucity of substantial prospective clinical research to support its physiologically plausible rationale.

To address the knowledge gaps surrounding supplementation with L and Z during pregnancy, we conducted a phase II, randomized clinical trial, the Lutein and Zeaxanthin in Pregnancy (L-ZIP) study (NCT03750968). We hypothesized that prenatal carotenoid supplementation would significantly impact ocular and systemic biomarkers of maternal and infant carotenoid status relative to a matched, standard-of-care prenatal supplement without added L and Z. Furthermore, we hypothesized that newborn infants with the highest ocular carotenoid status would have a more mature foveal structure.

Methods

A comprehensive summary of the study design and methodology for L-ZIP has been documented elsewhere.[27] Briefly, the L-ZIP clinical trial is a phase II, single-site, prospective, active-controlled, double-masked, randomized clinical trial conducted at the John A. Moran Eye Center, University of Utah, located in Salt Lake City, Utah, United States (US). The study employed a 1:1 allocation ratio to randomize low-risk pregnancy participants into 2 intervention groups: the Carotenoid Group and the Control Group. The L-ZIP trial was registered at ClinicalTrials.gov as NCT 03750968. It commenced in September 2019 when the first subject enrolled, and it concluded in January 2022 with the last participant visit.

Per the International Conference on Harmonization Guidance E6, all participants provided written informed consent before study enrollment and assessments. The L-ZIP study was approved by the University of Utah Institutional Review Board. The trial adhered to the Declaration of Helsinki principles, the International Conference on Harmonization Harmonized Tripartite Guidance for Good Clinical Practice (International Conference on Harmonization-Good Clinical Practice E6 [R1]), and the code of ethics governing participant enrollment, study assessment, and data protection. To ensure participant safety and monitor study progress, a Data Safety and Monitoring Committee, consisting of a maternal-fetal medicine specialist and a pediatric ophthalmologist, met every 6 months to review adverse events. No changes to study methods were deemed necessary after study initiation.

The L-ZIP inclusion criteria comprised pregnant women of all races and ethnicities with uncomplicated obstetric histories aged ≥18 years who planned to deliver at the University of Utah Hospital via vaginal or Caesarean delivery. Subjects were excluded from the study if they regularly (i.e., daily for the past 6 months) ingested carotenoid supplements with >0.5 mg of L and/or Z, had significant eye disease associated with MP abnormalities such as Stargardt disease, albinism, or macular telangiectasia type 2, or had any health conditions associated with high-risk pregnancies such as adolescent pregnancy, multifetal pregnancy, current or past history of diabetes, preeclampsia, previous premature delivery, drug abuse, or other significant medical illness. The screening assessments for eligibility included demographic information, visual function measures, and a comprehensive ophthalmic examination performed by a qualified ophthalmologist with a retinal subspecialty.

Following the determination of study eligibility, the study staff randomly assigned participants to the Carotenoid or Control Groups using a computer-generated random sequence in a 1:1 allocation ratio. All participants, clinicians, and staff involved in the trial were masked to the group assignment, as the study formulations had only coded identification labels. The Carotenoid Group participants received the active intervention opaque amber-colored softgels containing 10 mg L and 2 mg Z in safflower oil for daily consumption, while the Control Group received identical appearing softgels containing only the safflower oil vehicle. The active intervention dosage is identical to the carotenoid dosage used in the Age-Related Eye Disease Study 2 (AREDS2).[28] Kemin Health L.C., supplied the active and placebo softgels for this trial, and they were subjected to high-performance liquid chromatography analysis every 6 months to ensure purity and stability. All subjects in Carotenoid and Control Groups additionally received a commercially available standard-of-care prenatal multivitamin for daily consumption (*Prenatal Multivitamin + docosahexaenoic acid*, distributed by Walgreen Co). Each *Prenatal Multivitamin + docosahexaenoic acid* softgel contained 1200 μg retinol activity equivalent vitamin A, 60 mg vitamin C, 400 international units vitamin D3, 10 mg vitamin E, 1.5 mg vitamin B1, 1.7 mg vitamin B2, 18 mg niacin equivalent vitamin B3, 2.6 mg vitamin B6, 800 μg folic acid, 4 μg vitamin B12, 38 mg calcium, 27 mg iron, 25 mg zinc, and 250 mg docosahexaenoic acid.

The study participants were not subjected to any study-related dietary restrictions. However, participants were instructed to avoid carotenoid-containing supplements throughout the 6–8 month study duration. To ensure compliance, we regularly contacted the participants via phone calls and text messages and counted the remaining pills during each study visit. The study involved 4 visits in total and was categorized as the baseline first trimester (T1), which occurred before 14 weeks of gestational age, the second trimester (T2), between 22 and 26 weeks, T3, between

37 and 39 weeks, and the postpartum (PP) visit, which occurred within 2 weeks of delivery.

