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Lutein Supplementation Increases Breast Milk and Plasma Lutein Concentrations in Lactating Women and in Infant Plasma Concentrations but Does Not Affect Other Carotenoids[1–3]

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Abstract

Lutein is a carotenoid that varies in breast milk depending on maternal intake. Data are lacking with regard to the effect of dietary lutein supplementation on breast milk lutein concentration during lactation and subsequent plasma lutein concentration in breast-fed infants. This study was conducted to determine the impact of lutein supplementation in the breast milk and plasma of lactating women and in the plasma of breast-fed infants 2–3 mo postpartum. Lutein is the dominant carotenoid in the infant brain and the major carotenoid found in the retina of the eye. Eighty-nine lactating women 4–6 wk postpartum were randomly assigned to be administered either 0 mg/d of lutein (placebo), 6 mg/d of lutein (low-dose), or 12 mg/d of lutein (high-dose). The supplements were consumed for 6 wk while mothers followed their usual diets. Breast milk carotenoids were measured weekly by HPLC, and maternal plasma carotenoid concentrations were measured at the beginning and end of the study. Infant plasma carotenoid concentrations were assessed at the end of the study. No significant differences were found between dietary lutein + zeaxanthin intake and carotenoid concentrations in breast milk and plasma or body mass index at baseline. Total lutein + zeaxanthin concentrations were greater in the low- and high-dose–supplemented groups than in the placebo group in breast milk (140% and 250%, respectively; $P < 0.0001$), maternal plasma (170% and 250%, respectively; $P < 0.0001$), and infant plasma (180% and 330%, respectively; $P < 0.05$). Lutein supplementation did not affect other carotenoids in lactating women or their infants. Lactating women are highly responsive to lutein supplementation, which affects plasma lutein concentrations in the infant. This trial was registered at clinicaltrials.gov as NCT01747668. J. Nutr. doi: 10.3945/jn.114.192914.

Introduction

Research suggests that maternal dietary habits are related to breast milk composition, in both adequately nourished and malnourished populations. In malnourished populations, vitamin and mineral concentrations are affected in breast milk (1). In adequately nourished populations, the impact of maternal dietary habits can be seen for a variety of dietary components, such as cholinze (2), selenium (3), and FAs (4–6). With such knowledge, it is appealing to institute dietary changes to correct deficiencies and to improve the nutritional quality of breast milk. This strategy has shown early promise for some nutrients. For example, supplementing high-dose vitamin D_3 to lactating mothers improved serum 25-hydroxyvitamin D_3 status in both mothers and exclusively breast-fed infants (7,8). For other dietary components, such as carotenoids, the ability to supplement lactating mothers and to improve breast milk concentrations is unknown, although breast milk carotenoid concentrations correlate to maternal intake (9,10).

Carotenoids are pigments occurring naturally in plants that serve a number of functions in human tissues; however, they cannot be synthesized in the body and must come from the diet. Lutein may be particularly important for developing infants (11). For example, lutein and its isomers are the only carotenoids found in the neural retina (12) and absorb short-wave light in the neural retina, which provides protection from actinic damage and improves visual functions in short-wave dominant-viewing conditions in both infants and adults (13,14). Lutein also serves as an active antioxidant and may prevent age-related neural loss attributed to oxidative stress (15). Although there is no dietary recommendation for carotenoids, a prudent amount can be

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[3] Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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considered on the basis of the intake of individuals consuming the recommended amount of vegetables according to the *Dietary Guidelines for Americans*, which is 2.5 cups of vegetables/d, of which 1.5 cups/wk should be dark-green, for a 2000-kcal diet (16). Adults consuming the recommended vegetable servings had a lutein + zeaxanthin intake of 4–7 mg/d (17). Typical vegetables consumed in the U.S. diet, such as corn and green beans, are not very high in lutein with only 200–300 μg/100 g (18). Dark-green leafy vegetables, such as raw spinach and cooked kale, have higher lutein concentrations of 7–8 mg/100 g (18) but are less frequently consumed in the United States (19), resulting in low dietary lutein intakes of ≤2 mg/d (20).

In pregnant women, lutein is transferred across the placenta to the developing infant, and cord blood concentrations correlate highly with maternal plasma concentrations (21), which vary with dietary intakes (22). Lutein is highly concentrated in breast milk (23) and like other carotenoids is actively transported into breast milk (24). Although the specific transport mechanism has not yet been fully elucidated, carotenoids tend to be found in higher concentrations in early milk (<3 mo postpartum) when lipid concentrations are lower (25). The relation between intake and concentration may be culturally dependent, because a study of breast milk from women in 9 countries showed that women in China and Japan have the highest breast milk lutein + zeaxanthin concentrations, which are 3 times higher than those in women from the United Kingdom, the United States, and Australia (26). Because placental and breast milk lutein are dependent on maternal consumption, which is low in the United States, understanding the impact of lutein supplementation and the effects on the mother and infant is important. Therefore, as part of a larger multisite, prospective, randomized, placebo-controlled dose-response study to examine the impact of lutein, DHA (22:6n–3), and vitamin E supplementation in lactating women, the aims of this portion of the study were to determine the dose response to lutein supplementation, understand if lutein supplementation had an impact on concentrations of other carotenoids, and ascertain the relation of carotenoid content in breast milk and maternal and infant plasma.

Participants and Methods

Participants and study design. Eighty-nine U.S. mothers ≥18 y of age who had delivered a full-term singleton infant, who were 4–6 wk postpartum, and who had been continuously, successfully lactating and planned to continue breastfeeding for at least 6 wk were randomly assigned from 6 study centers over a 4-mo period (November–March). Mothers with a BMI ≥30 kg/m^2 or taking cholesterol medication and/or other medications affecting lipid absorption and/or transport were excluded from the study. If participants (n = 71) had been consuming a supplement with lutein, DHA, or vitamin E, they participated in a 10-d washout before starting the study and were instructed not to consume any supplements with these components during the study; only 1 participant (n = 1) had been previously consuming a supplement with lutein. Ninety percent (n = 74) of participants elected to consume a multivitamin/mineral tablet that was offered, which did not contain any of these components, but they were not given any additional dietary instruction.

