

A 12-Wk Egg Intervention Increases Serum Zeaxanthin and Macular Pigment Optical Density in Women¹

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Abstract

Two carotenoids found in egg yolk, lutein and zeaxanthin, accumulate in the macular retina where they may reduce photostress. Increases in serum lutein and zeaxanthin were observed in previous egg interventions, but no study measured macular carotenoids. The objective of this project was to determine whether increased consumption of eggs would increase retinal lutein and zeaxanthin, or macular pigment. Twenty-four females, between 24 and 59 y, were assigned to a pill treatment (PILL) or 1 of 2 egg treatments for 12 wk. Individuals in the PILL treatment consumed 1 sugar-filled capsule/d. Individuals in the egg treatments consumed 6 eggs/wk, containing either 331 μg (EGG 1) or 964 μg (EGG 2) of lutein and zeaxanthin/yolk. Serum cholesterol, serum carotenoids, and macular pigment OD (MPOD) were measured at baseline and after 4, 8, and 12 wk of intervention. Serum cholesterol concentrations did not change in either egg treatment group, but total cholesterol ($P = 0.04$) and triglycerides ($P = 0.02$) increased in the PILL group. Serum zeaxanthin, but not serum lutein, increased in both the EGG 1 ($P = 0.04$) and EGG 2 ($P = 0.01$) groups. Likewise, MPOD increased in both the EGG 1 ($P = 0.001$) and EGG 2 ($P = 0.049$) groups. Although the aggregate concentration of carotenoid in 1 egg yolk may be modest relative to other sources, such as spinach, their bioavailability to the retina appears to be high. Increasing egg consumption to 6 eggs/wk may be an effective method to increase MPOD. J. Nutr. 136: 2568–2573, 2006.

Introduction

Eggs are one of the most nutrient-dense foods regularly consumed in the human diet. In addition to several essential vitamins and minerals, eggs contain the carotenoids lutein and zeaxanthin. These carotenoids are derived from the hen's feed and deposited in the yolk, contributing to its yellow hue (1). Humans, like hens, cannot synthesize lutein and zeaxanthin. Rather, they are acquired in the diet by consuming egg yolks, fruits, and green-leafy vegetables. The amount of lutein and zeaxanthin consumed in the diet is associated with risk for various diseases, including cancer and heart disease (2). Additionally, lutein and zeaxanthin are associated with eye health due to their accumulation in the retina (3). In the retina, lutein and zeaxanthin are collectively called macular pigment because of their accumulation in macular cells and yellow coloration of the macular retina; hence, the term macula lutea.

The macular pigment may protect the central retina from disease by attenuating photostress. Lutein and zeaxanthin are deposited primarily in the anterior layers of the retina (4). Consequently, light incident on the central retina must pass

through the macular pigment before reaching the photopigments and posterior tissue. The macular carotenoids absorb wavelengths of visible light below ~ 520 nm. Ham et al. (5) showed that such wavelengths of light are more potentially harmful than longer wavelengths of light in that 10 times or more energy was needed to induce retinal damage at long wavelengths of visible light compared with shorter ones. The amount of light absorbed by macular pigment, measured as OD, varies among individuals (6). Relative to individuals with high macular pigment OD (MPOD),⁵ individuals with low MPOD may be at greater risk for retinal disease due to the greater levels of potentially harmful short-wavelength light reaching posterior ocular tissue. Bone et al. (7) found that eyes from individuals with age-related macular degeneration had significantly lower concentrations of macular carotenoids compared with eyes from individuals of similar age but free from disease.

If macular pigment protects the central retina, individuals at risk for retinal disease may benefit from lutein and zeaxanthin supplementation. Intervention studies have found changes in

¹ Supported in part by the Egg Nutrition Center and New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2296 from the New Hampshire Agricultural Experiment Station.

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⁵ Abbreviations used: CHD, coronary heart disease; EGG 1, treatment of 6 eggs/wk for 12 wk with 331 μg of lutein and zeaxanthin/yolk; EGG 2, treatment of 6 eggs/wk for 12 wk with 964 μg of lutein and zeaxanthin/yolk; HDL-C, HDL cholesterol; L+Z, combined serum lutein and zeaxanthin; LDL-C, LDL cholesterol; MPOD, macular pigment OD; PILL, treatment of 1 sugar-filled capsule/d for 12 wk; TC:HDL-C, ratio of total cholesterol to HDL-C; η_p^2 , partial eta squared.

