Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk[1–3]

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ABSTRACT
Background: The food matrix in which carotenoids are found affects their bioavailability. Lutein and zeaxanthin are abundant in egg yolks and accumulate in the macular region of the retina, where they may affect visual function.
Objective: We sought to determine whether plasma lutein and zeaxanthin concentrations are elevated after dietary supplementation with egg yolk.
Design: Eleven moderately hypercholesterolemic men and women consumed 2 separate baseline diets, which contained 29–33% of energy as total fat, with 20% of energy as either beef tallow or corn oil. These diets were supplemented with cooked chicken egg yolks (1.3 egg yolks/d for an intake of 10.4 MJ). Each subject consumed all 4 diets. Each diet was consumed for 4.5 wk, with a washout period of ≥2 wk between diet phases. At the end of each diet phase, fasting morning plasma samples were collected and stored for carotenoid analysis by HPLC. Commercial chicken egg yolks were analyzed for carotenoids and cholesterol.
Results: Egg yolk supplementation of the beef tallow diet increased plasma lutein by 28% ($P < 0.05$) and zeaxanthin by 142% ($P < 0.001$); supplementation of the corn oil diet increased plasma lutein by 50% ($P < 0.05$) and zeaxanthin by 114% ($P < 0.001$). Changes in plasma lycopene and β-carotene were variable, with no consistent trend. Egg yolk supplementation increased plasma LDL-cholesterol concentrations by 8–11% ($P < 0.05$).
Conclusions: Egg yolk is a highly bioavailable source of lutein and zeaxanthin. The benefit of introducing these carotenoids into the diet with egg yolk is counterbalanced by potential LDL-cholesterol elevation from the added dietary cholesterol. *Am J Clin Nutr* 1999;70:247–51.

KEY WORDS Humans, plasma, lutein, zeaxanthin, carotenoids, HPLC, nutrient absorption, chickens, egg yolk, bioavailability, cholesterol, LDL cholesterol

INTRODUCTION

The bioavailability of carotenoids is determined by characteristics of the food source and interactions with other dietary constituents. Studies with β-carotene and lycopene have shown that association with a lipid matrix increases the bioavailability of these carotenoids (1–3). Localization within a plant (eg, in chloroplasts or chromoplasts) and compounds that interfere with intestinal micelle formation (eg, pectin) can decrease carotenoid bioavailability (4, 5). To some extent, the inhibitory effects of the plant matrix can be overcome by cooking to break down the plant cell well and by decreasing food particle size (eg, by chopping and puréeing) (6). Mineral oil, which is not digestible, can interfere with the absorption of carotenoids (7).

Lutein and zeaxanthin have been identified as carotenoids that accumulate in the macular region of the human retina (8, 9) that may play a role in the prevention of age-related macular degeneration (10, 11) and some forms of cancer (12). Like many dietary carotenoids, lutein and zeaxanthin are not converted to vitamin A by human metabolism (13, 14). The yolks of chicken eggs produced in United States contain large amounts of lutein and zeaxanthin compared with other common dietary sources of carotenoids (15, 16). The chicken egg yolk is a matrix composed of digestible lipids, ie, cholesterol, triacylglycerol, and phospholipid (17). Lutein and zeaxanthin are dispersed in this matrix along with other fat-soluble micronutrients such as vitamins A, D, and E (15, 17). Lutein and zeaxanthin in egg yolks might be highly bioavailable because of their association with the lipid matrix of the egg yolk.

In middle-aged and older adults with moderate hypercholesterolemia, we previously investigated the effect on lipid profiles of adding dietary cholesterol in the form of egg yolk to diets enriched in polyunsaturated and saturated fat (18). In the same subjects, we examined here how the added egg yolk affected plasma concentrations of lutein and zeaxanthin.

SUBJECTS AND METHODS

Subjects

We examined the effects of dietary supplementation with egg yolk on plasma lutein and zeaxanthin concentrations in 11 subjects (6 men and 5 women) with a mean age of 62 y (range: 46–78 y),

TABLE 1
Proportions of total energy from different components of the isoenergetic study diets[1]