Study Outcomes

The L-ZIP study predefined maternal ocular biomarkers of carotenoid status and systemic carotenoid (skin and serum, as detailed in Report 1[29]) as its primary outcome measures.[27] The secondary outcome assessed infants' carotenoid status in the eyes and systemically (cord blood and skin), whereas the exploratory outcomes investigated the maternal visual performance and infants' foveal architecture. No changes to study outcome measures were made following trial initiation.

Demographic and Lifestyle Questionnaire

We obtained participant's demographic and lifestyle information, including contact details (i.e., name, date of birth, phone number [s], place of residence, and email address), self-declared race and ethnicity, occupation, medical history, ocular medical history, smoking habits (history and frequency), and alcohol consumption (average intake per week and frequency). We assessed maternal dietary carotenoid intake at each study visit using the LZQ™ quantitative food frequency questionnaire analyzed at Tufts University by Dr Elizabeth Johnson. The questionnaire contained ~ 90% of the L/Z foods consumed in the US, based on the National Health and Nutrition Examination Survey data.[18]

Visual Function

Best-corrected visual acuity (BCVA) assessment was by the logarithm of minimum angle of resolution ETDRS test charts viewed at 4 m. Likewise, CS was measured using Pelli−Robson CS test charts viewed at 1 m. Both tests of visual functions utilized Sloan optotypes following standardized protocols.

Pupillary Dilation

We dilated participants' pupils before carrying out MP imaging. For dilation of the mothers' eyes, we used a drop each of 1% tropicamide and 2.5% phenylephrine hydrochloride, and for infants, we used Cyclomydril™ drops containing 0.2% cyclopentolate hydrochloride and 1% phenylephrine hydrochloride. These eye drops are considered the standard-of-care for dilation during pregnancy and in newborns. The nondominant eye was preferably chosen as the study eye. However, in instances of poor-quality images in the nondominant eye, we used images from the dominant eye, knowing that there is a good correlation between each subject's left and right eyes in the absence of obvious pathology.

Maternal MP Measurement

Mothers' MP was measured using the dual-wavelength autofluorescence method on the Heidelberg Multicolor Spectralis (Heidelberg Engineering GmbH), which measures the attenuation of lipofuscin autofluorescence by blue absorbing MP. First, the examiner enters the participant's details into the Heidelberg Eye Explorer (HEYEX version 1.7.1.0) software. The participant then fixates on a target with the dilated study eye, ensuring good alignment and camera focus for quality retinal imaging. Autofluorescence images were collected as the macula was raster-scanned sequentially with alternating 486 nm (blue) and 518 nm (green) lasers.[30] Macular pigment images were obtained by digitally subtracting the green image from the blue image using appropriate correction factors to compensate for the absorption spectrum of the macular carotenoid pigment and then analyzed using a beta version of Heidelberg's proprietary MP analysis software after setting the zero point at 9° eccentricity from the point of fixation. We used 9° eccentricity to enable consistency and comparison with previous studies from our research group and to avoid the effect of the optic nerve and retinal blood vessels on MP measurements. The imaging instrument and software have proven highly reliable and reproducible, especially when measuring MP optical volume at 9° eccentricity (MPOV), which shows the total of all MP optical density (MPOD) values for all pixels with valid results within 9° eccentricity.[31]

Infant MP Measurement

We used blue light reflectometry in measuring infants' MP.[22] The absence of lipofuscin in infants' eyes makes MP measurement with autofluorescence imaging unsuitable. Therefore, a reflection-based method is necessary for infant MP assessment. We gently opened the swaddled baby's eyes with a pediatric lid speculum after topical anesthesia. Posterior pole 80° images centered on the fovea were obtained with the RetCam (Clarity Medical Systems, Inc) retinal camera using its optional blue light source while deliberately omitting the fluorescein angiography barrier filter in the collection light path. Color images were also obtained to facilitate crucial retinal landmark identification. The best quality blue-light reflectance images from the red, green, blue detector (with sharp focus and uniform background illumination) were downloaded and transferred to Java-based imaging software (ImageJ; National Institutes of Health), where the blue-light channel data were converted to 8-bit grayscale images. The average pixel intensity at the designated peripheral reference point relative to the fovea regions of the image (I_{max}) divided by pixel intensity at the fovea (I_{min}) was then used to calculate peak MPOD. Equation (1) provides details of the formula, assuming a double pass through the MP after reflection from the sclera. A scaling factor *(a)* was empirically determined to be 1.15 to account for the absorbance of the MP at 480 to 485 nm, the peak wavelength range of the blue-light source measured on 2 different RetCams with a high-resolution spectrometer (HR2000+; Ocean Optics), relative to the MP's peak absorbance at 460 nm derived from a published absorption spectrum of the primate MP. In this reflectance model, it was assumed that the media anterior to the retinal surface are optically clear and low in light scattering and that melanin pigmentation of the retinal pigment epithelium/choroid is uniform throughout the posterior pole.