MPOD following lutein supplementation with doses between 2.6 mg/d (8) and 30 mg/d (9). Aleman et al. (10), for example, reported a significant mean increase of ~ 0.08 log units after subjects consumed 20 mg of lutein supplements for 6 mo. Similar increases were observed in 3 mo using 30 mg of lutein supplements (9) or 11.2 mg of lutein and 0.6 mg of zeaxanthin from a combination of spinach and corn (11). Although the retinal loci measured in these studies were slightly different, the similar increases in MPOD with dissimilar doses of lutein suggest that lutein, and perhaps zeaxanthin, may be more bioavailable to the retina from spinach than from supplements. Recently, Chung et al. (12) observed a 33% higher serum lutein response after subjects consumed spinach compared with when they consumed an equal dose of lutein from supplements. The serum response after consuming either spinach or supplements, however, was significantly lower than the response to an equal amount of lutein from lutein-enhanced eggs. These findings suggest that lutein may be more bioavailable from a food matrix than from supplements. Further, increased consumption of eggs may increase not only serum, but macular, carotenoid concentrations. The objective of the current project was to determine whether consuming 6 eggs/wk for 12 wk could increase MPOD.

Subjects and Methods

Subject protocol. Twenty-four females, between 24 and 59 y, volunteered to participate in the study. Individuals were excluded from participation if they met any of the following criteria: egg allergy, history of heart or gastrointestinal disease, diabetes, current smoker, BMI >30 , or a total cholesterol to HDL cholesterol (HDL-C) ratio exceeding 5.0, the upper limit recommended by the American Heart Association. The subjects were assigned to 1 of 3 treatment groups: PILL ($n = 8$), EGG 1 ($n = 8$), and EGG 2 ($n = 8$). Individuals in the PILL treatment group were instructed to consume 1 pill/d, a gelatin capsule containing sugar, for 12 wk. Individuals in the EGG 1 and EGG 2 treatment groups were asked to consume 6 "treatment" eggs/wk for 12 wk. The lutein and zeaxanthin content of several locally available brands of eggs was analyzed to find 2 varieties with different doses of carotenoid. The treatment eggs administered to individuals in the EGG 1 group contained ~ 331 μg of lutein and zeaxanthin/yolk and were purchased from a local supermarket. The treatment eggs for the EGG 2 group contained ~ 964 μg of lutein and zeaxanthin/yolk and were purchased from a local, certified organic farm. Subjects in EGG 1 and EGG 2 received 1 dozen treatment eggs every 2 wk. They were instructed to consume no more than 2 treatment eggs/d and not go >2 d without eating the eggs. Subjects in the PILL group received a 1-mo supply of pills in an unmarked bottle each month. Empty pill bottles and egg cartons were collected at each laboratory visit. All subjects were told to continue their normal diet and use of multivitamins, as no subjects were consuming supplements containing lutein or zeaxanthin.

At baseline, height and weight were measured for the calculation of BMI (kg/m^2). Serum lipids, serum carotenoids, and MPOD were measured at baseline and after 4, 8, and 12 wk of intervention. The use of human subjects in this study was approved by the University of New Hampshire Institutional Review Board and complied with the Helsinki Declaration.

Measurement of serum cholesterol. Subjects fasted for 12 h prior to each laboratory visit. A trained phlebotomist collected a blood sample from the subject's antecubital vein into a lithium heparin Vacutainer tube (36-7871; Becton Dickinson). A 60- μL sample was drawn from the tube and injected into the well of a lipid test cassette (10-991) and analyzed by a Cholestech LDX instrument. In short, this system uses reflectance photometry to determine lipoprotein concentrations after 4 reagent pads react with the blood sample. In 9 subjects, 7 in EGG 1 and 2 in PILL, lipoprotein concentrations were measured using a Roche Cobas Mira Plus (Roche Instrument Center) analyzer because the Cholestech LDX was not available. With this instrument, lipoprotein concentrations were

measured using selective antibody reagents. Both instruments provided total cholesterol, HDL-C, and triglyceride concentrations. LDL cholesterol (LDL-C) was calculated with the Friedewald formula (total cholesterol - HDL-C - triglyceride/2.2). Total cholesterol was divided by HDL-C to yield a ratio (TC:HDL-C) associated with heart disease risk (13). The variability in measurements obtained with the Cobas Mira Plus is $\sim \pm 2\%$, whereas the measurement variability of cholesterol for the Cholestech LDX is $\sim \pm 7\%$.