Dietary component	Corn oil	Corn oil + egg yolk	Tallow	Tallow + egg yolk
Protein (% of energy)	17.4 ± 0.9	15.7 ± 0.4	16.1 ± 0.2	16.7 ± 0.5
Carbohydrate (% of energy)	53.3 ± 2.4	52.9 ± 0.8	51.2 ± 1.6	52.4 ± 2.1
Total fat (% of energy)	29.4 ± 1.5	31.5 ± 1.0	32.7 ± 1.1	30.8 ± 2.6
Fatty acids (% of energy)				
SFA	6.9 ± 0.60	7.4 ± 0.38	13.7 ± 0.58	12.1 ± 1.07
MUFA	9.0 ± 0.64	9.2 ± 0.27	12.1 ± 1.07	11.3 ± 0.68
PUFA	11.2 ± 0.52	11.9 ± 0.15	2.6 ± 0.35	3.4 ± 0.21
Cholesterol (mg/MJ)	20.4 ± 1.0	47.3 ± 3.4	26.1 ± 2.9	54.3 ± 5.0

[1] $\overline{x} \pm$ SD. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

who were enrolled in a study of the effects of dietary egg yolk supplementation on plasma lipid concentrations (18). The study was reviewed and approved by the committee on human subjects for Tufts University and the New England Medical Center. Plasma samples for carotenoid analysis were available from 11 of the 14 subjects enrolled in the study. The subjects were moderately hypercholesterolemic (LDL-cholesterol concentration: 4.34 ± 0.65 mmol/L) but were otherwise healthy. The subjects were nonsmokers and were not using any medications known to lower plasma lipid concentrations. Each subject consumed a full sequence of 4 separate diets in a crossover design. Each diet phase was 4.5 wk, with a washout period of ≥2 wk between phases, when the subjects consumed their usual diets.

Diets

Dietary fat provided 29–33% of the total energy for each diet. Diet phase 1 contained 20% of energy as corn oil, and diet phase 2 contained 20% of energy as beef tallow. Diet phase 3 was prepared by supplementing the beef tallow diet with cooked egg yolk (1.3 yolks/d for a 10.4-MJ intake), which was added to different food items throughout the day. Diet phase 4 was prepared by supplementing the corn oil diet with cooked egg yolk (1.3 yolks/d for a 10.4-MJ intake). The egg yolk supplement increased dietary cholesterol intake by ≈300 mg/d, when comparing each egg yolk–supplemented diet with its respective baseline diet. The protein, carbohydrate, and lipid profiles of each diet are described in **Table 1**. All diets were fed in amounts that maintained a constant body weight throughout the study period. All food and drink consumed during the study were prepared in a metabolic kitchen under the supervision of a dietitian.

Analysis of plasma lutein and zeaxanthin

During each dietary phase, morning blood samples were collected after a 14-h fast. Plasma was analyzed for total cholesterol, LDL cholesterol, and other lipids. Plasma aliquots were archived in sealed vials at −80°C for 3 y before analysis for carotenoids; plasma carotenoid concentrations have been shown to be stable under these storage conditions (19).

The plasma carotenoid concentrations reported here are those obtained after 4.5 wk of each defined diet. Plasma carotenoid concentrations during the 2 non-egg yolk–supplemented phases served as baseline values for each subject. Plasma lipid and lipoprotein measures and chemical analyses of the diets were described by Lichtenstein et al (18).

Lutein and zeaxanthin standards were provided by Hoffmann-La Roche (Nutley, NJ). β-Carotene was from Sigma Chemical Co

(St Louis) and lycopene was identified with a standard extracted from tomato paste. Cholesterol esterase (sterol esterase, EC 3.1.1.13; *Pseudomonas* sp.) and triacylglycerol lipase (EC 3.1.1.3; *Rhizopus*) were from Calbiochem (Torrey Pines, CA). HPLC-grade solvents were used (Fisher Chemical Co, Albany, NY).

HPLC was carried out with a Hewlett-Packard model 1100 gradient HPLC apparatus with a diode array detector (Avondale, PA) and equipped with a 300 mm × 4.6 mm column. The column was packed with Adsorbosphere-HS C_{18} (20% carbon load) with 3-μm particle size (Alltech Associates, Deerfield, IL). The column flow rate was 1 mL/min. The initial mobile phase was 81% acetonitrile:19% methanol:0.04% ammonium acetate (wt:vol), with a step gradient at 20 min to 30% isopropanol. Column temperature was maintained at 16.4°C with a water jacket and circulator.

Plasma carotenoids were analyzed by using a procedure adapted from the method of Handelman et al (20). Positive-displacement pipettors were used to dispense plasma samples and internal standard. For calibration, aliquots of carotenoid standards were carried through the full analytic procedure in parallel with the plasma samples. Plasma samples (100 μL) were mixed with 1 mL reagent containing 1 U cholesterol esterase and 100 U triacylglycerol lipase. The enzyme reagent was prepared in 0.1 mol sodium phosphate buffer/L, pH 7.0, with 0.1% Triton-X-100 (Sigma Chemical Co). Samples were allowed to digest at ambient temperature for 1 h. The samples were then mixed with 1 mL ethanol, 100 μL 5% sodium dodecyl sulfate, and 50 μL internal standard (*apo*-10′-carotenal-*O*-ethyl-oxime) in methanol (20). After vigorous mixing, the sample was extracted with 2 mL hexane and 2 mL diethyl ether. The hexane-ether supernate was evaporated to dryness and the residue dissolved in 100 μL methanol for analysis by reversed-phase HPLC. For the analysis, a 50-μL sample was injected onto the HPLC apparatus.