$$\mathrm{MPOD} = \left(\frac{1}{2}\right) \times (a) \times \log\left(\frac{I_{max}}{I_{min}}\right) \quad (1)$$

Infant Foveal Anatomy

The Bioptigen spectral-domain OCT (Leica Microsystems) is a US Food and Drug Administration-approved handheld portable unit used to image premature and full-term infants' foveal anatomy. Swaddled infants had their eyelids gently opened by a certified ophthalmic photographer or a pediatric ophthalmologist. A lid speculum to hold the eyelids and proparacaine to numb the eyes were used, if necessary, during imaging. Vertical and horizontal scans of the retina were then acquired. The OCT images of the infant preceded the contact images with the RetCam. The OCT images were then downloaded and transferred to Java-based imaging software (ImageJ; National Institutes of Health), where the various foveal parameters (i.e., foveal thickness, distance between peaks, temporal and nasal peak heights, pit depth, full width at half maximum [FWHM], depth at FWHM, peak average, foveal to parafoveal ratio, and ellipsoid zone [EZ] grading) were assessed. We used 1 eye per infant with the highest quality OCT image for statistical analysis to ensure consistent layer measurements. We

also developed a grading system for the infants' EZ as a measure of infant foveal maturity. Ellipsoid zone maturity was determined by the distance the EZ migrates toward the center of the fovea. Grade 1 through 4 is in order from least mature (1) to most mature (4).

Serum and Skin Carotenoids Assessment

We assessed study participants' serum and skin carotenoid status using high-performance liquid chromatography and resonance Raman spectroscopy, respectively. The detailed procedures have been previously documented in our published study protocol and in L-ZIP Report 1.[27,29]

Sample Size Calculation

Statistical power and sample size for one of the primary outcome measures, maternal skin carotenoids at birth, were determined using data from a previous study by Henriksen et al, in which the average maternal skin carotenoids ± standard deviation at birth was 34 000 ± 8300 resonance Raman units.[26] This value is 20% lower than the mean Moran Eye Center skin carotenoid of ∼42 500 resonance Raman units reported for our ancillary AREDS2 study.[32] Hence, with 0.90 power and 0.05 alpha level, a sample size of 20 per study group was adequate to detect and prevent a 20% decline during pregnancy. To accommodate for subsequent ineligibility due to premature birth, low birth weight, or other problems (∼10%) and to plan for expected noncompliance and loss to follow-up (∼20%), a goal of enrolling 30 subjects in each study group was deemed appropriate.

Statistical Analysis

All data analyses for this study utilized Stata 14.0 software (StataCorp). Only participants who completed the first and last study visits (n = 41) were included in the study analysis, as specified in the L-ZIP protocol. Between-group differences at baseline were analyzed using an independent t-test for continuous variables and the chi-squared tests for categorical variables. Repeated measures analysis of variance was used for between-group comparisons of change in outcome variables over time. Bonferroni adjustments were made for multiple comparisons. Paired t-tests compared the carotenoid status of infants whose mothers were in the 2 study groups. Correlational analysis determined the relationships between maternal and infant ocular carotenoid status. Statistical significance was set at $P < 0.05$ for all analyses.

Results

Enrollment and Baseline Characteristics

Enrollment for the L-ZIP Trial began in September 2019 and ended in July 2021. Our Consolidated Standards of Reporting Trials flow diagram is presented in Fig S1. Despite the coronavirus disease 2019 pandemic leading to many eligible participants declining enrollment, participants who enrolled consistently adhered to the study schedule, resulting in a dropout rate of <15% in both study groups. Due to the high level of engagement and compliance, enrollment was terminated at 47 randomized subjects, as it was clear that the per protocol target of 20 subjects per group would be achieved. The maternal demographic and baseline characteristics of the 41 study participants (Carotenoid Group, n = 21, and Control Group, n = 20) who completed the PP study visits are summarized in Table 1.

Compliance and Adverse Events

Of the 41 subjects who completed their PP visits, only 1 mother missed her T3 visit because of unexpected early labor. Subjects' adherence to their assigned study supplement assessed by pill counting was 90%, 85%, and 76% at the T2, T3, and PP visits, respectively. Supplement compliance did not differ significantly between the Carotenoid and the Control Groups over the study period. One Carotenoid Group infant missed their skin carotenoid assessment, and 2 cord blood samples were missing for each experimental group. Additionally, 9 infants in each study group missed retinal imaging due to coronavirus disease restrictions, low-quality images, or parental refusal to consent for infant dilation and retinal imaging.