When an eligible participant was enrolled, a sealed envelope with the treatment group assignment that had been prepared by the sponsor from computer-generated randomization schedules by a pseudo-random permuted block algorithm was opened by the site coordinator to determine the participant's treatment group. Participants were assigned to 1 of 3 groups and were asked to consume 2 study capsules daily in the morning with food for 6 wk. Two doses were chosen to examine a dose response on the basis of the prudent intake amount previously mentioned (17) as well as on an interim USDA report demonstrating that 12 mg/d lutein increased breast milk and plasma concentrations in lactating women (27). The groups were as follows: *1*) placebo with 0 mg/d of lutein, *2*) a low dose of 6 mg/d lutein, or *3*) a high dose of 12 mg/d lutein (Floraglo Lutein; Kemin Industries). The zeaxanthin content of the experimental capsule was ~1.6% lutein (96 μg/capsule). The composition of the study capsules is shown in **Table 1**. The placebo capsules were identical in appearance to the experimental capsules and contained no lutein or zeaxanthin. Study group identity was not disclosed to study staff or participants.

At the beginning of the study, participants provided a baseline breast milk sample and optional blood sample before starting the supplement as well as weekly breast milk samples during the supplementation period and an optional blood sample at the end of the study. Breast milk was collected either at the research site or at home and kept under refrigeration for <12 h before transporting to the research site. An optional infant blood sample was also collected at the end of the study for determination of carotenoids as described below. All blood samples were collected at the research site. Infant birth weight and length were also collected. For those infants providing a blood sample, an infant food record was collected. Weekly 3-d food records were kept by each participant to determine the dietary intake of lutein as described below. This study was conducted in accordance with Good Clinical Practice and International Conference of Harmonization and was approved by the Copernicus Group Institutional Review Board. Written informed consent was obtained from all participants before enrollment. Only carotenoid data are currently presented.

Breast milk and blood sample collections. Participants were instructed that a midmilk sample of ~20 mL should be collected from 1 breast starting ~5 min after the infant had begun suckling or the breast had begun to be pumped. Milk collection was recommended at the same time of the day, ideally between 1300 and 1700 h, with at least a 2-h time gap since the previous feeding with that breast. Carotenoid concentrations from a single, midafternoon breast milk sample have been shown to be representative of the mean 24-h concentration of carotenoids in breast milk (28). Random venous blood samples (~6 mL in mothers and ~4 mL in infants) were collected in sodium heparin vacuum tubes, inverted twice, and centrifuged within 1 h of collection at 1000–1300 *g* for 10 min to separate the plasma. The plasma layer was transferred into Eppendorf tubes and stored at −20°C for no more than 6 wk until analysis was performed as described below.

Carotenoid analysis. All samples were stored below −20°C and shipped frozen for analysis (Craft Technologies). Carotenoids were analyzed as previously described (29). Briefly, breast milk was saponified for 60 min at 70°C in 16% KOH containing pyrogallol blanketed with nitrogen. Samples were extracted with hexane/tetrahydrofuran, washed, and dried in a centrifugal evaporator. Residues were redissolved in ethyl acetate and diluted with acetonitrile/isopropanol (90:10 v:v) before HPLC. Plasma samples were diluted in water containing EDTA and ascorbic acid and then precipitated with ethanol containing Tocol [3,4-Dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol; Matreya]. The denatured plasma was extracted, dried in a centrifugal evaporator, dissolved in ethyl acetate, and diluted in acetonitrile/isopropanol (90:10 v:v). Breast milk and plasma concentrations of carotenoids were measured by using reverse-phase HPLC with diode array detection at 450 nm. Tocol monitored at 296 nm was used as an internal standard. Carotenoid

TABLE 1 Composition of study supplements[1]

Nutrient	Placebo capsule	Experimental capsule
Energy, *kcal*	5	5
Fat, *g*	0.5	0.5
DHA (22:6n–3), *mg*	0	200
Lutein, *mg*	0	6
d-α-Tocopherol, *IU*	0	30
dl-α-Tocopheryl acetate, *IU*	50.6	0
Soybean oil, *mg*	650	0

[1] The placebo group consumed 2 placebo capsules, the low-dose group consumed 1 placebo and 1 experimental capsule, and the high-dose group consumed 2 experimental capsules.

concentrations were calculated by external standards by using peak areas and adjusted for internal standard recovery.

Dietary analysis. All participants completed an FFQ modified from a previously published FFQ (30) that focused on lutein-containing foods. Participants also completed a 3-d food record each week and were instructed to write down all food and beverages consumed on any 3 d. The flexibility of any 3 d, compared with requiring 2 weekdays and 1 weekend day, was to increase compliance of recording intakes; a mean of ~24% of weekend days was reported, which is very similar to the percentage of days of the week that are weekend days (28.6%). Guidance and instruction were provided to the participants on completing the 3-d food record and for estimating serving sizes using commonly accepted portion sizes. All food records were analyzed by using The Food Processor (ESHA Research), and, whenever possible, USDA food values were used to most accurately capture lutein content of food.

Sample size and statistical analysis. The sample size was obtained from the software package nQuery Advisor 5.0 (Statistical Solutions Ltd.). The NHANES database from 2003 to 2004 was used to derive an estimate for the common SD for plasma lutein, which for women 18–45 y of age is 4.6 μg/dL. This value was used as the assumed common SD for breast milk because breast milk was not in the NHANES report and a sample size of 20 in each treatment group has 80% power to detect a difference in mean breast milk concentrations of lutein of at least 4.2 μg/dL by using a 2-group *t* test with a 0.05 2-sided significance level. The plasma lutein value was used as an estimate for breast milk concentrations of lutein. Because of general non-normality of carotenoid data, a log transformation was often used before analysis to improve model fit. ANOVA was used in baseline comparisons of continuous variables, whereas Cochran-Mantel-Haenszel test statistics were used in baseline comparisons of categorical variables. Carotenoids were compared between treatment groups by using ANOVA techniques. If there was an overall significant treatment group effect, then least-squares means were compared between each pair of treatment groups and adjusted for multiple comparisons by using the step-down Bonferroni adjustment. Relations between treatment groups were explored by using linear regression. Comparisons between paired data were analyzed by using *t* tests and Pearson's correlations, as appropriate. SAS version 9.2 was used to perform the statistical analyses.