Measurement of serum carotenoids. A second blood sample was collected at each visit for carotenoid analyses. Blood was collected from the antecubital vein into a Vacutainer SST Gel and Clot Activator tube (36-6511 Becton Dickinson). After clotting for 30 min, blood was centrifuged at $3000 \times g$ at 4°C for 15 min (Beckman Centrifuge TJ-6; Beckman Coulter). Serum was drawn off and stored in aliquots at -80°C until analyzed.

Aliquots were prepared following the technique of Handelman et al. (14). In brief, the serum samples were precipitated with ethanol containing internal standard and extracted into hexane. The extraction procedure was performed twice and the layers from both extractions were combined in amber vials. The sample was completely evaporated under nitrogen, and then resuspended in 200 μL ethanol. An aliquot of 20 μL was removed for analysis with reversed-phase HPLC. Two separate analyses of each sample were performed.

Serum concentrations of lutein, zeaxanthin, and β -carotene were quantified with a Hewlett-Packard/Agilent Technologies 1100 Series HPLC system (Waldbronn). The photodiode array detector was set at 452 nm and a C_{18} analytical column (4.6- \times 250-mm Bakerbond Mallinckrodt Baker) was used to separate the analytes. The mobile phase had a flow rate of 1.0 mL/min and consisted of 90% acetonitrile and 10% methanol buffered with 0.1% ammonium acetate (Solvent A). From the start of the run to min 18, 100% Solvent A was running. Beginning at min 18, the mobile phase transitioned, for 4 min, to 70% solvent A and 30% Solvent B, 100% isopropanol. This gradient was maintained for 23 min, after which transition to 100% solvent A occurred over 1 min. Quantification was performed with peak area ratios to internal standards and by simultaneously analyzing laboratory standards and external standards. Accuracy of the method to quantify carotenoids was assessed using the National Institute of Standards and Technology (NIST) Standard Reference Material SRM 986. The variability for replicate analyses of carotenoids was $< \pm 2\%$.

Measurement of egg yolk carotenoids. The concentration of lutein and zeaxanthin in randomly selected treatment eggs was analyzed with a technique described by Handelman et al. (15). In short, egg yolks were separated from the albumin and homogenized with 150 mL of $1 \times$ PBS. A 1-mL aliquot was collected and placed on a dry bath incubator heat block (11-718-6, Fisher Scientific) at 60°C for 60 min. Samples were then precipitated with ethanol containing internal standard and extracted into a 50:50 mixture of hexane and ethyl ether. The extraction procedure was performed twice, with layers from both extractions combined in amber vials. The sample was completely evaporated under nitrogen and then resuspended in 200 μL ethanol. An aliquot of 20 μL was removed for HPLC analysis. Two separate analyses of each sample were performed. The same reverse-phase gradient HPLC system used for serum carotenoid analyses was used for egg carotenoid analyses.

Measurement of MPOD. Heterochromatic flicker photometry was used to measure MPOD (16). In this technique, the difference in short-wavelength sensitivity between a foveal locus, where macular pigment is present, and a parafoveal locus, where macular pigment is undetectable or negligible, is used to estimate MPOD. In the fovea, sensitivity to a short-wavelength light was measured with a centrally fixed disc that subtended 1° of visual angle. Sensitivity in the nasal parafovea was measured with a 2° disc centered at 7° eccentricity by the subject's fixation of a small point of light. Both discs were composed of a 460-nm test light that alternated in square-wave with a 540-nm reference light (1.7 log trolands), and superimposed on a 6° , 470-nm background (1.5 log trolands). The radiance of the test light, relative to the reference light, necessary to eliminate a subject's perception of flicker was determined 6 times for both targets. The mean log radiance of these measures was used to calculate MPOD. Specifically, the mean log energy of the 6 measures

in the parafovea was subtracted from the mean log energy of the 6 foveal measures to yield MPOD. Two measures of MPOD were obtained for each subject at baseline, 4, 8, and 12 wk. One measure was performed on the day of the blood draw and a second was performed within 3 d. The mean of these 2 measurements was used as a subject's MPOD for the corresponding visit (e.g., baseline). The variability observed between the 2 measurements was $\sim\pm 0.03$ log units. An optical system designed by Macular Metrics and described by Wooten et al. (17) was used to conduct the measurements.

