Consumption of 2 and 4 egg yolks/d for 5 wk increases macular pigment concentrations in older adults with low macular pigment taking cholesterol-lowering statins\textsuperscript{1–3}

Rohini Vishwanathan, Elizabeth F Goodrow-Kotyla, Billy R Wooten, Thomas A Wilson, and Robert J Nicolosi

ABSTRACT

Background: Lutein and zeaxanthin may reduce the risk of dry, age-related macular degeneration because of their photo-oxidative role as macular pigment.

Objective: The present study evaluated serum lutein, zeaxanthin, and macular pigment optical density (MPOD) responses at 0.25\textdegree, 0.5\textdegree, and 1\textdegree retinal eccentricities to the consumption of 2 and 4 egg yolks/d by older adults taking cholesterol-lowering medications.

Design: Subjects consumed foods containing 2 followed by 4 egg yolks/d for 5 wk each with a 4-wk egg-free period at baseline and between the 2 interventions.

Results: Changes in MPOD (n = 37) with egg yolk consumption were inversely associated (P < 0.05) with baseline MPOD. Subjects with low-baseline MPOD (defined as MPOD ≤ 0.5 at 0.25\textdegree, ≤ 0.4 at 0.5\textdegree, and ≤ 0.35 at 1\textdegree) showed increases of ≤ 50% (P < 0.05) with 4 egg yolks at the 3 retinal eccentricities. MPOD increased by 31% (P = 0.059) at 0.5\textdegree with 2 egg yolks. Serum lutein increased by only 16% and 24% (P < 0.05) compared with increases of 36% and 82% (P < 0.001) in serum zeaxanthin (n = 52) after consumption of 2 and 4 egg yolks, respectively. Serum HDL cholesterol increased by 5% (P < 0.05) after consumption of 2 and 4 egg yolks. Serum LDL cholesterol did not change with either egg yolk treatment.

Conclusions: Consumption of 4 egg yolks/d, and possibly of 2 egg yolks/d, for 5 wk benefited macular health in older adults with low MPOD. Serum HDL cholesterol increased without an increase in LDL cholesterol in this study population, most of whom were taking cholesterol-lowering statins. Am J Clin Nutr 2009;90:1272–9.

INTRODUCTION

Age-related macular degeneration (AMD) is ranked as the leading cause of vision loss in the elderly, affecting 30–50 million individuals worldwide\textsuperscript{(1)}. The Age-related Eye Disease Study (AREDS) found that 20% of untreated individuals with dry AMD progressed to the advanced stage at a rate of 4%/y over a 5-y study period\textsuperscript{(2)}. Thus, preventing or reducing the progression of dry AMD is a critical factor in preventing vision loss.

Although there are no established standard medical treatments for dry AMD, lutein and/or antioxidant supplements are now being prescribed\textsuperscript{(3)}. Dry AMD is thought to be caused by a breakdown of light-sensitive cells in the macula, a region 5–6 mm in diameter in the posterior pole of the retina, which results in gradual loss of central vision\textsuperscript{(4)}. Continuous exposure, specifically to blue radiation (430 nm) of light, triggers oxidative reactions and results in the breakdown of retinal epithelial cells beneath the macula\textsuperscript{(5)}. Lutein, zeaxanthin, and meso-zeaxanthin—a lutein isomer—accumulate in the macula and are collectively referred to as macular pigment (MP)\textsuperscript{(6)}. With an absorption maximum of 460 nm, MP acts as an efficient high-energy blue-light filter that protects the underlying retinal cells\textsuperscript{(7)}. In addition, because lutein and zeaxanthin are potent antioxidants, they are also reported to protect the retina from oxidative stress\textsuperscript{(8)}.

Egg interventions in our laboratory and others\textsuperscript{(8–10)} showed significant increases in serum lutein and zeaxanthin concentrations. Interventions with food sources of lutein and zeaxanthin, such as spinach and corn, were shown to increase MP optical density (MPOD) in healthy adults\textsuperscript{(11, 12)}. Studies have also shown MP enrichment through consumption of lutein supplements with concentrations ranging from 2 to 30 mg/d, and lutein supplements in combination with other antioxidants\textsuperscript{(13–16)}. Lutein and zeaxanthin from natural sources, such as spinach, eggs, and corn, especially eggs, appear to be equally or more bioavailable than from supplements\textsuperscript{(8, 12)}. In a collaborative effort with our laboratory, the only study to date that has measured the effect of egg consumption on MPOD, Wenzel et al\textsuperscript{(17)} reported that the consumption of 6 eggs/wk for 12 wk effectively increased MPOD in women aged 24–59 y. Positive results from our earlier study with 1 egg/d\textsuperscript{(10)} led us to design the current study with increased egg consumption of up to 4 egg yolks/d to evaluate MPD in addition to serum lutein and zeaxanthin responses. Other egg intervention studies that used >1 egg/d only measured lipoprotein profiles\textsuperscript{(18, 19)}.

