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ORIGINAL ARTICLE

Effect of n-3 fatty acid enriched eggs and organic eggs on serum lutein in free-living lacto-ovo vegetarians

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Background/Objective: Lutein is a xanthophyll found in the chloroplasts of dark green leafy vegetables, chromoplasts of fruits, and egg yolk. Dietary, serum and macular lutein are inversely related to the risk of age-related macular degeneration. Although the lutein from egg is known to be more bioavailable than that from spinach, not much is known about lutein bioavailability from n-3 fatty acid enriched eggs and organic eggs, both of which are increasingly available to consumers.
Subjects/Methods: We determined the effects of feeding n-3 fatty acid-enriched eggs and organic eggs on serum lutein, zeaxanthin and β-carotene in 20 healthy lacto-ovo-vegetarian (LOV) adults using a single-blind, randomized, crossover study design with a 4-week washout between treatments: six organic eggs or six n-3 fatty acid enriched eggs per week or no egg control for 8weeks each.
Results: Serum lutein was significantly higher in both egg treatments ($P<0.009$) compared with the control, but was not different between the two egg treatments. Serum β-carotene was also higher in the egg groups compared with control but only approached significance ($P=0.066$). Serum zeaxanthin increased in both egg treatments compared with control but did not reach statistical significance ($P=0.139$).
Conclusion: n-3 fatty acid enriched eggs and organic eggs may both significantly increase serum lutein in healthy LOV consuming a predominately plant-based diet.
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Keywords: n-3 fatty acid egg; organic egg; age-related macular degeneration; lutein

Introduction

It has been established that the risk of age-related macular degeneration is inversely related to lutein concentration in the diet, serum and the macula (Nolan *et al*., 2007; Johnson *et al*., 2008). Lutein is a carotenoid found in high concentrations in dark green leafy and *Brassica* species vegetables (Lienau *et al*., 2003), and is an antioxidant that protects the macula from light-initiated oxidative damage (Nolan *et al*., 2007). Zeaxanthin and lutein are frequently found together, because zeaxanthin is a stereoisomer of lutein. In the human eye, lutein is found in the macula, retinal fluid (Chan *et al*., 1998), rod outer segment membranes (Rapp *et al*., 2000) and the lens of the eye (Olmedilla *et al*., 2003). In plants, lutein resides in the chloroplasts of vegetables and leaves, and in the chromoplasts of fruits and vegetables (van Het Hof *et al*., 2000). Raw spinach, one of the richest sources of lutein, contains about 12.2 mg of lutein plus zeaxanthin per 100 g, whereas most regular eggs typically contain about 0.15–0.44 mg lutein/100 g yolk (USDA Nutrient Database, U.S. Department of Agriculture, Agricultural Research Services (2008)). Even though intake of both green leafy vegetables and eggs have shown to increase serum lutein levels in healthy individuals, the bioavailability seems higher from eggs (Chung *et al*., 2004), possibly owing to the lipid matrix of the egg yolk (Handelman *et al*., 1999; Surai *et al*., 2000; Chung *et al*., 2004; Clark *et al*., 2006; Herron *et al*., 2006).

One of the recent public health messages for the prevention of cardiovascular disease is to increase the intake of long-chain n-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid in the daily diet. Fatty fish

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is one of the richest and most commonly used source of these fatty acids (Kris-Etherton *et al*., 2000), however, this is not a preferable option for vegetarians and those who avoid certain types of fish because of taste or heavy metal toxicity (Mozaffarian and Rimm, 2006). In recent years, the n-3 fatty acid enriched eggs have become increasingly available to consumers and may be a viable source of n-3 fatty acids for lacto-ovo vegetarians (LOV). In addition to providing these long chain n-3 fatty acids, these eggs can also be used as a dietary source of lutein. DHA is an important fatty acid and like lutein, it is found in the retina. The combination of lutein and DHA may protect against age-related macular degeneration (Johnson *et al*., 2008). So far, there has been only one study on lutein bioavailability from n-3 fatty acid enriched eggs, which was conducted on 40 healthy Scottish adults consuming a mixed omnivore diet (Surai *et al*., 2000). The n-3 fatty acid enriched egg used in their study contained nearly 12–15 times the lutein of regular eggs.