Statistical analysis. Subjects' serum cholesterol, serum carotenoids, and MPOD at baseline were plotted as histograms and fit with a normal curve. All data appeared to be normally distributed based on a visual inspection of the data. Pearson product-moment correlations were performed using baseline subject characteristics (i.e., age and BMI), lipoprotein concentrations, serum carotenoid concentrations, and MPOD. A 1-way ANOVA and Tukey's HSD procedure were used to compare between-group means at baseline. Within-group changes in lipoproteins, serum carotenoids, and MPOD were assessed using one-factor repeated measures ANOVA. For significant tests ($P < 0.05$), the strength of association was calculated with partial eta squared (η_p^2). All analyses were performed with SPSS 13.0 and Origin 7.0. Data are reported as means \pm SEM.

Results

At baseline, neither serum lutein ($r = 0.34$, $P = 0.09$) nor serum zeaxanthin ($r = 0.2$, $P = 0.90$) concentrations were related to MPOD in this sample. Serum total cholesterol was not correlated with lutein ($r = 0.39$, $P = 0.06$), zeaxanthin ($r = 0.14$, $P = 0.51$), or MPOD ($r = 0.324$, $P = 0.12$). In contrast, HDL-C was related to both combined serum lutein and zeaxanthin (L+Z; $r = 0.40$, $P = 0.04$) and MPOD ($r = 0.44$, $P = 0.03$). Serum L+Z was also related to LDL-C ($r = 0.42$, $P = 0.04$) and triglyceride ($r = 0.50$, $P = 0.01$) concentrations. Serum β -carotene, age, and BMI were not related to serum lipid concentrations, serum lutein and zeaxanthin or MPOD. Subject characteristics and serum lipoprotein and serum carotenoid concentrations did not differ among the 3 treatment groups at baseline (Table 1). The baseline MPOD, however, differed between the EGG 1 and EGG 2 groups ($P < 0.05$).

Serum total cholesterol significantly increased in the PILL group ($P = 0.042$, $\eta_p^2 = 0.31$) but not in the EGG 1 ($P = 0.83$) or EGG 2 ($P = 0.47$) groups; the changes in the PILL group

TABLE 1 Baseline data for EGG 1, EGG 2, and PILL treatment groups¹

Variable	EGG 1	EGG 2	PILL
Age, y	34.1 \pm 0.79	35.8 \pm 4.89	28.4 \pm 5.40
BMI	23.1 \pm 1.09	21.3 \pm 1.40	23.1 \pm 1.34
Serum lipids, mmol/L			
Total cholesterol	4.76 \pm 0.26	4.86 \pm 0.42	4.66 \pm 0.32
HDL-C	1.65 \pm 0.11	1.61 \pm 0.12	1.63 \pm 0.11
LDL-C	2.55 \pm 0.24	2.78 \pm 0.30	2.57 \pm 0.26
Triglycerides	0.98 \pm 0.11	1.00 \pm 0.19	1.00 \pm 0.13
TC:HDL-C	2.9 \pm 0.18	3.0 \pm 0.14	2.9 \pm 0.21
Serum carotenoids, μ mol/L			
Lutein	0.389 \pm 0.07	0.428 \pm 0.09	0.505 \pm 0.11
Zeaxanthin	0.101 \pm 0.02	0.057 \pm 0.01	0.096 \pm 0.02
Lutein + zeaxanthin	0.490 \pm 0.09	0.485 \pm 0.09	0.601 \pm 0.12
β -Carotene	1.797 \pm 0.77	0.442 \pm 0.10	0.912 \pm 0.19
MPOD	0.18 ^a \pm 0.02	0.37 ^b \pm 0.06	0.27 ^{ab} \pm 0.04