Results

Participant characteristics. Of the 89 participants enrolled in the study, 7 were excluded for reasons documented in **Figure 1**. The

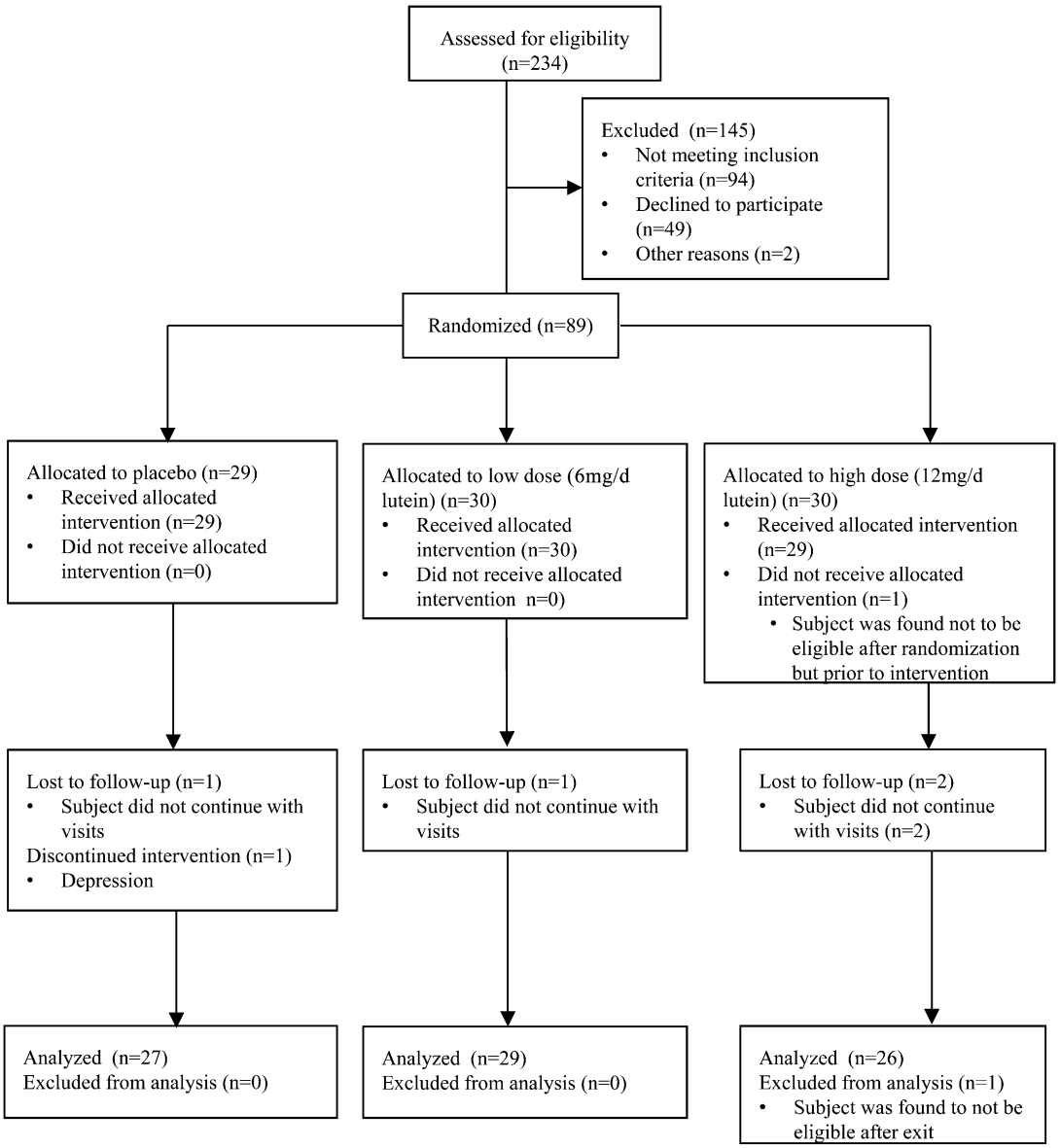

FIGURE 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

TABLE 2 Maternal and infant demographic characteristics[1]

	Placebo (n = 27)	Low-dose (n = 29)	High-dose (n = 26)
Maternal characteristics			
Ethnicity			
Not Hispanic	27 (100)	29 (100)	26 (100)
Race			
White	25 (93)	23 (79)	24 (92)
Black	2 (7)	4 (14)	2 (8)
Other	0	1 (3)	0
White/black	0	1 (3)	0
Current smoker	2 (7)	0	1 (4)
Consumes alcohol	8 (30)	3 (10)	7 (27)
Age at enrollment, y	29.4 ± 1.1	29.7 ± 0.9	27.8 ± 0.8
Prepregnancy BMI, kg/m^2	24.0 ± 0.61	24.7 ± 0.56	23.1 ± 0.54
Infant characteristics			
Gender			
M	15 (56)	15 (52)	12 (46)
F	12 (44)	14 (48)	14 (54)
Race			
White	25 (93)	21 (72)	23 (88)
Black	2 (7)	4 (14)	1 (4)
White/black	0	2 (7)	2 (8)
White/Asian	0	1 (3)	0
White/other	0	1 (3)	0
Ethnicity			
Hispanic	2 (7)	1 (3)	0
Not Hispanic	25 (93)	28 (97)	26 (100)
Birth weight, g			
Mean ± SEM	3432 ± 94.2	3562 ± 88.3	3496 ± 70.0
Median	3459	3600	3459
SD	489	475	357
Minimum, maximum	2608, 4706	2835, 4508	2920, 4224

[1] Values are n (%) or means ± SEMs unless otherwise indicated. There were no significant differences between the 3 groups.