Consumer preference for organic egg is also steadily increasing (Oberholtzer *et al*., 2006), partly driven by production methods used or perceived health benefits. It seems that the fatty acid composition of organic eggs is very similar to that of regular eggs although the saturated fat, especially stearic acid, may be slightly higher in the organic egg yolk (Samman *et al*., 2009). So far, there have not been any studies on lutein content or bioavailability of lutein from organic eggs.

Thus, the objective of our study was to determine lutein bioavailability (assessed by serum lutein levels) from n-3 fatty acid enriched eggs and organic eggs in comparison with a no-egg control in healthy LOV.

Materials and methods

Subjects

Healthy LOVs were selected through a multistage screening process that included filling out screening questionnaires and interviews with investigators. Subjects were selected if they met the inclusion criteria: between 21–90 years of age, had no existing medical conditions, did not take multiple vitamins or supplements, did not smoke, did not consume alcohol, were willing to follow the diet restrictions and instructions of the study and were LOV for $\geqslant 3$ months. Subjects were recruited mostly from southern California and surrounding areas. For obtaining 80% power for all analyses with $\alpha = 0.05$, the minimum sample size was determined to be 18. A total of 26 subjects were recruited to account for potential dropouts. All study participants signed a written informed consent, approved by the Institutional Review Boards at Loma Linda University, Loma Linda, CA, USA and California State Polytechnic University, Pomona, CA, USA.

Study design

This study was a completely balanced, randomized cross-over design of three 8-week dietary treatments separated by 4-week washout periods. Subjects were free-living and were randomly assigned to one of the six possible sequences of the following dietary interventions: six organic eggs/week, six n-3 fatty acid enriched eggs/week and control (no eggs). Subjects were recruited in two cohorts: one started the study in the fall and the other started in the spring. Whole fresh eggs were donated from Chino Valley Ranchers, Arcadia, CA, USA. Organic eggs were used in the study instead of regular eggs owing to the subject's preference for eggs from vegetarian-fed chickens. Both treatment eggs came from the same Ranchers and chickens were fed with a very similar feed except some which were fed with a soy-based feed (organic eggs) and others with a flaxseed-based feed (n-3 fatty acid enriched eggs).

The subjects received diet counseling from a registered dietitian at Loma Linda University campus, every 2 weeks throughout the study. Subjects were asked not to consume any eggs except those provided in the study and to avoid over-consumption of high lutein-containing foods, such as broccoli, spinach, kale, parsley, avocados, corn, turnip or collard greens, and corn oil during the egg treatment periods. Subjects were permitted to eat the above restricted fruits and vegetables during the 4-week washout and during the no-egg (control) treatment, and kept a diary for noting deviations from dietary protocol or for writing down medications consumed during the study.

At the beginning and the end of each dietary treatment, 12 h fasting blood was drawn. After collection, non-refrigerated centrifugation was performed for 10 min at 2700 r.p.m., the serum was aliquoted in vials and stored at $-80\,^{\circ}C$ until analysis.

Dietary intake analyses

Subjects' dietary intake information was obtained by three 24-h recalls during each treatment using Nutrition Data System-Research software (Nutrition Coordinating Center, Division of Epidemiology 2004 by the Regents of the University of Minnesota). The analytical file for this report was generated using Nutrition Data System-Research software 2007 database to obtain updated lutein and zeaxanthin data. Combined lutein and zeaxanthin totals are available, but individual dietary amounts of each are not. The 24-h recalls and diet diaries were used to determine subject compliance and the 24-h recalls were used to assess the diet composition.

Biochemical analyses

The determination of carotenoids from serum involved the extraction into hexane using established techniques by Guiliano *et al*. (1993), followed by separation and quantification using high-performance liquid chromatography. For β-carotene and lutein, high-performance liquid chromatography analysis was conducted on a Shimadzu (Carlsbad, CA, USA) system, which included the LC-10AT pump, SIL-10 AD

auto-injector and SPD 10A UV/VIS, and the wavelength was set at 450 nm. The column used was a Supelco (Bellefonte, PA, USA) Supelcosil CL-18 (25 cm × 4.6 mm × 5 μm). The mobile phase was acetonitrile:methanol:methylene chloride (70:10:20 vol:vol) with 0.13% (by vol) of triethylamine and 0.01% (wt:vol) of ammonium acetate at a flow rate of 1 ml/min (Rock *et al*., 1997). This method quantifies β-carotene but does not separate lutein from zeaxanthin.