¹ Values are means \pm SEM ($n = 8$). Means in a row with superscripts without a common letter differ, $P < 0.05$.

followed a cubic trend ($P = 0.047$, $\eta_p^2 = 0.45$) (Table 2). HDL-C did not change in any treatment group (EGG 1: $P = 0.22$; EGG 2: $P = 0.35$; PILL: $P = 0.35$). Consequently, the ratio of total cholesterol to HDL-C (TC:HDL-C) did not change in the EGG 1 ($P = 0.31$) or EGG 2 ($P = 0.58$) treatment groups. In contrast, the increased total cholesterol in the PILL group resulted in a TC:HDL-C ratio that followed a cubic trend ($P = 0.001$, $\eta_p^2 = 0.82$) and differed from baseline ($P < 0.001$, $\eta_p^2 = 0.83$). Serum LDL-C concentrations did not change from baseline (EGG 1: $P = 0.47$; EGG 2: $P = 0.75$; PILL: $P = 0.07$). Like total cholesterol, triglycerides did not change in the EGG 1 ($P = 0.33$) or EGG 2 ($P = 0.50$) groups, but did increase in the PILL group ($P = 0.02$, $\eta_p^2 = 0.74$) and appeared to follow a cubic trend ($P = 0.04$, $\eta_p^2 = 0.45$).

Although serum total cholesterol did not significantly change from baseline in either egg group, treatment was discontinued in 2 subjects in EGG 1 and 1 subject in EGG 2 after 2 mo of intervention because their serum total cholesterol exceeded 5.1 mmol/L. The increase of total cholesterol in these subjects was ~ 0.83 mmol/L, representing a 17% increase. Their HDL-C concentration, likewise, increased $\sim 17\%$, or 0.28 mmol/L. As a result, their TC:HDL-C ratio did not change from baseline. Their ratio at baseline and after 2 mo of intervention was 3.2. Total cholesterol decreased 1 mo after cessation of treatment in all 3 subjects, and HDL-C decreased in 2 subjects. The decreases in total cholesterol and HDL-C were 0.61 mmol/L and 0.07 mmol/L, respectively.

After 12 wk of intervention, serum lutein concentration was 0.477 ± 0.06 μ mol/L in EGG 1 ($P = 0.014$), 0.540 ± 0.01 μ mol/L in EGG 2 ($P = 0.52$), and 0.457 ± 0.11 μ mol/L in the PILL group ($P = 0.56$). In contrast to serum lutein, serum zeaxanthin significantly increased from baseline in both egg treatment groups (EGG 1: $P = 0.04$, $\eta_p^2 = 0.30$; EGG 2: $P = 0.01$, $\eta_p^2 = 0.46$) (Figure 1). Serum zeaxanthin did not change in the PILL group ($P = 0.17$). Serum L+Z, like serum lutein (alone), increased in the EGG 1 group ($P = 0.07$) but did not change in the EGG 2 ($P = 0.33$) and PILL ($P = 0.45$) groups. At wk 12, serum β -carotene concentration was 2.16 ± 0.86 μ mol/L in the EGG 1 group ($P = 0.56$), 0.607 ± 0.13 μ mol/L in the EGG2 group ($P = 0.26$), and 0.859 ± 0.23 μ mol/L in the PILL group ($P = 0.53$).

In both EGG 1 ($P = 0.001$, $\eta_p^2 = 0.53$) and EGG 2 ($P = 0.04$, $\eta_p^2 = 0.30$), MPOD significantly increased from baseline (Figure 2). The increases in MPOD followed a linear trend (EGG 1: $P = 0.007$, $\eta_p^2 = 0.67$; EGG 2: $P = 0.042$, $\eta_p^2 = 0.47$). No change in MPOD was observed in the PILL group ($P = 0.51$).