82 participants who successfully completed the study and had data included in the analysis are described in **Table 2.** All were similar in age and BMI. Participants' mean self-reported intake of study supplements was ~99% compliant with the protocol to consume 2 pills/d for 6 wk and did not differ between the 3 groups (data not shown). There was no reported difference in dietary intake of lutein-containing foods in the month before starting the study as assessed by the FFQ, and there was no significant difference in dietary intake of lutein throughout the study as determined by self-reported 3-d food records (data not shown). All but 1 mother consented to provide an optional blood sample at the beginning and end of the study, and 61% of mothers consented to provide an optional blood sample from their infant at the end of the study; however, only 34% of samples were usable due to either not enough sample for analysis, mother withdrawing consent before blood draw, or a failed attempt at obtaining blood from the infant. The characteristics of the infants are summarized in Table 2. There were similar numbers of female and male infants, and mean birth weights were similar among the 3 groups. There was no difference in adverse events between the 3 groups in the mothers and infants, and none were related to the study intervention.

Characteristics of carotenoids in breast milk and plasma. At baseline for all participants, total lutein + zeaxanthin was the most abundant carotenoid in breast milk, whereas total lycopene was the most abundant in maternal plasma, and total lutein + zeaxanthin ranked fifth in maternal plasma. The overall distribution of carotenoids was different between maternal plasma and infant plasma (placebo group only), and the carotenoid distribution of infant plasma (placebo group only) more closely matched that of breast milk (**Fig. 2***A*, *B*). There was a significant correlation between breast milk and maternal plasma carotenoids at baseline (total lutein + zeaxanthin: r = 0.57; total lycopene: r = 0.51; total β-carotene; r = 0.59; P < 0.0001). There was a decline in breast milk (**Fig. 3***A*) and maternal plasma (Fig. 3*B*) non–lutein + zeaxanthin carotenoids over time. In breast milk, total lycopene decreased by 21%; there was no change in total β-carotene. In plasma, total lycopene and total β-carotene decreased by 28% and 22%, respectively. Over time, there was a significant decrease in all non–lutein + zeaxanthin carotenoids in maternal plasma (**Supplemental Table 1**), whereas only a few non–lutein + zeaxanthin carotenoids demonstrated significant decreases in breast milk concentration (**Supplemental Table 2**). In the placebo group, there was a trend toward a decrease in total lutein + zeaxanthin in breast milk over time (P = 0.08), and no change in maternal plasma.

Change in carotenoids with lutein supplementation. At the beginning of the study there was no difference in total lutein + zeaxanthin concentrations in breast milk (**Fig. 4***A*) or maternal plasma (Fig. 4*B*) between the 3 groups. After 6 wk of supplementation, total lutein + zeaxanthin in breast milk was 140% and 250% greater in the low- and high-dose groups, respectively,

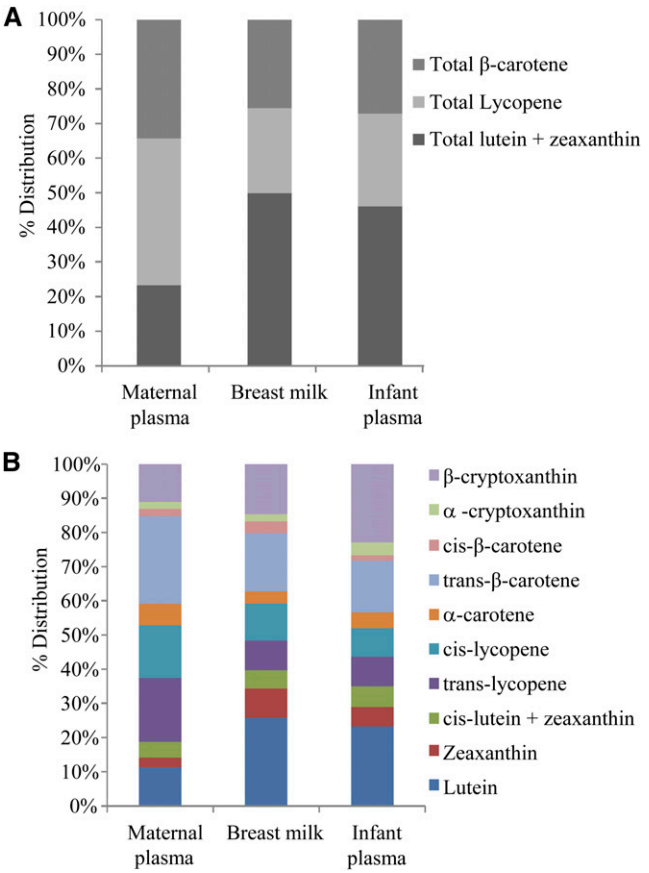

FIGURE 2 Distribution of total (*A*) and individual (*B*) carotenoids in maternal plasma and breast milk of the women at baseline and in the plasma of infants in the placebo group at 6 wk. Values are means and represented as percentages of carotenoids in the tissue (maternal plasma, n = 79; breast milk, n = 82; infant plasma, n = 11).