Egg yolk analysis for lutein, zeaxanthin, and cholesterol

Commercial chicken eggs were obtained in the fall of 1997 from 6 different grocery stores in the Boston metropolitan area. The eggs were classified as large, except for one egg that was extra large. A 50-μL sample of each egg yolk was mixed in an 8-mL borosilicate glass vial with 1 mL distilled water, 1 mL ethanol, and 100 μL 30% aqueous potassium hydroxide. This mixture was heated for 1 h at 60°C to saponify the lipids and hydrolyze the carotenol esters. The saponified egg yolk sample was extracted in the same way as was the plasma sample and analyzed by HPLC for carotenoid content. To determine the fraction of lutein and zeaxanthin that was not esterified, egg yolk was also analyzed for carotenoids by HPLC without the saponification step.

TABLE 2
Carotenoid content of representative chicken egg yolks

Egg	Lutein	Zeaxanthin	Lutein	Zeaxanthin
	μg/yolk		*μg/mg cholesterol*	
1	435	316	1.41	1.02
2	242	164	1.25	0.84
3	218	195	0.98	0.88
4	433	316	1.66	1.21
5	278	176	1.41	0.89
6	150	100	0.77	0.56
$\bar{x} \pm SD$	292 ± 117	213 ± 85	1.19 ± 0.32	0.87 ± 0.23

For cholesterol analysis of the egg yolk, a 50-μL sample of each yolk was diluted with 1 mL buffer, pH 7.4 (10 mmol sodium phosphate/L, 100 mmol NaCl/L). A 20-μL aliquot of dilute egg yolk was analyzed with 1 mL enzymatic cholesterol reagent (Boehringer Mannheim, Indianapolis), by using the Trinder (21) chromophore (phenol and 4-amino-phenazine).

Statistical analysis of data

The data were analyzed by using repeated-measures analysis of variance with type of fat (corn oil or beef tallow) and egg yolk (yes or no) as within-subject factors. Tukey's honestly significant difference test was used for post hoc analyses. All calculations were performed by using SYSTAT for WINDOWS, version 7.0.1 (SPSS Inc, Chicago). Data are reported as means ± SDs.

RESULTS

Egg yolk lutein, zeaxanthin, and cholesterol contents

The lutein and zeaxanthin content of the eggs was 292 ± 117 and 213 ± 85 μg/yolk, respectively (**Table 2**). The lutein and zeaxanthin content was 1.19 ± 0.32 and 0.87 ± 0.23 μg/mg cholesterol, respectively. Lutein and zeaxanthin in the eggs were predominantly nonesterified; their content in nonsaponified yolks was 90% of the amount measured after saponification. Lycopene was not be detected in these egg yolks and the β-carotene content was 3.6 ± 1.5 μg/yolk. The cholesterol content of the commercial egg yolks was 14.7 ± 0.8 mg/g yolk, a value comparable with that reported by Bair and Marion (22).

Increased dietary intake of lutein and zeaxanthin during egg yolk supplementation

We estimated that addition of 300 mg egg yolk cholesterol to a diet that provided 10.4 MJ/d would require 1.3 egg yolks. This amount of yolk would add 380 μg lutein and 280 μg zeaxanthin to the diet each day (Table 2). Reliable determination of the lutein and zeaxanthin content of mixed total diet samples archived at −80°C could not be obtained.

Response of plasma carotenoids to egg yolk supplementation

No significant differences were noted in plasma carotenoid concentrations during the baseline tallow and corn oil diets; lutein concentrations tended to be higher and β-carotene tended to be lower during the tallow diet than during the corn oil diet (**Table 3**). Egg yolk supplementation of the beef tallow diet increased plasma lutein concentrations by 28%, from 0.333 ± 0.089 to 0.427 ± 0.114 μmol/L, relative to the baseline diet. Zeaxanthin increased by 142%, from 0.048 ± 0.012 to 0.116 ± 0.027 μmol/L, relative to the baseline tallow diet (Table 3). Egg yolk supplementation of the corn oil diet increased plasma lutein by 50%, from 0.269 ± 0.083 to 0.403 ± 0.114 μmol/L, relative to the baseline diet. Zeaxanthin increased by 114%, from 0.049 ± 0.012 to 0.105 ± 0.023 μmol/L, relative to the baseline corn oil diet (Table 3).