The carotenoid composition of the eggs distributed to subjects in the EGG 1 group varied considerably. In the 46 eggs randomly selected for carotenoid analyses, the lutein

TABLE 2 Changes in serum lipid concentrations from baseline to wk 12 of intervention for the EGG 1, EGG 2, and PILL treatment groups¹

Serum lipids	mmol/L		
	EGG 1	EGG 2	PILL
Total cholesterol	0.16 \pm 0.21	0.26 \pm 0.23	0.27 ² \pm 0.21
HDL-C	0.10 \pm 0.07	0.06 \pm 0.10	-0.09 \pm 0.07
LDL-C	0.10 \pm 0.15	0.13 \pm 0.21	0.30 \pm 0.17
Triglycerides	-0.11 \pm 0.08	0.13 \pm 0.18	0.11 ² \pm 0.07
TC:HDL-C	-0.06 \pm 0.10	0.05 \pm 0.21	0.17 \pm 0.04

¹ Values are mean changes from baseline ($n = 8$).

² Different, $P < 0.05$, from baseline (see Table 1).

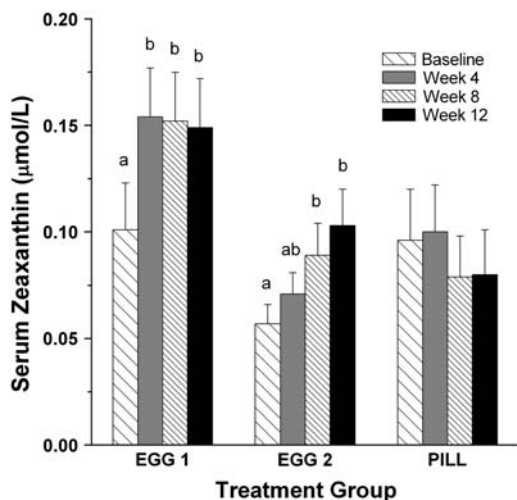


Figure 1 Serum zeaxanthin concentration for each treatment group at baseline and after 4, 8, and 12 wk of intervention. Values are means \pm SEM, $n = 8$. Within-group means without a common letter differ, $P < 0.05$.

content ranged from 63.74 μg to 655.71 $\mu\text{g}/\text{yolk}$, with a mean concentration of $197.14 \pm 131.4 \mu\text{g}$. The zeaxanthin content ranged between 61.31 μg and 271.95 $\mu\text{g}/\text{yolk}$, with a mean concentration of $133.35 \pm 56.70 \mu\text{g}$. In contrast, the lutein content of the 22 eggs analyzed from the EGG 2 treatment ranged from 436.65 μg to 833.81 $\mu\text{g}/\text{yolk}$, with a mean concentration of $598.92 \pm 131.7 \mu\text{g}$. The zeaxanthin content ranged between 187.49 μg and 705.39 $\mu\text{g}/\text{yolk}$, with a mean concentration of $364.98 \pm 177.8 \mu\text{g}$. The mean lutein to zeaxanthin ratio was 1.44:1 in EGG 1 yolks and 1.69:1 in EGG 2 yolks.

Discussion

The increase in total cholesterol observed in the PILL group was unexpected, although other researchers have noted considerable fluctuations in cholesterol among individuals consuming a regular diet (18,19). On the other hand, a change in total cholesterol among some egg consumers was anticipated given the relatively high cholesterol content of eggs. Significant increases

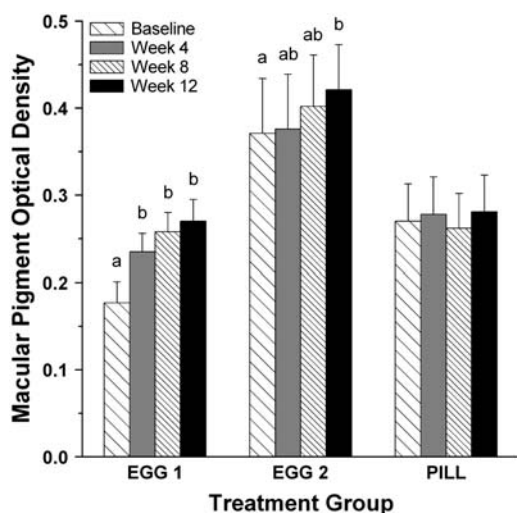


Figure 2 MPOD for each treatment group at baseline and after 4, 8, and 12 wk of intervention. Values are means \pm SEM, $n = 8$. Within-group means without a common letter differ, $P < 0.05$.

in total cholesterol were observed in other egg interventions with 2 (20), 3 (21), and 4 (12) eggs/d. In the current study, a slight, but nonsignificant (EGG 1: $P = 0.83$; EGG 2: $P = 0.47$) change in total cholesterol occurred after subjects consumed an average of 0.85 eggs/d. It may be that egg consumption influences serum cholesterol in a dose-dependent manner (22), with significant changes occurring with 2 or more eggs/d.