Systemic adverse events reported during the L-ZIP study were not considered unusual occurrences during pregnancy by the investigators and the Data Safety and Monitoring Committee (documented in Report 1[29]). For all 47 randomized subjects, no ocular adverse events were recorded in either the mothers or their children beyond 5 Control infants and 2 Carotenoid infants who had macular cysts on OCT that resolved spontaneously upon subsequent examination.

Maternal MPOV during the Study Period

Following the initiation of supplementation, we found a statistically significant increase in MPOV in the Carotenoid Group mothers compared with the Control Group mothers at each study visit (Table 2 and Fig 2). Within the Carotenoid Group, MPOV showed a remarkable steady increase from T1 to PP, and there was a statistically significant difference in MPOV at T2, T3, and PP compared to T1. In contrast, MPOV in the Control Group did not change significantly throughout pregnancy, and we found no statistically significant MPOV change relative to T1 at the T2, T3, and PP visits. Figs S3–S5 show each participant's serum L + Z concentrations, skin carotenoids, and MPOV in response to the study intervention at every visit, respectively.

Infant MPOD and Systemic Carotenoid Status

Infants whose mothers were in the Carotenoid Group (n = 12) had 20% higher MPOD levels than those in the Control Group (n = 11), although this was not statistically significant ($P = 0.242$), possibly due to the small number of subjects and low neonatal MP levels. Infants' skin carotenoids and serum L + Z significantly increased in the Carotenoid Group relative to the Control Group ($P \leq 0.0001$). These results are graphically presented in Figure 6.

Association Between PP Maternal and Infant Systemic and Ocular Carotenoid Status

Regardless of the study group assignment, we determined the interrelationships between PP maternal and infant ocular and systemic carotenoid concentrations. We found a positive significant correlation between PP MPOV and maternal skin carotenoids ($r = 0.48$, $P = 0.0014$), L + Z concentrations ($r = 0.54$, $P < 0.0001$), and serum total carotenoids

Table 1. Baseline Maternal Demographics, Lifestyle, and Ocular Carotenoid Concentrations of the Carotenoid and the Control Groups

Variables	Carotenoid Group (n = 21)	Control Group (n = 20)	*P*-Value
Age, years	30.8 ± 3.1	29.1 ± 3.7	0.114
BMI, kg/m^2	23.9 ± 3.7	25.4 ± 4.6	0.259
Race, n (%)			1.000
White	21 (100.0)	20 (100.0)	
Non-White	0 (0.0)	0 (0.0)	
Ethnicity, n (%)			0.972
Non-Hispanic	20 (95.2)	19 (95.0)	
Hispanic	1 (4.8)	1 (5.0)	
Smoking habits, n (%)			1.000
Never smoked	21 (100.0)	20 (100.0)	
Smoked	0 (0.0)	0 (0.0)	
Alcohol frequency, n (%)			0.323
Never	20 (95.2)	20 (100.0)	
Occasional	1 (4.8)	0 (0.0)	
Diet, estimated intake of L and Z, ng/mL			
L	4.0 ± 5.7	3.4 ± 3.1	0.689
Z	0.3 ± 0.3	0.2 ± 0.2	0.308
L + Z	4.3 ± 5.9	3.6 ± 3.1	0.656
Visual Function			
BCVA	88.4 ± 1.3	89.3 ± 1.1	0.602
CS	1.68 ± 0.1	1.69 ± 0.1	0.843
MPOV	8809 ± 3001	7002 ± 2502	0.154

BCVA = best corrected visual acuity; BMI = body mass index; CS = contrast sensitivity; L = lutein; MPOV = macular pigment optical volume; Z = Zeaxanthin.
Data shown are mean ± standard deviation for continuous data and percentages for categorical data.

($r = 0.53$, $P = 0.0004$). Likewise, infants' skin carotenoids correlated significantly with umbilical cord blood L + Z concentrations ($r = 0.61$, $P = 0.0001$) and serum total carotenoids ($r = 0.61$, $P = 0.0001$). Also, PP MPOV significantly correlated with infants' skin carotenoids ($r = 0.38$, $P = 0.0145$), umbilical cord blood L + Z ($r = 0.48$, $P = 0.0030$), and serum total carotenoid concentrations ($r = 0.47$, $P = 0.0033$). On the other hand, infants' MPOD was weakly correlated with infants' systemic carotenoids and PP maternal systemic and ocular carotenoids ($r = -0.19 - 0.09$, $P \geq 0.38$).