The objective of this study was to evaluate serum lutein and zeaxanthin concentrations and MPOD changes in response to 5-

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\textsuperscript{2} RJJN was supported by the American Egg Board, Egg Nutrition Center, Washington, DC, and the Massachusetts Lions Eye Research Fund Inc, New Bedford, MA.

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wk sequential interventions of 2 and 4 egg yolks/d in older adults whose preexisting high blood cholesterol concentrations were controlled by cholesterol-lowering medications. Because most of the population aged ≥60 y take some sort of cholesterol-lowering medication, we hypothesized that increased egg yolk consumption could effectively raise MPOD without adversely affecting the lipoprotein cholesterol profile in these individuals.

**SUBJECTS AND METHODS**

**Subjects**

Subjects aged ≥60 y who had been taking a cholesterol-lowering medication for ≥3 mo before the study began were recruited. Recruitment for the study began in the spring of 2005. Other inclusion criteria were the ability of subjects to undergo blood collection and the willingness to consume foods containing the equivalent of 2 and 4 egg yolks/d for 5 wk each. Subjects taking multivitamins containing lutein switched to multivitamins without lutein 4 wk before initiation of the study until completion of the study. There were no restrictions on the consumption of lutein- and zeaxanthin-containing vegetables or fruit, which were measured through dietary records. The goal was to study the addition of egg yolks to the participant’s normal diet and not an egg intervention in isolation. Baked food products such as cookies and muffins were allowed throughout the study as long as they did not contribute significant amounts of egg yolk, such as quiche.

All subjects who were recruited into the study signed an informed consent form and obtained written approval from their primary care physician as a criterion for study participation. Study subjects were recruited from senior citizen centers, University of Massachusetts Lowell faculty and staff, and a random subset of the free-living population in the Greater Lowell, MA, area. The use of human subjects for this study was approved by the University of Massachusetts, Lowell, Institutional Review Board. All subjects were given a cash incentive and a certificate of participation at the end of the study.

**Study design**

The 5-mo study period consisted of 4 phases. All subjects started with a 4-wk baseline phase, which was followed by the consumption of foods containing 2 egg yolks/d for 5 wk. This was followed by a 4-wk washout phase, and then subjects consumed foods containing 4 egg yolks/d for 5 wk. Subjects were instructed to refrain from eating eggs or egg yolk–rich products (other than study eggs or foods) during the entire study period; egg whites were allowed. On day 1, a demographics questionnaire and a Mini-Mental State Examination (MMSE) were administered to all subjects. The MMSE was used to indicate any abnormalities in the subject’s cognitive ability (20). The subjects were familiarized with the MP apparatus, shown an instructional video that explained the task, and then underwent a practice session (data not included). MPOD measurements were done at the University of Massachusetts, Lowell, at the end of each of the 4 phases. The subjects filled out a 7-d diet record (7DDR) once during each phase, which was analyzed by using EvalEat version 1.2 (Pearson Education Inc, San Francisco, CA) and also served as a measure of compliance.

Different food items containing 2 and 4 egg yolks from nonenriched, large, store-bought eggs were prepared by Aramark Corporation, which is the food service supplier for the University of Massachusetts, Lowell. The food choices included baked custard, fruit smoothies, waffles, deviled eggs, chocolate mousse, blueberry pancakes, caramel flan, potato puff, egg foo young, tomato chive scramble, breakfast wraps, quiche, pasta salad, potato salad, and egg salad. The foods were delivered to the participant’s home twice each week. Food containers were collected, and weekly phone calls were made to the subjects to measure compliance.

**Blood collection**

A total of 9 blood samples were collected during the study (at the beginning of the study, at weeks 2 and 4 of the baseline and washout phases, and at weeks 3 and 5 of the egg-intervention phases). Serum was separated by centrifugation at 1500 × g for 12 min at 4°C, and multiple aliquots were stored at −80°C until analyzed. Serum lipid and lipoprotein-cholesterol concentrations were analyzed from each blood sample and the means from the 2 samples from each phase were used for statistical analyses. Serum lutein and zeaxanthin concentrations were measured only from the blood sample collected at the end of each phase.