In previous egg interventions, subjects were described as either hyporesponders or hyperresponders based on their change in serum cholesterol from baseline (23). Herron et al. (24), for example, defined hyperresponders as individuals whose serum total cholesterol increased $>0.06 \text{ mmol/L}$ for each additional 100 mg/d of dietary cholesterol. Individuals with an increase $<0.06 \text{ mmol/L}$ or a decrease in serum cholesterol were considered hyporesponders. Assuming the cholesterol content of the treatment eggs was similar to other eggs, about 200 mg, subjects in EGG 1 and EGG 2 could be described as hyperresponders if their change in serum cholesterol exceeded 0.102 mmol/L [$(0.85 \text{ eggs/d} \times 200 \text{ mg/egg} \div 100 \text{ mg}) \times 0.06 \text{ mmol/L}$]. Based on this calculation, 7 of the 16 (43%) egg consumers were hyperresponders. A similar percentage of hyperresponders was reported by Herron et al. (24). Three of the 7 hyperresponders in the current project were removed from treatment after 2 mo because their serum total cholesterol exceeded 5.1 mmol/L. The other hyperresponders' total cholesterol changed similarly, increasing about 0.71 mmol/L, but did not exceed the 5.1 mmol/L threshold for discontinuation of treatment. Of note, the change in serum L+Z was 8 times greater in the 7 hyperresponders than in the 9 hyporesponders, an effect recently reported by Clark et al. (25).

Lutein and zeaxanthin are circulated through the bloodstream bound to lipoproteins. Consequently, serum concentrations of lutein and zeaxanthin may be directly related to serum lipoprotein concentrations (26–28). In the current study, serum L+Z was linearly related to both HDL-C and LDL-C at baseline. A direct relation between lipoproteins and serum L+Z suggests that changes in lipoprotein concentrations may affect serum concentrations of L+Z (28). Indeed, there was a linear relation ($r = 0.523$, $n = 16$, $P = 0.03$) between changes in HDL-C from baseline and changes in serum L+Z from baseline in individuals who consumed eggs. No relation was observed between changes in LDL-C and changes in serum L+Z ($r = 0.350$, $n = 16$, $P = 0.18$). These findings are not surprising, because lutein and zeaxanthin may be primarily transported by HDL-C (29). Egg consumption, then, may increase the carrying capacity of carotenoids in serum by increasing HDL-C concentration. Additionally, egg consumption may increase carotenoid carrying capacity in serum by increasing the number of large HDL-C particles. Recently, Greene et al. (30) found an increase in HDL-C particle size following egg intervention. Moreover, both serum lutein and serum zeaxanthin concentrations during intervention were linearly related to HDL-C particle size.

In addition to serum lipoprotein concentrations, competition for carotenoids among body tissues may affect the amount of carotenoid available to serum and the retina (31). Compared with individuals with low BMI, individuals with high BMI may have a greater number of peripheral binding sites for carotenoids and, consequently, attenuated serum and retinal responses to intervention. In a cross-sectional study, Broekmans et al. (32) reported significant negative correlations between BMI and serum lutein concentration and between BMI and MPOD. In the current study, BMI was not related to baseline carotenoid status or changes in serum and retinal carotenoids. The influence of

BMI, however, may only impact individuals with a BMI >27 (33) or 29 (34). Only 1 egg consumer had a BMI >27 and volunteers were excluded from the project if their BMI was >30.