Visual Function

Comparing maternal BCVA and CS between the Carotenoid and Control Groups, we found no statistically significant difference between the 2 study groups following supplementation ($P = 1.000$, for all). Although subjects in the Carotenoid Group had slightly enhanced visual acuity (i.e., a letter improvement) relative to the Control Group, the difference was not statistically significant.

Infants' OCT

For our exploratory aim, we compared foveal maturity between infants whose mothers were in the Carotenoid and the Control groups. Figure 7 (panel A) shows all retinal parameters examined in this study, and Figure 7 (panel B) illustrates the EZ grading in order of least mature (1) to most mature (4). We observed similar foveal architecture of the left and right eye for all infants in the study. Hence, we used 1 eye per infant with the highest-quality OCT image for our analysis. Although no statistically significant difference was observed in all retinal parameters (i.e., foveal

Table 2. Change in Maternal Ocular Carotenoid Status From Baseline to PP Between Study Groups Using Repeated Measures Analysis of Variance

Variable	Carotenoid Group (n = 21)	Control Group (n = 20)	Mean Difference	*P*-Value
MPOV				
T1	8809 ± 655	7002 ± 559	1807 ± 866	0.154
T2	10 191 ± 750	7420 ± 591	2771 ± 876	0.008*
T3	11 622 ± 662	6914 ± 479	4708 ± 876	<0.001*
PP	12 244 ± 688	7343 ± 470	4901 ± 866	<0.001*

MPOV = macular pigment optical volume; PP = postpartum; T1 = first trimester/baseline; T2 = second trimester; T3 = third trimester.
The data shown are the mean ± standard error.
*A statistically significant difference between the 2 study groups at 0.05 level. The mean difference is Bonferroni adjusted.

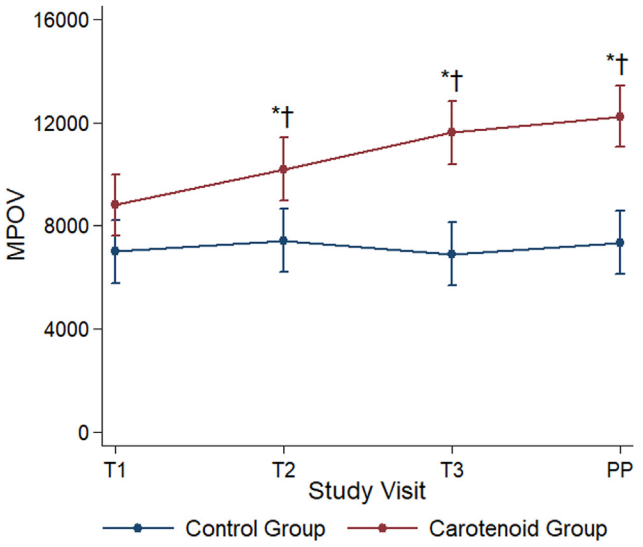

Figure 2. Changes in maternal MPOV between the Carotenoid (n = 21) and the Control (n = 20) Groups over the study duration. There was a statistically significant difference between the Carotenoid and the Control Group at all study time points following study intervention initiation (*, $P < 0.001$). Also, MPOV was significantly increased relative to T1 at the T2, T3, and PP visits (†, $P < 0.001$) in the Carotenoid Group, whereas within the Control Group, MPOV did not change significantly throughout pregnancy. Error bars represent 95% confidence interval. MPOV = macular pigment optical volume; PP = postpartum; T1 = first trimester/baseline; T2 = second trimester; T3 = third trimester.

thickness, the distance between peaks, temporal and nasal peak heights, pit depth, FWHM, depth at FWHM, peak average, foveal to parafoveal ratio, and EZ grading, see Table 3), we noticed a trend for improvement in parameters related to foveal maturity in infants whose mothers were in the Carotenoid Group compared with those in the Control Group.

Discussion

The L-ZIP trial sought to determine the effect of prenatal carotenoid supplementation on mothers and their infants' systemic and ocular carotenoids during a 6 to 8 month study period. This report highlights the ocular effects of prenatal carotenoid supplementation in the mothers and their babies, as the systemic effects have been reported elsewhere.[29] Following prenatal carotenoid supplementation, we observed a significant increase in MPOV in the Carotenoid Group mothers relative to participants in the Control Group at all study time points. Also, infants whose mothers were in the Carotenoid Group showed trends for increased MPOD and foveal maturity compared with those in the Control Group. Infants in the Carotenoid Group also had significantly increased measurements of skin and serum carotenoids.