**Serum lutein and zeaxanthin**

*Serum*

Aliquots of serum collected at the end of each phase were analyzed for carotenoids for each subject after the subject completed the study. This was done by using an Agilent model 1100 (Agilent Technologies, Santa Clara, CA) HPLC apparatus with a diode array detector after 100 μL serum was extracted as per the procedure described by Handelman et al (21). The enzyme reagent used for the extractions was prepared by using cholesterol esterase and triacylglycerol lipases (Calbiochem, San Diego, CA), which breakdown lipoprotein fractions and release the carotenoids normally carried within these fractions. The internal standard used was Tocol (22). The HPLC column was a 300 mm × 4.6 mm Adsorbosphere HS C18 with a 3-μm particle size and 60-Å pore size (Grace Davison Discovery Sciences, Deerfield, IL) that was maintained at 18.5°C during analysis. A gradient elution system was used at 1 mL/min with an initial mobile phase consisting of 25% acetonitrile and 75% of a mixture containing 0.4% ammonium acetate, 50% acetonitrile, and 50% methanol. After a gradient at 20 min to 20% isopropanol, the initial conditions were resumed at 40 min for equilibration before the next sample was analyzed.

*Egg yolks*

A cohort of eggs used to prepare the foods was obtained from Aramark and was diluted in 0.1 mol phosphate buffer/L and extracted in a similar manner as for serum using 6 N KOH instead of an enzyme reagent (21). Twenty-five eggs were randomly selected during the course of the study and analyzed on different days to calculate the average concentration of lutein and zeaxanthin in the egg yolks.
MPOD

MPOD was measured based on the principle of heterochromatic flicker photometry with a Macular Metrics Densitometer at the start of the study and at the end of each phase (23–25). This is a reliable, noninvasive, psychophysical technique that has been shown to generate meaningful MPOD data in older subjects (23). The technique uses the blue-light absorbing property of xanthophylls in the macula to measure pigment optical density. Measurements were made in the right eye at 4 foveal retinal eccentricities (0.25°, 0.5°, 1°, and 1.75°), where MP peaks, and at one parafoveal eccentricity (7°) in the temporal retina, where MP is minimal. Most studies measure only the right eye, because MPOD is similar in both eyes (26). A total of 5 readings were taken at each stimulus, and the average values were used to calculate MPOD at each of the 4 retinal eccentricities.

Serum lipids and lipoprotein cholesterol

Serum lipid and lipoprotein-cholesterol concentrations were measured immediately after centrifugation with a Cobas Mira Plus Clinical Chemistry Autoanalyzer (Roche, Branchburg, NJ). Serum total cholesterol (TC) (27) concentrations were measured enzymatically with the Infinity Cholesterol Reagent procedure from Thermo Electron (Melbourne, Australia). Serum triglyceride concentrations (28) were measured enzymatically with the Infinity-Triglyceride Reagent procedure from Thermo Electron. Serum HDL cholesterol was measured directly with the Infinity HDL Cholesterol Reagent procedure from Thermo Electron (Melbourne, Australia). Serum triacylglyceride concentrations (28) were measured enzymatically with the Infinity Cholesterol Reagent procedure from Thermo Electron. The concentration of serum LDL cholesterol was measured immediately after centrifugation with a Cobas Mira Plus Clinical Chemistry Autoanalyzer (Roche, Branchburg, NJ). Serum TC, HDL cholesterol, and triglycerides are maintained by partici-

Electron. The technique uses the blue-light absorbing property of xan-
thophylls in the macula to measure pigment optical density. Measurements were made in the right eye at 4 foveal retinal eccentricities (0.25°, 0.5°, 1°, and 1.75°), where MP peaks, and at one parafoveal eccentricity (7°) in the temporal retina, where MP is minimal. Most studies measure only the right eye, because MPOD is similar in both eyes (26). A total of 5 readings were taken at each stimulus, and the average values were used to calculate MPOD at each of the 4 retinal eccentricities.

Statistical analysis

Sigma Stat version 3.1 (Jandel Scientific, San Rafael, CA), an SPSS statistical software package, was used for all data analyses. All serum concentrations and MPOD values are expressed as means ± SEMs, and statistical significance was set at $P < 0.05$. Percentage changes and any significant effects of the egg interventions were compared with phase I (baseline)—no egg phase using a repeated-measures one-factor analysis of variance (29). When differences were observed, Tukey’s test was used. Pearson product moment correlation was performed to obtain associations between all of the variables measured.