Average increases in plasma lutein and zeaxanthin concentrations in both egg yolk–supplemented diet phases were 0.114 and 0.062 μmol/L, respectively. The magnitude of these changes correlated with the carotenoid content of the yolks, which contained 37% more lutein than zeaxanthin (Table 2). Plasma concentrations of β-carotene and lycopene were not significantly affected by the diets (Table 3).

Examination of individual responses to egg carotenoid intake showed an increase in plasma zeaxanthin in every subject after egg yolk supplementation. Individual changes in plasma lutein, zeaxanthin, lycopene, and β-carotene concentrations after egg yolk supplementation of the tallow diet are shown in **Figure 1**; similar results were obtained with the corn oil diet (data not shown).

Relative concentrations of plasma cholesterol and carotenoids

The addition of egg yolk to the corn oil–based diet increased plasma cholesterol by 5%, from 5.02 ± 0.52 to 5.31 ± 0.67 mmol/L. The addition of egg yolk to the tallow-based diet also increased plasma cholesterol by 5%, from 5.64 ± 0.77 to 5.85 ± 0.88 mmol/L. Much of the increase was in LDL cholesterol, which increased 8–11% after addition of egg yolk to the diet. However, these increases in plasma cholesterol concentrations would not account for the larger average increase in plasma lutein (39%) and zeaxanthin (128%) after the egg yolk–supplemented diets compared with the baseline diets.

DISCUSSION

The lipid matrix of the egg yolk provides a vehicle for the efficient absorption of dietary lutein and zeaxanthin. Consumption of

TABLE 3
Plasma carotenoid concentrations at baseline and after egg yolk supplementation[1]

	Corn oil	Corn oil + egg yolk	Tallow	Tallow + egg yolk
	μmol/L			
Lutein	0.269 ± 0.083	0.403 ± 0.114[2]	0.333 ± 0.089	0.427 ± 0.114[2]
Zeaxanthin	0.049 ± 0.012	0.105 ± 0.023[3]	0.048 ± 0.012	0.116 ± 0.027[3]
Lycopene	0.390 ± 0.147	0.392 ± 0.196	0.325 ± 0.173	0.420 ± 0.182
β-Carotene	0.642 ± 0.256	0.691 ± 0.345	0.538 ± 0.290	0.696 ± 0.295

[1] $\bar{x} \pm SD$; $n = 11$ for each diet.
[2,3] Significant main effect compared with respective baseline diet (ANOVA): [2] $P < 0.05$, [3] $P < 0.01$.
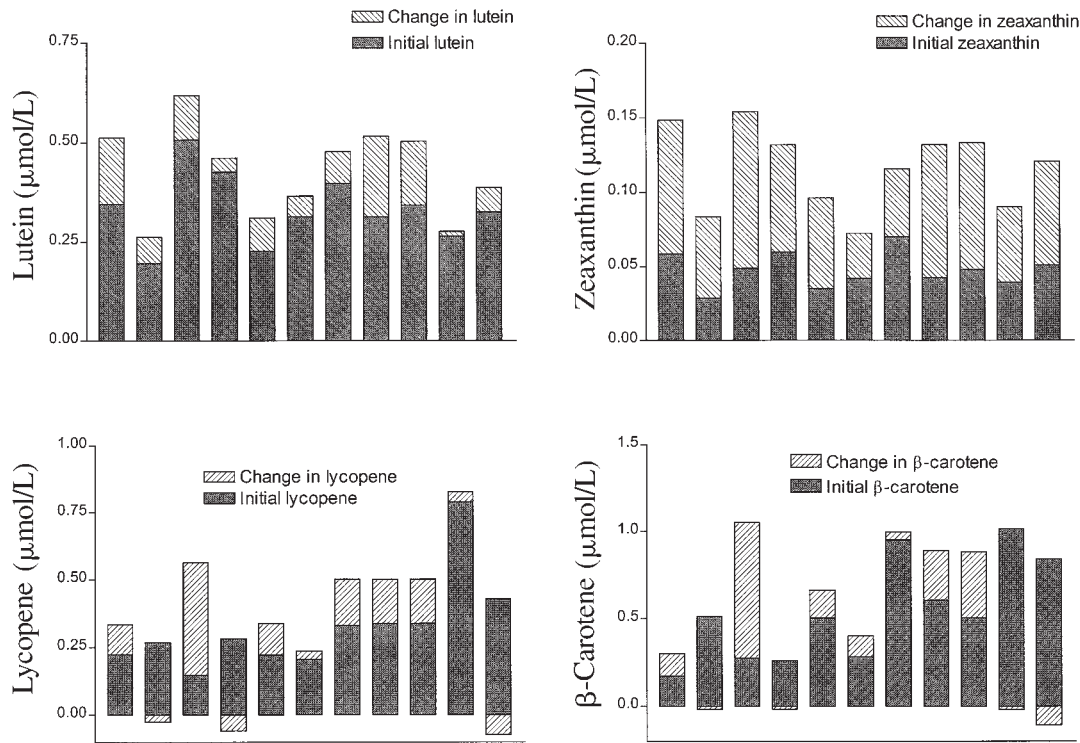
FIGURE 1. Changes in plasma carotenoids for each of the 11 subjects after addition of egg yolk to the beef tallow–based diet. Similar changes for each subject were observed when egg yolk was added to the corn oil–based diet.