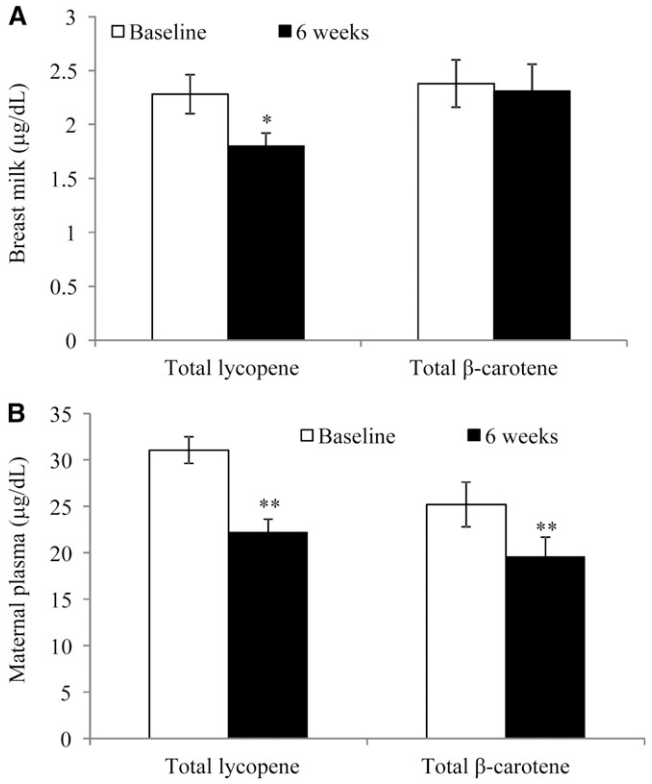

FIGURE 3 Breast milk (*A*) and maternal plasma (*B*) total lycopene and total β-carotene concentrations in the women at baseline and after 6 wk of supplementation with 0, 6, or 12 mg/d of lutein. Values are means ± SEMs, n = 78–82. Different from baseline: $^{*}P < 0.05$, $^{**}P < 0.0001$.

compared with placebo. The lutein + zeaxanthin concentration in breast milk tended to be 47% greater in the high-dose group than in the low-dose group ($P = 0.06$). Maternal plasma lutein + zeaxanthin was 170% and 250% greater in the low- and high-dose groups, respectively, compared with the placebo group, and that in the high-dose group was 35% greater than that in the low-dose group ($P < 0.05$). The change over the 6 wk of supplementation was significantly greater in the low- and high-dose groups compared with the placebo group, and that in the high-dose group was greater than that in the low-dose group in breast milk (Fig. 4*A*) and maternal plasma (Fig. 4*B*). There was a significant, positive linear relation between the 3 groups for both breast milk and maternal plasma (**Fig. 5***A*, *B*). The infants of mothers consuming the low- and high-dose supplements did not differ from one another, but had 180% and 330% greater concentrations of plasma total lutein + zeaxanthin, respectively, at the end of the study compared with the placebo group (28.4 ± 5.02 and 44.3 ± 10.7 vs. 10.3 ± 1.50 μg/dL, $P < 0.05$). There were no differences in other nonlutein carotenoids in infant plasma (**Supplemental Table 3**). Breast milk total lutein + zeaxanthin was significantly correlated with both maternal ($r = 0.74$, $P < 0.0001$) and infant ($r = 0.81$, $P < 0.0001$) plasma at the end of the study. Maternal and infant plasma total lutein + zeaxanthin were also significantly correlated ($r = 0.82$, $P < 0.0001$). The strength of the correlation between breast milk and maternal plasma total β-carotene increased over time [$r = 0.59$, $P < 0.0001$ (beginning); $r = 0.71$, $P < 0.0001$ (end)], whereas the strength of the correlation between breast milk and maternal plasma for total lycopene decreased [$r = 0.51$, $P < 0.0001$ (beginning); $r = 0.26$, $P = 0.02$ (end)]. Infant plasma total β-carotene was significantly correlated with breast milk ($r = 0.93$, $P < 0.0001$) and maternal plasma total β-carotene ($r = 0.70$, $P < 0.0001$); total lycopene demonstrated a significant correlation between infant plasma and breast milk ($r = 0.56$, $P < 0.005$).

Discussion

This is the first longitudinal study, to our knowledge, to report the breast milk and plasma concentrations of carotenoids 2–3 mo postpartum and to investigate the effect of lutein supplementation during this period. Most lutein supplementation trials have evaluated vision-related outcomes and demonstrated improvement in visual function in healthy participants (31), patients with age-related macular degeneration (15), and patients with age-related cataracts (32). This supplementation trial in lactating women demonstrates that, similar to other lutein supplementation trials in healthy adults (31), there is a dose-dependent response. Both doses of lutein significantly increased breast milk and maternal plasma total lutein + zeaxanthin compared with placebo, and there was a dose response in maternal plasma total lutein + zeaxanthin and a trend towards dose response in breast milk. One previous interim USDA report showed similar results demonstrating

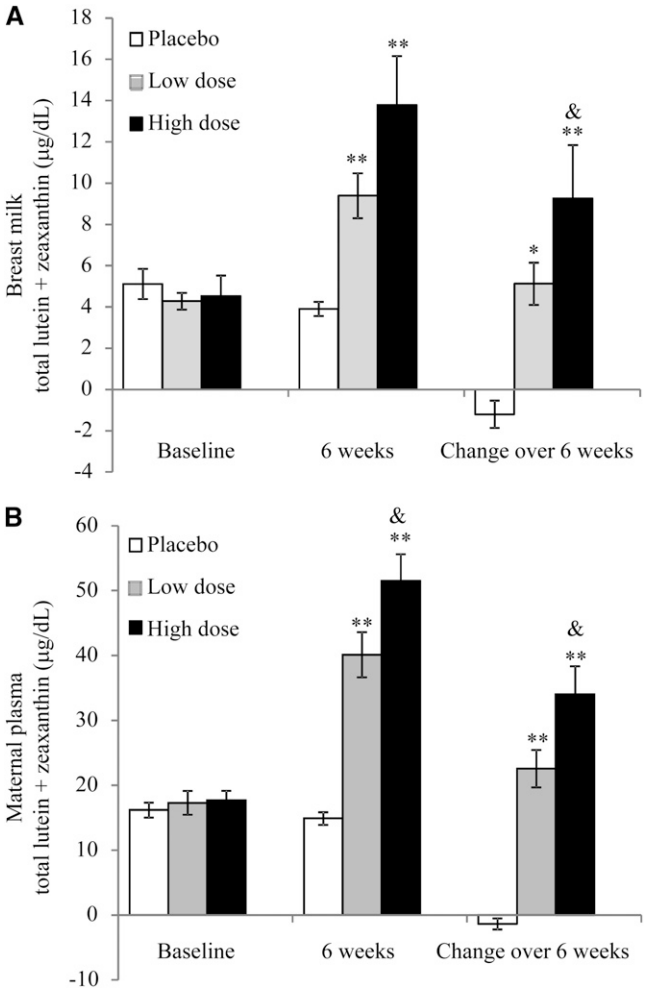

FIGURE 4 Breast milk (*A*) and maternal plasma (*B*) total lutein + zeaxanthin concentrations in women in the placebo, low-dose, and high-dose groups. Values are means ± SEMs, n = 24–29. Different from baseline: $^{*}P < 0.05$, $^{**}P < 0.0001$. Different between treatment groups: $^{\&}P < 0.05$.