RESULTS

Subject characteristics and diet

Of the 56 subjects recruited into the study, 4 were unable to complete the study: 2 because of unexpected vacations, 1 because they stopped taking their cholesterol-lowering medication per their physician, and 1 because of gastrointestinal discomfort during the 2-egg phase. None of the subjects were removed from the study by their primary care physicians because of adverse changes in their serum cholesterol profile during the egg interventions. Only 37 subjects underwent MPOD measurements because the device was not calibrated at the start of the study, and 3 subjects with a diagnosis of drusen/dry AMD were unable to undergo the measurements. Demographic data of the 52 subjects at baseline are shown in Table 1. An MMSE score of 28.5 (normal score: 26–30) indicates that all subjects had normal communication and memory and were capable of carrying out their day-to-day activities (20). Ninety-four percent of subjects were taking a statin, 4% a statin-ezetimibe combination, and 2% ezetimibe for high blood cholesterol. Of the statin consumers, 52% were taking atorvastatin, 25% simvastatin, 10% lovastatin, and 4% fluvastatin, pravastatin, and rosuvastatin. The dose of statins ranged from 5 to 80 mg.

The 7DDR showed significant increases from baseline in dietary cholesterol during the 2- and 4-egg yolk phases, whereas dietary cholesterol increased significantly with 4 egg yolks compared with 2 egg yolks (Table 2). Total caloric intake was significantly higher during the 2-egg yolk phase than during all other phases. Carbohydrate and fiber intakes were significantly lower during the 4-egg yolk phase than during all other phases. Whereas total and saturated fat intake with 4 egg yolks was not significantly greater than that with 2 egg yolks, it was significantly greater during both egg yolk phases than at baseline. Monounsaturated fat intake during the 4-egg yolk phase was significantly greater than at baseline. There were no significant differences in micronutrient intake during any of the phases (data not shown). Although the 7DDR analysis program did not calculate carotenoid intake, the dietary records were reviewed to evaluate the lutein- and zeaxanthin-rich foods consumed during the 4 phases. Spinach, broccoli, and corn were the only other foods frequently consumed during the study that had substantial amounts of lutein and zeaxanthin. The mean intakes of these...
Egg yolks contributed ≈6.6 and 13.2 mg total lutein and zeaxanthin per week during the 2- and 4-egg yolk phases, respectively, whereas the other foods contributed ≈2 mg/wk during all 4 phases (30). The 7DDR also served as a measure of compliance as egg yolk consumption was recorded.

**Serum lutein and zeaxanthin**

Serum lutein increased 16% \((P < 0.050)\) after the 2-egg yolk phase and 24% \((P < 0.001)\) after the 4-egg yolk phase compared with the baseline phase \((n = 52; \text{Table 3})\). Serum zeaxanthin increased 36% \((P < 0.001)\) after the 2-egg yolk phase and 82% \((P < 0.001)\) after the 4-egg yolk phase compared with the baseline phase. There was a 36% \((P < 0.001)\) increase in the serum zeaxanthin concentration after the 4-egg yolk phase compared with the 2-egg yolk phase. The increase in serum lutein after the 4-egg yolk phase was not statistically different from the 2-egg yolk phase. No differences were observed in serum lutein and zeaxanthin concentrations between the baseline and washout phases.

**MPOD**

No significant changes were observed in mean MPODs \((n = 37)\) after both the 2- and 4-egg yolk phases compared with baseline at any retinal eccentricity (Table 3). However, MPOD responses to the 2- and 4-egg yolk phases were found to be inversely associated \((P < 0.05)\) with baseline MPOD at retinal eccentricities of both 0.25 and 0.5. The 0.5 correlation graphs are shown as an example in Figure 1. At 1°, the correlation was significant only at the 4-egg yolk phase. Because no correlation was observed at 1.75°, the data are not further discussed.

On the basis of this observation, the subjects were then divided into 2 subgroups by baseline MPOD. The mean baseline MPOD for the high-baseline MPOD subgroup \((>0.5 \text{ at } 0.25, >0.4 \text{ at } 0.5, \text{ and } >0.35 \text{ at } 1°)\) was greater \((P < 0.001)\) than that of the low-baseline MPOD subgroup \((\leq0.5 \text{ at } 0.25, \leq0.4 \text{ at } 0.5, \text{ and } \leq0.35 \text{ at } 1°)\). The cutoff values were chosen on the basis of previously published MP studies and given that MPOD decreases with increasing eccentricity (9). The high-baseline MPOD subgroup had no change in MPOD during the 2- and 4-egg yolk phases compared with the baseline phase at any eccentricity.