Regardless of BMI, the dose of carotenoid obviously affects the relative amount of carotenoid absorbed and ultimately available to various tissues. In studies that measured both serum and macular carotenoids, significant increases were reported with supplement doses between 2.6 mg/d (8) and 30 mg/d (9) of lutein and in a food intervention with 11.2 mg/d of lutein and 0.6 mg/d of zeaxanthin (11). The approximate daily dose of L+Z in the current study was 283.6 $\mu\text{g}/\text{d}$ from the EGG 1 treatment and 826.2 $\mu\text{g}/\text{d}$ from the EGG 2 treatment. Despite these relatively small doses of carotenoids, the increases in MPOD were similar to previous carotenoid interventions (8,10,11), which suggests that the lutein and zeaxanthin in egg yolk may be more bioavailable to the macula than from other sources. Unlike vegetables and supplements, carotenoids in egg yolks are packaged in a lipid matrix that may facilitate absorption (15). No previous egg interventions measured changes in MPOD, although studies reported increases in serum lutein and zeaxanthin following interventions with 1.3 yolks/d (15), 3 liquid whole eggs/d (21,24,25,30), and 4 lutein-enhanced eggs/d (12).

As observed in other intervention studies (8,10,11), some subjects did not appear to respond to the increased consumption of carotenoids. Hammond et al. (11), in a carotenoid intervention, identified 3 different response patterns of subjects: 1) individuals who responded with increases in both serum and MPOD (i.e., retinal responders); 2) individuals who responded with an increase in serum but not the retina (i.e., retinal nonresponders); and 3) individuals who did not respond in either serum or MPOD (i.e., serum and retinal nonresponders). In the current study, 8 egg consumers could be described as retinal responders, 4 as retinal nonresponders, and 1 as a serum and retinal nonresponder. Three egg consumers, however, appeared to have a different response pattern and might be described as serum nonresponders. In these subjects, MPOD, but neither serum lutein nor zeaxanthin, increased from baseline. Four of the cholesterol hyperresponders were retinal responders, with the other 3 being retinal nonresponders. The subjects who either did not respond to treatment or showed only a change in the retina were cholesterol hyporesponders.

The mean increase in MPOD was greater in the EGG 1 group than in the EGG 2 group. One possible reason for the difference was the number of retinal responders in each group. Six subjects in the EGG 1 group (5 serum and retinal responders and 1 retina-only responder) showed an increase in MPOD, whereas 5 subjects (3 serum and retinal responders and 2 retina-only responders) showed an increase in the EGG 2 group. Another possible reason for the different retinal responses was MPOD at baseline, which significantly differed between the 2 groups. If the amount of carotenoid in the retina inversely affects the accumulation of additional carotenoids, then changes in the EGG 2 group may have been limited more than changes in the EGG 1 group. In fact, there was an inverse relation between baseline MPOD and change in MPOD among egg consumers ($r = -0.590$, $n = 16$, $P = 0.016$) (Figure 3). Future carotenoid interventions may benefit from pseudo-randomizing subjects to avoid the possible effects of 2 treatment groups with dissimilar MPOD at baseline.

In summary, eggs are a nutrient-dense food, rich in essential vitamins and minerals, as well as carotenoids, but may be avoided in the diet because of their relatively high cholesterol content. In the current study, neither mean total cholesterol nor TC:HDL-C significantly increased following a 12-wk egg

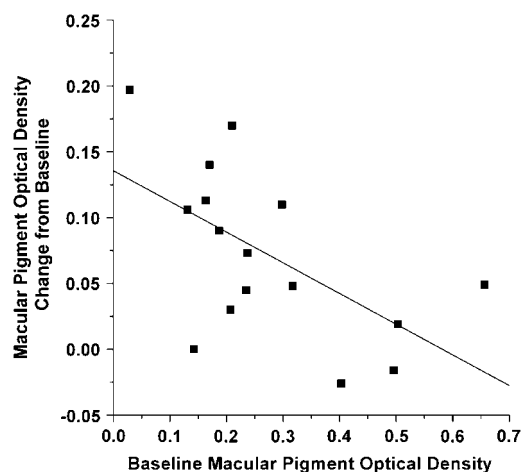


Figure 3 Relation between MPOD at baseline and the difference between MPOD at baseline and after 12 wk of egg intervention. The line represents a linear fit of the data, $r = -0.59$; $P = 0.01$.

intervention. Serum zeaxanthin, but not serum lutein, increased in both egg treatment groups, as did MPOD. These findings suggest that the relatively small concentration of zeaxanthin found in egg yolk may be highly bioavailable to the retina.

Acknowledgments

The authors thank Adele Marone for drawing the blood samples and Brooke Gowdy-Johnson for technical assistance.

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