Our study intervention, consisting of 10 mg L and 2 mg Z, was comparable in dosage to the carotenoid concentrations administered in the AREDS2 study[28] and was well tolerated by our participants. Pill count compliance was high, and the low dropout rate indicated the intervention was well-received. Throughout the study, we observed no serious adverse events attributable to the intervention, and all recorded adverse events were consistent with those typically observed during routine pregnancies. This is consistent with the US Food and Drug Administration's position that carotenoids such as L and Z are Generally Recognized as Safe for human adults and children.

A finding that merits consideration is the sustained increase in MPOV in the Carotenoid Group relative to the Control Group throughout the study. This result compares favorably to past studies that showed increased MP among supplemented participants enrolled in age-related macular degeneration trials.[10,28,32–35] Although no significant depletion of ocular carotenoids occurred in either study group, this observation could be attributable to the fact that

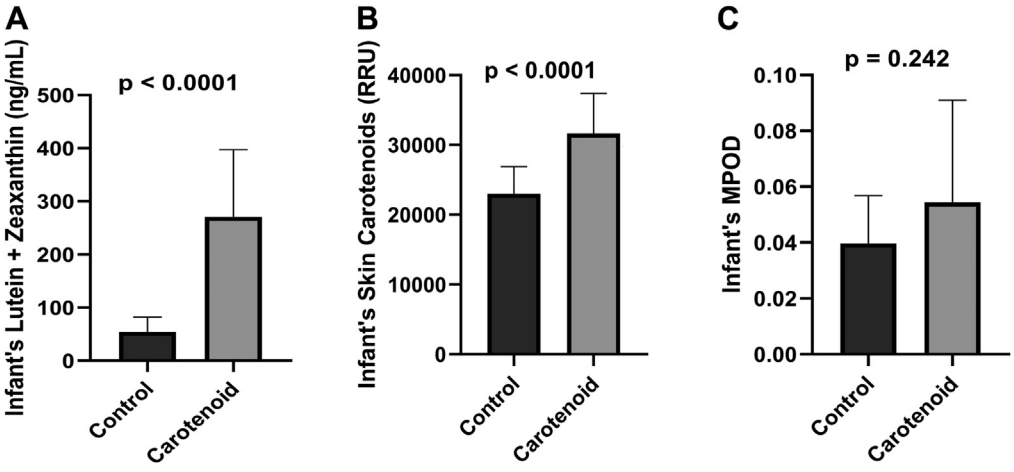

Figure 6. Systemic and ocular carotenoid status in infants whose mothers were in the Carotenoid and the Control Group. Infant serum lutein + zeaxanthin and skin carotenoid status increased significantly in the Carotenoid Group relative to the Control Group ($P < 0.0001$). Infants in the Carotenoid Group had a 20% increase in MPOD ($P = 0.242$). For **A**, Control (n = 18) and Carotenoid (n = 19); **B**, Control (n = 20) and Carotenoid (n = 20); **C**, Control (n = 11) and Carotenoid (n = 12). MPOD = macular pigment optical density; RRU = resonance Raman units.