**Table 3**

Concentrations of serum lutein, zeaxanthin, lipids, and lipoprotein cholesterol and macular pigment optical density (MPOD) at the end of the baseline, 2-egg yolk, washout, and 4-egg yolk phases \((n = 52)\)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 Egg yolks/d</th>
<th>Washout</th>
<th>4 Egg yolks/d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lutein (μmol/L)</strong></td>
<td>0.142 ± 0.010 (^a)</td>
<td>0.164 ± 0.010 (^b)</td>
<td>0.137 ± 0.010 (^a)</td>
<td>0.176 ± 0.013 (^b)</td>
</tr>
<tr>
<td><strong>Zeaxanthin (μmol/L)</strong></td>
<td>0.033 ± 0.003 (^a)</td>
<td>0.045 ± 0.003 (^b)</td>
<td>0.033 ± 0.002 (^a)</td>
<td>0.060 ± 0.004 (^b)</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>4.67 ± 0.11 (^a)</td>
<td>4.68 ± 0.11 (^b)</td>
<td>4.38 ± 0.11 (^b)</td>
<td>4.84 ± 0.11 (^b)</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.48 ± 0.02 (^a)</td>
<td>1.56 ± 0.06 (^b)</td>
<td>1.45 ± 0.05 (^a)</td>
<td>1.56 ± 0.05 (^b)</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>2.50 ± 0.08 (^c)</td>
<td>2.41 ± 0.08 (^b)</td>
<td>2.27 ± 0.08 (^b)</td>
<td>2.60 ± 0.09</td>
</tr>
<tr>
<td><strong>TC: HDL ratio</strong></td>
<td>3.24 ± 0.10</td>
<td>3.13 ± 0.10</td>
<td>3.11 ± 0.1</td>
<td>3.32 ± 0.14</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.50 ± 0.09</td>
<td>1.55 ± 0.09</td>
<td>1.52 ± 0.09</td>
<td>1.48 ± 0.09</td>
</tr>
<tr>
<td><strong>MPOD ((n = 37))</strong></td>
<td>(0.25^a)</td>
<td>0.55 ± 0.04</td>
<td>0.55 ± 0.04</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(0.5^a)</td>
<td>0.49 ± 0.04 (^c)</td>
<td>0.52 ± 0.04</td>
<td>0.45 ± 0.03 (^a)</td>
</tr>
<tr>
<td></td>
<td>(1^a)</td>
<td>0.35 ± 0.03 (^b)</td>
<td>0.37 ± 0.03 (^b)</td>
<td>0.31 ± 0.03 (^a)</td>
</tr>
</tbody>
</table>

\(^a\) All values are means ± SEMs. Values in a row with different superscript letters are significantly different, \(P < 0.05\) (one-factor repeated-measures ANOVA followed by Tukey’s test).
retinal eccentricity. Subjects with a baseline MPOD ≤ 0.50 (n = 17) at 0.25° showed a 6% increase in mean MPOD after the 2–egg yolk phase and a 48% (P < 0.001) increase after the 4–egg yolk phase (Figure 2). Subjects with a baseline MPOD ≤ 0.40 (n = 16) at 0.5° showed a 31% (P = 0.059) increase in MPOD after the 2–egg yolk phase and a 54% (P < 0.001) increase after the 4–egg yolk phase (Figure 2). These were the same subjects who had a low baseline MPOD (≤0.50) at 0.25°. Subjects with a baseline MPOD ≤ 0.35 (n = 21) at 1° showed a 15% increase in MPOD after the 2–egg yolk phase and a 50% increase (P < 0.05) after the 4–egg yolk phase (Figure 2). Sixteen of these 21 subjects also had a low baseline MPOD at 0.25° and 0.5°, and one subject had a low baseline MPOD at 0.25° only. No significant correlations were observed between serum lutein, zeaxanthin, and MPOD at the 4 retinal eccentricities during any of the phases (n = 37; data not shown).

Age, sex, and BMI

No significant correlations existed between the age of the subjects and serum lutein and zeaxanthin concentrations and age and MPOD during any of the 4 phases. There were no significant differences in serum lutein and zeaxanthin concentrations between men (n = 22) and women (n = 34) at any of the 4 phases. Although, overall, MPOD values tended to be higher for women (n = 22) than for men (n = 15), with a difference of up to 0.14 units at baseline and washout, they were not statistically significant. Seventy percent of the subjects had a BMI ≥ 27 (Table 1). Serum lutein, zeaxanthin, and MPOD were not correlated with BMI during the 4 phases.