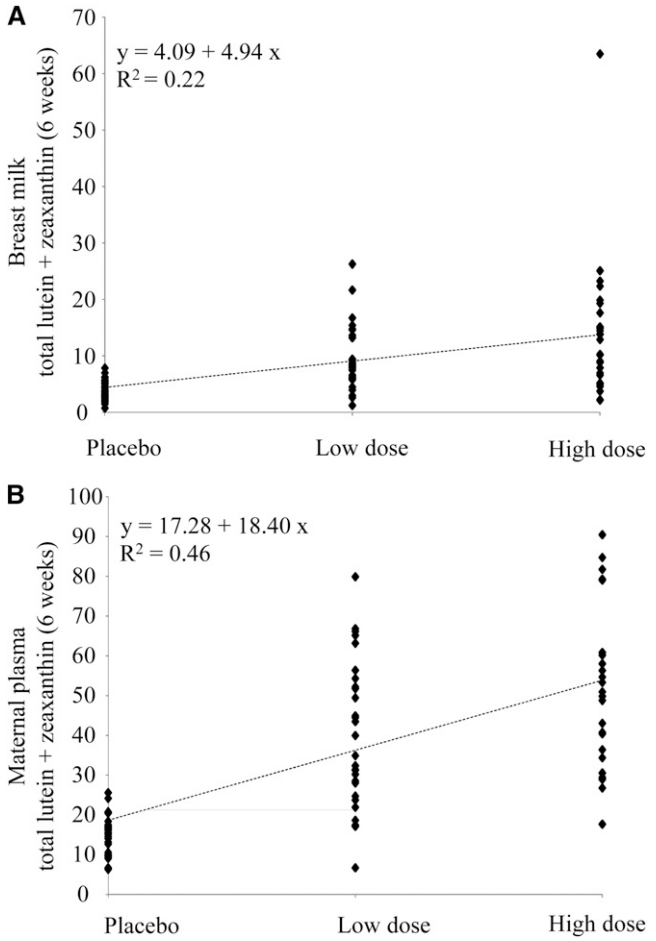

FIGURE 5 Regression analysis of breast milk (*A*) and maternal plasma (*B*) total lutein + zeaxanthin.

~200% and 300% greater concentrations in maternal plasma and breast milk lutein, respectively, after 3 wk of taking a 12-mg/d supplement (27). Infants of mothers administered both doses of lutein demonstrated a significant increase in plasma total lutein + zeaxanthin compared with placebo, and there was a strong trend (P = 0.06) toward significance between doses. Supplementation did not affect other non–lutein + zeaxanthin carotenoids in breast milk or plasma. Nonetheless, at 2–3 mo postpartum, all non–lutein + zeaxanthin carotenoids had significant decreases over time in plasma, and in breast milk only a few had significant decreases over time. Previous studies reported that breast milk carotenoids are stable after ~1 mo postpartum (33,34). To our knowledge, this is the first report to demonstrate a decrease in circulating carotenoids in lactating women.

Dietary lutein + zeaxanthin intake did not differ between the 3 groups and was within the intakes reported in other studies (9,33). The range of lutein + zeaxanthin intake was large, and those with the highest intakes reported consuming dark, leafy greens, mainly spinach, on the majority of the self-reported 3-d food records. The distribution of carotenoids in breast milk was different than in the U.S. population in a previous multinational study, which reported β-carotene as the most abundant carotenoid in breast milk and lutein as the most abundant in the diet (26). The current study demonstrates that, at baseline, total lutein + zeaxanthin was the most abundant carotenoid in breast milk and lycopene was the most abundant carotenoid in the diet followed by β-carotene. Canfield et al. (26) did not report lycopene in the diet, so it is unclear how the dietary intakes in these study populations differ. The baseline concentrations in breast milk of the major carotenoids are in agreement with some previous reports (34). However, in other studies (26), total lutein + zeaxanthin, lycopene, and β-cryptoxanthin concentrations were lower and α-carotene concentrations were higher, whereas the concentration of β-carotene was similar to that in the current study. It is likely that the increased variability in the collection time in Canfield et al. (26) (1–12 mo postpartum) resulted in the difference in our study with a more discrete period of 2–3 mo postpartum. Other studies in lactating women (35) and adolescent girls (36) reported lower values of breast milk carotenoids than in the current study. Although there is no clear distinction for the differences, these studies and the current data all report a wide range of carotenoid concentrations, demonstrating the variability of these constituents in breast milk. Plasma carotenoids in lactating women are comparable to those in adult participants as previously reported by the Institute of Medicine (37). Reports of carotenoids in breast-fed infants (35,38) had lower mean plasma lutein than in the placebo group in the current study. β-Carotene concentrations were similar to those reported in Mackey et al. (38), but lycopene concentrations were higher in the current study; additionally, infant plasma carotenoids in the current study were higher than in others (39). Because of the issues outlined in the Participants and Methods section in obtaining infant blood samples, there was a relatively small sample size for the infants, which may account for some of the differences in previous research.

Across the study, significant correlations between breast milk concentrations and plasma concentrations were demonstrated. As expected, the correlation between breast milk and maternal plasma total lutein + zeaxanthin increased in magnitude over time with supplementation. Carotenoids in the infant were significantly correlated with concentrations in the mother, both for breast milk and plasma. The strongest correlations were seen between infant plasma and breast milk for total lycopene and β-carotene and were very similar between infant plasma and breast milk and maternal plasma for total lutein + zeaxanthin. Total lutein + zeaxanthin and β-carotene had similar, strong correlations. The current study is in agreement with previous studies demonstrating a correlation between infant intake of carotenoids and plasma concentrations (35,38).