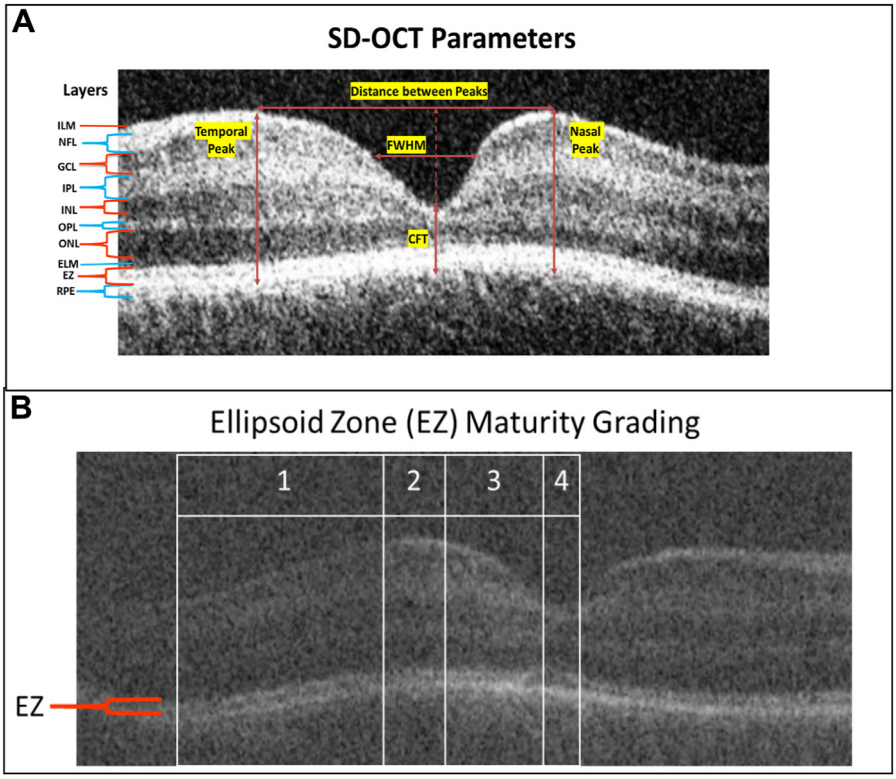

Figure 7. Infant's retinal parameters (**A**) and the EZ grading (**B**). **B,** A retina with a complete EZ with a grade of 4 evidenced by the merging of nasal and temporal EZs at the center of the fovea. CFT = central foveal thickness; ELM = external limiting membrane; EZ = ellipsoid zone; FWHM = full width at half maximum; GCL = ganglion cell layer; ILM = inner limiting membrane; INL = inner nuclear layer; IPL = inner plexiform layer; ONL = outer nuclear layer; OPL = outer plexiform layer; NFL = nerve fiber layer; RPE = retinal pigment epithelium; SD-OCT = spectral-domain OCT.

Table 3. Infants' Ocular Parameters of Maturity at Postpartum Between the Carotenoid and the Control Groups

Parameter	Carotenoid Group (n = 13)	Control Group (n = 11)	Mean Difference	*P*-Value
CFT (μm)	130.4 ± 6.3	129.2 ± 6.6	1.2 ± 9.2	0.90
Distance between peaks (μm)	698.1 ± 33.0	655.4 ± 15.5	42.7 ± 38.7	0.28
Temporal peak height (μm)	305.7 ± 5.5	292.6 ± 8.3	13.1 ± 9.7	0.19
Nasal peak height (μm)	309.5 ± 6.5	308.1 ± 11.0	1.4 ± 12.3	0.91
Pit depth (μm)	183.9 ± 9.2	168.0 ± 7.6	16.0 ± 12.2	0.20
FWHM (μm)	221.4 ± 13.0	212.3 ± 8.0	9.2 ± 16.0	0.57
Depth x FWHM (μm^2)	44 065.4 ± 4832.9	35 389.5 ± 1545.5	8675.8 ± 5459.0	0.13
Peak average (μm)	307.6 ± 4.8	300.4 ± 9.4	7.2 ± 10.1	0.48
FP ratio	0.42 ± 0.02	0.43 ± 0.02	−0.01 ± 0.03	0.83
EZ grading*	192.5†	107.5†	275.9‡	0.07

CFT = central foveal thickness; EZ = ellipsoid zone; FP = foveal to parafoveal; FWHM = full width at half maximum.
The data shown are the mean ± standard error.
CFT is the inner aspect of the inner limiting membrane to the inner aspect of the retinal pigment epithelium at the foveal center.
Distance between peaks is the horizontal measure spanning from the temporal foveal peak to the nasal foveal peak.
Temporal peak height is the measure of foveal thickness at the center of the temporal peak.
Nasal peak height is the measure of foveal thickness at the center of the nasal peak.
Peak average is the average of nasal and temporal peak heights.
Foveal pit depth is the measure of the lowest inner limiting membrane aspect to the height of the intersection of nasal and temporal peaks.
FWHM is the pit width measured at half of the foveal pit depth.
*Mann–Whitney U test for EZ grading.
†Median.
‡U statistic.

mothers we enrolled were generally healthy and nutritionally informed, as shown by healthy baseline body mass indexes, minimal consumption of alcohol, no cigarette smoking, and high baseline consumption of carotenoid-rich foods (~4 mg/day of L + Z versus the American average of 1−2 mg/day). Hence, our hypothesized systemic maternal carotenoid depletion in T3 did not occur to a degree that could deplete their ocular store of carotenoids. We, however, expect to find MP depletion if we replicate this trial in a nutritionally compromised population. The considerable increase in maternal MP in the Carotenoid Group compared with the Control Group may be beneficial to maternal visual performance and general health in the long term, especially with carotenoids known to be protective against eye and other systemic diseases via their antioxidant and antiinflammatory activities.[7,8]

The study also evaluated maternal visual function (BCVA and CS) in the Carotenoid and Control Groups at each time point. While the differences in BCVA and CS between study groups were not statistically significant, the study showed a letter improvement in BCVA in mothers in the Carotenoid Group compared with the Control Group. These findings suggest that prenatal carotenoid supplementation may have a positive effect on BCVA and CS in mothers due to carotenoids' role in protecting photoreceptors from blue and ultraviolet light damage. However, further research is needed to determine the potential long-term effects on measures of maternal visual function, such as glare disability and photostress recovery time.