History of lutein supplementation

Of the 52 subjects, 10 subjects who were taking a lutein-containing multivitamin switched to an alternate vitamin supplement for the study duration. The mean serum lutein concentration at baseline for these 10 subjects was not significantly different from that of the remaining 42 subjects, who had no history of taking lutein supplements. One of the 10 subjects did not undergo MP measurement. The mean MPOD at baseline for the 9 subjects was not significantly different from that of the 28 subjects who had no history of taking lutein supplements. Four of these 9 subjects had a low MPOD at baseline and were in the low-baseline MPOD subgroup despite prior supplement intake. The observed high-baseline MPODs in this study population thus cannot be attributed to a history of lutein supplementation.

Serum lipids and lipoprotein cholesterol

Serum TC did not change significantly from baseline after the 2– and 4–egg yolk phases (n = 52; Table 3). Serum HDL cholesterol increased 5% (P < 0.05) from baseline during both the 2– and 4–egg yolk phases. Serum LDL cholesterol tended to decrease (−4%) after the 2–egg yolk phase and tended to increase (4%) after the 4–egg yolk phase compared with baseline, but neither change was significant. However, an 8% (P < 0.05) increase was observed between the 2– and 4–egg yolk phases. A significant decrease from baseline was observed in serum TC and LDL cholesterol during the washout phase. No significant change in serum triglycerides was observed between any of the phases. Also, the TC/HDL cholesterol ratio was maintained between 3.0 and 3.5 during the 4 phases (Table 3).
Correlations between serum lipoproteins, lutein, zeaxanthin, and MPOD

Serum lutein and zeaxanthin concentrations were positively associated (P < 0.05) with serum TC, LDL cholesterol, and HDL cholesterol during the 2- and 4-egg yolk phases (Table 4). At baseline and during the washout phases, a positive association (P < 0.05) was observed only between serum lutein and serum TC, HDL cholesterol, and LDL cholesterol. Serum zeaxanthin was positively associated (P < 0.05) with serum LDL cholesterol during the washout phase only.

A positive correlation (P < 0.05) was observed between serum HDL cholesterol and MPOD at the 0.5° retinal eccentricity during both the 2- and 4-egg yolk phases. At 1° and 1.75°, a positive correlation (P < 0.05) was observed only during the 2-egg yolk phase; no correlation was observed at 0.25°.

No correlation was observed between the dose of statins and serum LDL cholesterol, HDL cholesterol, lutein, or zeaxanthin during the egg yolk interventions. The type of statin did not appear to affect the observed percentage changes in serum LDL responses to the egg yolk interventions. The type of statin did not appear to affect the observed percentage changes in serum LDL cholesterol, HDL cholesterol, lutein, and zeaxanthin during the egg yolk phases.

**DISCUSSION**

MPOD and serum lutein and zeaxanthin responses in older adults to a short-term intervention of up to 4 nonenriched, store-bought egg yolks has not been studied before. The magnitudes of the increases in serum zeaxanthin with 2 and 4 egg yolks/d for 5 wk of 36% and 82%, respectively, were remarkably greater than those observed for serum lutein, which were 16% and 24% respectively. The lower-than-expected serum lutein responses compared with zeaxanthin may have been due to several reasons. One reason could be that baseline lutein concentrations were 4.5 times those of serum zeaxanthin, which may be important because baseline concentrations of carotenoids predict their responses to dietary interventions (17). The greater response to dietary zeaxanthin may have also been due to higher amounts of dietary zeaxanthin may have also been due to higher amounts of zeaxanthin in the egg yolks used in the present study (mean: 230 μg/yolk) compared with previously published data (mean: 94 μg/yolk) (10). Wenzel et al (17) reported similarly high zeaxanthin amounts in eggs and a greater serum zeaxanthin response. Even though a wide range was observed in egg yolk lutein and zeaxanthin concentrations, large store-bought eggs were used for the preparation of foods in an effort to depict a real-life scenario. Similar egg yolk lutein and zeaxanthin ranges were observed in the treatment eggs used by Wenzel et al (17). Greater than 70% of the population in the current study was overweight, which may have caused a greater accumulation of lutein than of zeaxanthin in adipose tissue, thereby diminishing the serum lutein response (31). However, correlations between serum lutein/zeaxanthin and BMI in the current study were not significant, possibly because of sample size.