1.3 cooked egg yolks/d (per 10.4-MJ diet, typical American energy consumption) over 4.5 wk led to increased plasma lutein and zeaxanthin concentrations in 11 subjects. This supplement of 1.3 egg yolks contained ≈380 μg lutein and 280 μg zeaxanthin. In a previous investigation (23), a dietary supplement of 60 g cooked spinach/d was provided to increase the daily zeaxanthin intake by 300 μg, but no increase in plasma zeaxanthin was observed. Consumption of 150 g cooked corn/d, containing 300 μg zeaxanthin, also did not increase plasma zeaxanthin (23). Plasma lutein concentrations can be elevated by consumption of dark-green vegetables (23, 24), which contain 5–10 mg lutein/(100 g) serving (16). Plasma lutein can also be increased by consumption of a dietary lutein supplement (25); zeaxanthin supplements may also be effective in increasing plasma zeaxanthin.

Carotenoids in the plant matrix are generally associated with either chloroplasts or chromoplasts. The carotenoids in egg yolk are in a digestible lipid matrix consisting of cholesterol (200 mg/yolk), phospholipids (1 g/yolk), and triacylglycerols (4 g/yolk) (17, 26). Such a lipid matrix may be optimal for carotenoid absorption from the diet.

There was a 2-wk interval between phase 3 (beef tallow plus egg yolk) and phase 4 (corn oil plus egg yolk), during which subjects consumed their usual diets. The measurement of plasma carotenoids at the end of phase 4 was therefore 6.5 wk later than the measurement at the end of phase 3. When Micozzi et al (24) fed a broccoli supplement providing 6 mg lutein/d, plasma lutein values returned to baseline 4 wk after the dietary broccoli supplement was stopped. Therefore, most of the increase in plasma lutein and zeaxanthin concentrations seen at the end of phase 4 was due to lutein consumed during that period, but some contribution from increased lutein and zeaxanthin intakes during phase 3 cannot be ruled out.

The addition of egg yolk to the daily diet of the subjects in this study (1.3 egg yolks/d) was associated with an 8–11% increase in LDL-cholesterol concentrations (18). For middle-aged men in the United States with plasma cholesterol concentrations of 5.15 mmol/L, the 25-y incidence of ischemic heart disease mortality is 12% (27). If plasma cholesterol is increased to 5.45 mmol/L, comparable with the changes observed in this study with egg yolk supplementation, the 25-y risk is increased to 14% (27). Because plasma carotenoid concentrations frequently show a slow but steady increase after addition of carotenoid to the diet, we hypothesize that beneficial increases in plasma lutein and zeaxanthin might result from long-term consumption of egg yolks in amounts less than the 1.3 eggs/d fed in this 5-wk study, with a correspondingly lower elevation of plasma cholesterol and ischemic heart disease risk.

Lutein and zeaxanthin are specifically accumulated in the macular region of the retina (8, 9, 20), where they bind to the retinal protein tubulin (28). Zeaxanthin is specifically concentrated at the macula, whereas lutein is distributed throughout the retina (8, 9, 20). Dietary lutein and zeaxanthin may prevent age-related macular degeneration (10), a visual disorder that affects 20% of Americans >75 y of age (29). The protective effects of lutein and zeaxanthin against age-related macular degeneration should be examined in controlled, prospective trials in which plasma concentrations of these carotenoids can be elevated by modification of dietary intake.

In conclusion, egg yolk provides a highly bioavailable source of lutein and zeaxanthin. The benefit of introducing these carotenoids into the diet with egg yolk is counterbalanced by potential elevation of LDL-cholesterol concentrations from the added dietary cholesterol.

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