Despite infants MPOD not being statistically different between the 2 study groups, we observed a 20% increase in MPOD in the Carotenoid Group relative to the Control Group. This result indicates that prenatal carotenoid supplementation is bioavailable to potentially provide an early start for its lifelong physiological and protective roles for the developing retina and infant vision.[26,36,37]

Infants in the Carotenoid and Control groups showed differences in parameters related to foveal maturity status; however, these differences were nonsignificant. Normal foveal structural features indicative of maturity includes extrusion of plexiform layers, foveal pit presence, EZ lengthening, and ONL widening.[38,39] Conversely, typical markers of foveal immaturity include shallower foveal depression, the presence of persisting inner retinal layers, thinner retinal layers, and the absence of photoreceptor sublayers.[40] The EZ, an important factor for photoreceptor health and function, grows in a centripetal fashion toward the foveal center.[41] Infants in the Carotenoid Group showed a trend to more advanced EZ grades on average than the Control group ($P = 0.07$), suggestive of matured foveal development. Further research with larger numbers of subjects will be necessary to conclusively elucidate prenatal carotenoid supplementation's role in infants' foveal maturity.

The L-ZIP trial highlights the importance of studying optimal maternal and infant ocular xanthophyll status throughout pregnancy, and it is the first study to report on the effect of prenatal carotenoid supplementation on infants' foveal maturity and architecture using validated techniques and devices, indicating a potential benefit of prenatal L and Z supplementation on infants' retinal development. We have recently demonstrated that prenatal carotenoid supplementation significantly inhibits oxygen-induced retinopathy in a mouse model of retinopathy of prematurity, and we speculate that prenatal carotenoid supplementation could have similar beneficial effects for preterm infants.[42] A major limitation of the L-ZIP trial lies in the fact that we were underpowered to investigate infants' MP and foveal architecture in a statistically significant manner, but our results will be relevant to design and power of future large multicenter studies in higher risk populations. Also, no potential cognitive or visual function benefits of prenatal carotenoid supplementation were assessed in the infants, so we intend to conduct a future follow-up study once the infants are old enough to undergo reliable testing. Additionally, most of our study participants were Whites, so our findings may not be generalizable to other populations. We intend to replicate this trial in diverse groups in future studies.

In conclusion, prenatal carotenoid supplementation improved maternal systemic and ocular status significantly throughout pregnancy and significantly influenced her newborn infant's carotenoid status as well. Improved maternal and infant ocular and systemic carotenoid status could have lifelong positive impacts for eye health and visual performance. Furthermore, improved infants' foveal maturity and MPOD resulting from prenatal carotenoid supplementation could provide novel insights into mitigating the burden of retinopathy of prematurity and inform the design of future studies. Lutein and Z supplementation at AREDS2 doses during pregnancy was safe and well tolerated and deserves further study in large-scale clinical trials to encourage its addition to standard-of-care prenatal vitamins.

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Footnotes and Disclosures

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Disclosures:

All authors have completed and submitted the ICMJE disclosures form.

Dr Bernstein has been a consultant and speaker for Kemin Health. There are no conflicts of interest for any other authors.

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HUMAN SUBJECTS: Human subjects were included in this study. Per the International Conference on Harmonization (ICH) Guidance E6, all participants provided written informed consent before study enrollment and assessments. The L-ZIP study was approved by the University of Utah Institutional Review Board. The trial adhered to the Declaration of Helsinki principles, the ICH Harmonized Tripartite Guidance for Good Clinical Practice (IHC-GCP E6 [R1]), and the code of ethics governing participant enrollment, study assessment, and data protection.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Bernstein, Varner

Analysis and interpretation: Bernstein, Hartnett, Harrison, Addo, Gorka, Sharifzadeh, Gorka

Data collection: Allman, Hoffman, Addo, Gorka, Bernstein

Obtained funding: Bernstein

Overall responsibility: Addo, Gorka, Allman, Harrison, Sharifzadeh, Hoffman, Hartnett, Varner, Bernstein

Abbreviations and Acronyms:
AREDS2 = Age-Related Eye Disease Study 2; **BCVA** = best corrected visual acuity; **CS** = contrast sensitivity; **EZ** = ellipsoid zone; **FWHM** = full width at half maximum; **L** = lutein; **L-ZIP** = Lutein and Zeaxanthin in Pregnancy; **MP** = macular pigment; **MPOD** = macular pigment optical density; **MPOV** = macular pigment optical volume; **PP** = postpartum; **T1** = first trimester; **T2** = second trimester; **T3** = third trimester; **US** = United States; **Z** = zeaxanthin.

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