The MPOD response was observed with egg yolk interventions only when subjects were divided into subgroups. Subjects with a low-baseline MPOD responded to the 5-wk interventions of 2 and 4 egg yolks/d with significant increases in MPOD. The nonresponsiveness of subjects with high baseline MPOD may indicate saturation of retinal cells in the macula, resulting in a slower uptake of additional lutein and zeaxanthin (13). Wenzel et al (17) showed an increase in MPOD at a retinal eccentricity of 1° with a 1-egg intervention after only 12 wk in individuals whose baseline MPOD was 0.37 ± 0.06 (high), whereas an increase was observed by 4 wk in individuals whose baseline MPOD was 0.18 ± 0.02 (low). The low and high baseline MPOD subgroups were based on MPOD data collected from previously conducted studies (16, 17, 32–34). Johnson et al (16) and Burke et al (32) measured MPOD responses at central retinal eccentricities and showed the mean MPOD to be 0.35 at 0.5° and 0.63 (range: 0.45–0.65) at 0.4°. Four other studies showed the mean baseline MPOD to be in the range of 0.08–0.40 at 1° (17, 32–34).

Higher dietary amounts of lutein and zeaxanthin in 4 egg yolks together with greater serum lutein and zeaxanthin responses likely contributed to the greater increase in MPOD after the 4-egg yolk phase than after the 2-egg yolk phase. However, the 31% increase in MPOD at 0.5° during the 2-egg yolk phase, although not statistically significant, was clinically relevant because the mean MPOD increased from 0.26 at baseline to 0.34 (ie, by 0.08 units). The ratio of lutein to zeaxanthin within 0.25 mm (∼0.25°) of the fovea is 1:2.4; whereas at the periphery (∼1°)

**TABLE 4**

Pearson correlations (r) between serum lutein, zeaxanthin, lipid, and lipoprotein-cholesterol concentrations and serum HDL cholesterol and macular pigment optical density (MPOD) at 0.25°, 0.5°, and 1° retinal eccentricities during the baseline, 2-egg yolk, washout, and 4-egg yolk phases

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 Egg yolks/d</th>
<th>Washout</th>
<th>4 Egg yolks/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum lutein (n = 52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.364⁷</td>
<td>0.463⁷</td>
<td>0.158⁴</td>
<td>0.468⁷</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.433⁷</td>
<td>0.517⁷</td>
<td>0.296⁴</td>
<td>0.365⁷</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.315⁷</td>
<td>0.415⁷</td>
<td>0.279⁴</td>
<td>0.488⁷</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.182</td>
<td>−0.304⁴</td>
<td>−0.277⁴</td>
<td>−0.224</td>
</tr>
<tr>
<td>Serum zeaxanthin (n = 52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.202</td>
<td>0.375⁴</td>
<td>0.107</td>
<td>0.439⁴</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.241</td>
<td>0.379⁴</td>
<td>0.217</td>
<td>0.429⁴</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.188</td>
<td>0.390⁴</td>
<td>0.243⁴</td>
<td>0.440⁴</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.116</td>
<td>−0.302⁴</td>
<td>−0.226</td>
<td>−0.274⁴</td>
</tr>
<tr>
<td>Serum HDL cholesterol and MPOD (n = 37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25°</td>
<td>0.144</td>
<td>0.302</td>
<td>0.295</td>
<td>0.25⁴</td>
</tr>
<tr>
<td>0.5°</td>
<td>0.247</td>
<td>0.342⁴</td>
<td>0.257</td>
<td>0.326⁴</td>
</tr>
<tr>
<td>1°</td>
<td>0.284</td>
<td>0.362⁴</td>
<td>0.315</td>
<td>0.231</td>
</tr>
</tbody>
</table>

⁷ P < 0.05 on the basis of Pearson product moment correlation.
and 1.75°) it is 2:1 (6). The significant central increases in MPOD during the 2- and 4-egg yolk phases may also have been an effect of the greater serum zeaxanthin response compared with lutein. A greater central macular lutein and zeaxanthin accumulation at 0.5° during the 2-egg yolk phase and not at 0.25° may also be explained by the observed positive association between serum HDL cholesterol and MPOD. Lutein and zeaxanthin transport to the retina has been previously linked to the HDL lipoprotein (35). MPOD increases in the 2- and 4-egg yolk phases (≈946 μg and 2 mg lutein and zeaxanthin, respectively) were greater than MPOD increases of 39% and 21% after 140 d of supplementation with 30 mg lutein (14). The MPOD increases in the present study were also much higher than the 4-wk increase of 4–5% observed in a supplementation study of 10 mg lutein (11).