Breast milk lutein is highly variable and dependent on maternal intake. A previous report (40) examining lutein supplementation and macular pigment density demonstrated that some individuals do not respond to lutein supplementation, so-called lutein nonresponders. Interestingly, the study by Hammond et al. (40) had 3 response profiles that differentiated between nonresponse in tissue (macular pigment), plasma, or both. The current study demonstrates that there is also a varying response to lutein supplementation during lactation in both maternal plasma and breast milk. For example, 1 participant in the high-dose group had a particularly high breast milk total lutein + zeaxanthin value at the end of the study (63.5 μg/dL). This individual's samples were analyzed at 2 different time points from 2 different aliquots, and similarly high values were obtained. In addition to variability in response between participants, variability between "compartments" was also seen in this study, as was seen previously (40). For example, maternal plasma had a significant increase in lutein + zeaxanthin concentration with supplementation that explained ~46% of the variance. In those same participants, only ~22% of the variance in breast milk lutein + zeaxanthin was attributable to supplementation. Breast milk acts more like a "tissue" in this case and demonstrates similar response variability to other

tissues, such as retina, suggesting that "tissue" and "serum" nonresponse is also present when breast milk is the "tissue" of interest. Unlike in Hammond et al. (40), however, intake of the study supplements was determined from self-report (a limitation of this study); therefore, these results are suggestive of a "nonresponder" problem, but not conclusive. Of all the participants in the treatment groups with values similar to the placebo group at the end of the study, only 3 were likely to have lower than reported compliance on the basis of data from the other nutrients in the supplement (data not shown). The remaining participants with low lutein concentrations at baseline had generally low concentrations of lutein even with supplementation or were likely nonresponders (n = 4), only 1 of which was solely a breast milk nonresponder.

Lutein is the dominant carotenoid in the adult and infant brain (41,42), and lutein and its isomers are the only carotenoids found in the neural retina (12) and have been suggested to be important for the developing brain (11). As previously mentioned, lutein is also the dominant carotenoid in the neocortex, where it may serve additional functions, such as improved neural efficiency (43). For example, a number of studies have related lutein and zeaxanthin concentrations in the neural retina, as a biomarker of cortical lutein concentrations, to improved function in the visuomotor system (44), improved executive function (45), and, when supplemented, to improved working memory capacity (46). Therefore, the goal of this study was to determine whether lutein supplementation in lactating mothers increased breast milk lutein concentrations and provided corresponding plasma increases in their infants. Whether or not lutein can affect cognitive development early in life is not yet known; nonetheless, breast milk or infant formula is the sole source of nutrition during this critical period of development. Given the relation between intake of lutein by the breastfeeding mother, infant plasma concentrations, and the importance of lutein as an antioxidant and in eye health, it is prudent that lactating women consume adequate lutein from the diet or supplements to ensure adequate concentrations in breast milk for infant growth and development.

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C.L.S. and B.J.M. designed the project; C.L.S. conducted the research and had primary responsibility for the final content; C.L.S. and J.S.O. analyzed the data; and C.L.S. and L.M.R. wrote the manuscript . All authors read and approved the final manuscript.

References

1. Picciano MF. Nutrient composition of human milk. Pediatr Clin North Am 2001;48:53–67.
2. Fischer LM, da Costa KA, Galanko J, Sha W, Stephenson B, Vick J, Zeisel SH. Choline intake and genetic polymorphisms influence choline metabolite concentrations in human breast milk and plasma. Am J Clin Nutr 2010;92:336–46.
3. Valent F, Horvat M, Mazej D, Stibilj V, Barbone F. Maternal diet and selenium concentration in human milk from an Italian population. J Epidemiol 2011;21:285–92.
4. Samur G, Topcu A, Turan S. Trans fatty acids and fatty acid composition of mature breast milk in Turkish women and their association with maternal diet's. Lipids 2009;44:405–13.
5. Rist L, Mueller A, Barthel C, Snijders B, Jansen M, Simoes-Wust AP, Huber M, Kummeling I, von Mandach U, Steinhart H, et al. Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. Br J Nutr 2007;97:735–43.
6. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr 2007;85:1457–64.
7. Basile LA, Taylor SN, Wagner CL, Horst RL, Hollis BW. The effect of high-dose vitamin D supplementation on serum vitamin D levels and milk calcium concentration in lactating women and their infants. Breastfeed Med 2006;1:27–35.
8. Wagner CL, Hulsey TC, Fanning D, Ebeling M, Hollis BW. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. Breastfeed Med 2006;1:59–70.
9. Cena H, Castellazzi AM, Pietri A, Roggi C, Turconi G. Lutein concentration in human milk during early lactation and its relationship with dietary lutein intake. Public Health Nutr 2009;12:1878–84.
10. Webb AL, Aboud S, Furtado J, Murrin C, Campos H, Fawzi WW, Villamor E. Effect of vitamin supplementation on breast milk concentrations of retinol, carotenoids and tocopherols in HIV-infected Tanzanian women. Eur J Clin Nutr 2009;63:332–9.
11. Zimmer JP, Hammond BR Jr. Possible influences of lutein and zeaxanthin on the developing retina. Clin Ophthalmol 2007;1:25–35.
12. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. Invest Ophthalmol Vis Sci 1984;25:674–85.
13. Frick JE, Dengler M, Hammond BR. Effects of dietary intake of lutein and zeaxanthin on maturation of the human visual system. Argo Food Industry Hi Tech 2009;20:18–20.
14. Hammond BR Jr., Fletcher LM, Elliott JG. Glare disability, photostress recovery, and chromatic contrast: relation to macular pigment and serum lutein and zeaxanthin. Invest Ophthalmol Vis Sci 2013;54:476–81.
15. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, Pei K, Tsipursky M, Nyland J. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST Study (Lutein Antioxidant Supplementation Trial). Optometry 2004;75:216–30.
16. USDA; U.S. Department of Health and Human Services. *Dietary Guidelines for Americans 2010*. 7th ed. Washington: U.S. Government Printing Office, 2010.
17. Kruger CL, Murphy M, DeFreitas Z, Pfannkuch F, Heimbach J. An innovative approach to the determination of safety for a dietary ingredient derived from a new source: case study using a crystalline lutein product. Food Chem Toxicol 2002;40:1535–49.
18. Perry A, Rasmussen HM, Johnson EJ. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. J Food Compos Anal 2009;22:9–15.
19. Ervin R. Healthy Eat Index-2005 total and component scores for adults aged 20 and over: National Health and Nutrition Examination Survey, 2003–2004. Hyattsville (MD): National Center for Health Statistics; 2011.
20. George SM, Thompson FE, Midthune D, Subar AF, Berrigan D, Schatzkin A, Potischman N. Strength of the relationships between three self-reported dietary intake instruments and serum carotenoids: the Observing Energy and Protein Nutrition (OPEN) Study. Public Health Nutr 2012;15:1000–7.
21. Yeum KJ, Ferland G, Patry J, Russell RM. Relationship of plasma carotenoids, retinol and tocopherols in mothers and newborn infants. J Am Coll Nutr 1998;17:442–7.
22. Curran-Celentano J, Hammond BR Jr., Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. Am J Clin Nutr 2001;74:796–802.
23. Khachik F, Spangler CJ, Smith JC Jr., Canfield LM, Steck A, Pfander H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 1997;69:1873–81.
24. Macias C, Schweigert FJ. Changes in the concentration of carotenoids, vitamin A, alpha-tocopherol and total lipids in human milk throughout early lactation. Ann Nutr Metab 2001;45:82–5.
25. Lietz G, Mulokozi G, Henry JC, Tomkins AM. Xanthophyll and hydrocarbon carotenoid patterns differ in plasma and breast milk of women supplemented with red palm oil during pregnancy and lactation. J Nutr 2006;136:1821–7.
26. Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J, Goldman WJ, Pramuk K, Reyes H, Sablan B, et al. Multinational study of major breast milk carotenoids of healthy mothers. Eur J Nutr 2003;42:133–41.
27. Connor WE, Lowensohn R, Connor S, Wei W. The placental and mammary transfer of lutein and zeaxanthin into the fetus and the breast-fed infant. Oregon Health and Science University; National Institute of Food and Agriculture; 2006.