A decrease in MPOD was observed after the 4-wk washout phase in the low-baseline MPOD subgroups (Figure 2). This is a valuable finding because previous reports suggest that MPOD stays high for a long time after supplementation (12, 14). However, no previous studies evaluated MPOD responses to 5-wk interventions or to consumption of up to 4 nonenriched store-bought egg yolks. Hammond et al (12) used spinach and corn, whereas Wenzel et al (17) used eggs and a longer supplementation period of 15 wk. Other supplemental studies used comparatively high lutein doses of 10–30 mg/d (14, 15). The current study showed that short-term MPOD manipulation may be possible with eggs in a population with low baseline MPOD. In the high baseline MPOD subgroup, the decrease in MPOD during washout was significantly lower than that at baseline (20%: P < 0.05) at 0.25° and 0.5°. This may have been due to the 8% (P < 0.05) decrease in serum HDL cholesterol at washout compared with baseline in this subgroup (data not shown). The high baseline MPOD subgroup also consumed 3–3.5 mg nonegg lutein and zeaxanthin (from spinach, corn, and broccoli) during baseline as opposed to only 1 mg during the washout phase. The unexpected decrease in MPOD values at washout to lower than baseline values also minimized any experimenter bias.

The National Health and Nutrition Examination Survey (NHANES) 2005–2006 data showed that the following percentages of adults have a total blood cholesterol of ≥240 mg/dL: 1) 15.5% of men and 31.2% of women aged 55–64 y, 2) 8.8% of men and 23.4% of women aged 65–74 y, and 3) 9.6% of men and 15.1% of women aged ≥75 y (36). The above percentages represent a population taking cholesterol-lowering medications and also an age group more susceptible to AMD. In light of the recent findings of the JUPITER trial, the percentage of statin users (rosuvastatin) may not be limited to individuals with high LDL cholesterol alone, because it was shown to protect individuals with a normal lipid profile but a high C-reactive protein concentration (37). The present study is the only one to date that evaluated serum lipoprotein responses to dietary cholesterol (or egg) consumption in a population taking statins (94%). It is an interesting finding that 2 egg yolks/d significantly raised serum HDL cholesterol without affecting serum LDL cholesterol in this statin-using population. These findings are in keeping with 2 other egg-intervention studies with 3 eggs/d, which also showed significant increases in serum HDL cholesterol after egg intervention (18, 19). However, a significant increase in serum LDL cholesterol from baseline was not observed in the current study, even with 4 egg yolks/d, probably because of the action of statins, although it is important to note that the intervention period in the current study lasted only 5 wk. The implications of the TC:HDL ratio observed during both the 2- and 4-egg yolk phases in the present study (Table 3), a better predictor of coronary heart disease risk, was well below the upper limit of 4.5 recommended by the American Heart Association (38). This finding contradicts the results of a meta-analysis that showed increases in the TC:HDL ratio with dietary cholesterol (39) but agrees with the findings of Hu et al (40).

To summarize, consumption of 2 and 4 egg yolks/d for 5 wk improved MPOD at a retinal eccentricity of 0.5°, whereas consumption of 4 egg yolks/d improved MPOD at retinal eccentricities of 0.25° and 1° in an older population with low MPOD who were taking statins. Also, consumption of 2 and 4 egg yolks/d for 5 wk caused tremendous increases in serum zeaxanthin compared with serum lutein. The other beneficial effect of consuming 2 and 4 egg yolks/d in a population taking statins was the significant increase in serum HDL cholesterol but no change in serum LDL cholesterol compared with baseline. We are presently evaluating the effect of consuming 12 eggs/wk for 1 y on the progression of dry AMD in a population with early- to mid-stage dry AMD. Another important finding was the addition of high baseline MPOD as an exclusion criterion for short-term lutein or zeaxanthin intervention studies.

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The authors’ responsibilities were as follows—RJN and TAW: designed the study and interpreted the data; RV: performed the MP measurements, analyzed the serum carotenoids, analyzed and interpreted the data, recruited the participants, and prepared the manuscript; EFG-K: recruited the participants, coordinated the study, and analyzed serum lipid and lipoprotein cholesterol; and BRW: helped set up and calibrate the macular metrics densitometer, trained RV to make the MP measurements and interpreted the MP data. None of the authors had a conflict of interest to disclose.

REFERENCES