28. Giuliano AR, Nieilson EM, Yap HH, Baier M, Canfield LM. Quantitation of and inter/intra-individual variability in major carotenoids of mature human milk. J Nutr Biochem 1994;5:551–6.
29. Craft NE. Chromatographic techniques for carotenoid separation in *Current Protocols in Food Analytical Chemistry*. New York: Wiley; 2001.
30. Cena H, Roggi C, Turconi G. Development and validation of a brief food frequency questionnaire for dietary lutein and zeaxanthin intake assessment in Italian women. Eur J Nutr 2008;47:1–9.
31. Ma L, Lin XM, Zou ZY, Xu XR, Li Y, Xu R. A 12-week lutein supplementation improves visual function in Chinese people with long-term computer display light exposure. Br J Nutr 2009;102:186–90.
32. Olmedilla B, Granado F, Blanco I, Vaquero M. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. Nutrition 2003;19:21–4.
33. Gossage CP, Deyhim M, Yamini S, Douglass LW, Moser-Veillon PB. Carotenoid composition of human milk during the first month postpartum and the response to beta-carotene supplementation. Am J Clin Nutr 2002;76:193–7.
34. Jackson JG, Lien EL, White SJ, Bruns NJ, Kuhlman CF. Major carotenoids in mature human milk: longitudinal and diurnal patterns. J Nutr Biochem 1998;9:2–7.
35. Bettler J, Zimmer JP, Neuringer M, DeRusso PA. Serum lutein concentrations in healthy term infants fed human milk or infant formula with lutein. Eur J Nutr 2010;49:45–51.
36. de Azeredo VB, Trugo NM. Retinol, carotenoids, and tocopherols in the milk of lactating adolescents and relationships with plasma concentrations. Nutrition 2008;24:133–9.
37. Institute of Medicine. Dietary Reference Intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington: National Academy of Sciences; Institute of Medicine Food and Nutrition Board; 2000.
38. Mackey AD, Albrecht D, Oliver J, Williams T, Long AC, Price PT. Plasma carotenoid concentrations of infants are increased by feeding a milk-based infant formula supplemented with carotenoids. J Sci Food Agric 2013;93:1945–52.
39. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Carotenoid supply in breast-fed and formula-fed neonates. Eur J Pediatr 2000;159:86–90.
40. Hammond BR Jr., Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, Snodderly DM. Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 1997;38:1795–801.
41. Johnson EJ, Vishwanathan R, Schalch W, Poon L, Wittwer J, Johnson MA, Hausman D, Davey A, Green R, Gearing M, et al. Brain levels of lutein (L) and zeaxanthin (Z) are related to cognitive function in centenarians. *FASEB J* 2011;25:975.21.
42. Vishwanathan R, Kuchan M, Sen S, Johnson EJ. Lutein is the predominant carotenoid in infant brain: preterm infants have decreased concentrations of brain carotenoids. J Pediatr Gastroenterol Nutr 2014 (Epub ahead of print; DOI:10.1097/MPG.0000000000000389).
43. Renzi LM, Hammond BR Jr. The relation between the macular carotenoids, lutein and zeaxanthin, and temporal vision. Ophthalmic Physiol Opt 2010;30:351–7.
44. Renzi LM, Bovier ER, Hammond BR. A role for the macular carotenoids in visual motor response. Nutr Neurosci 2013;16:262–8.
45. Vishwanathan R, Iannaccone A, Scott TM, Kritchevsky SB, Jennings BJ, Carboni G, Forma G, Satterfield S, Harris T, Johnson KC, et al. Macular pigment optical density is related to cognitive function in older people. Age Ageing 2014;43(2):271–5.
46. Johnson EJ, McDonald K, Caldarella SM, Chung HY, Troen AM, Snodderly DM. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. Nutr Neurosci 2008;11:75–83.