For the separation of lutein and zeaxanthin, high-performance liquid chromatography was carried out as described by Bone *et al*. (2003), using a Phenomenex (Torrance, CA, USA) Ultracarb octadecylsilane column (25 cm × 4.6 mm × 5 μm). The mobile phase was acetonitrile:methanol (85:15) with 0.1% triethylamine. The flow rate was 1 ml/min and detection was at 451 nm. All analyses were carried out in duplicate. The amounts of β-carotene, lutein and zeaxanthin were determined by comparing their chromatogram peak areas with that of authentic external standards (lutein standard: Sigma-Aldrich, St Louis, MO, USA; zeaxanthin and β-carotene standards: Chromadex, Santa Ana, CA, USA). Intra-individual and day-to-day CV's of study samples were <5%. The determination of carotenoids in egg yolk was performed using the same methods used for the serum analyses. A total of three organic and three n-3 fatty acid enriched eggs were used for determining the carotenoids in egg yolk.

Body weight

Subjects were asked to maintain the same level of physical activity throughout the study period and record any deviations from this protocol in a diary, which was reviewed by the registered dietitian at each clinic visit. Body weight was measured at study baseline and at the end of each treatment period (Tanita TBF 310-GS scale, Tanita Corporation of America Inc., Arlington Heights, IL, USA).

Quality control

Participants consumed all treatment foods provided and refrained from eating non-protocol foods. Quality control and compliance was ensured among study participants by the following means: (1) the registered dietitian counseled the participants throughout the study; (2) participants maintained a diary in which they recorded any deviations from the study protocol, which was reviewed by the registered dietitian; (3) three 24-h telephone recalls were administered during each treatment period to assess dietary intake.

Statistical analyses

Before analysis, all serum carotenoid outcome variables were normalized by log-transformation. Significant differences in serum carotenoids among dietary treatments were determined by using mixed linear models containing fixed-effect terms for diet and treatment period, a covariate for the serum carotenoid level at the period baseline and a random-effect term for subject. To the above model, age, BMI and dietary cholesterol intake were added to see if they were related to the observed changes in serum carotenoids.

After normalization by log-transformation, significant differences in nutrient intake among dietary treatments were determined by using two-way fixed-effects ANOVA models containing terms for treatment and subject that allowed for heterogeneity of variance among the diets.

In all analyses, when paired comparisons were performed, *P*-values were adjusted for multiple comparisons by using Tukey–Kramer method. All statistical analyses were performed using the SAS mixed procedure (SAS/STAT software, version 9.1 of the SAS System for Windows, 2003, SAS Institute Inc., Cary, NC, USA). Results are presented as least square means and 95% confidence intervals after back transformation to original units of measure. Results are considered significant when *P*-values were <0.05.

Results

Subject characteristics

A total of 26 subjects were recruited and among them 20 completed the study ($n=16$ females and $n=4$ males). Five subjects left the study during the first diet period for family or job-related reasons, and one left the study during the second diet period for medical reasons. The average age of subjects was 38 ± 3 years, average BMI 23 ± 1 kg/m^2, mean serum cholesterol 4.77 ± 0.89 mmol/l and mean triacylgycerol 1.09 ± 0.57 mmol/l. The average time for which the subjects were LOV was 18.9 ± 13.8 years.

Diet composition and egg lutein content

The dietary intakes for energy, carbohydrate, total fat, protein and fiber were not significantly different between the treatments (Table 1). As expected, the mean dietary β-carotene for the n-3 fatty acid enriched egg (2287 μg) and organic egg (2479 μg) treatments were almost twice than that of the control group (1062 μg), and were statistically significant ($P=0.0124$). Dietary lutein and zeaxanthin intake was also higher in both the n-3 fatty acid enriched egg and the organic egg treatments as compared with the control ($P=0.0001$). Dietary cholesterol intake was significantly higher ($P<0.0001$) on both the egg treatments compared with the control, which established compliance to the egg treatment regime.

The n-3 fatty acid and carotenoid composition of the treatment eggs are given in Table 2. As expected, the n-3 fatty acid (alpha linolenic acid, eicosapentaenoic acid and DHA) content was higher in the n-3 fatty acid enriched egg compared with the organic egg. The carotenoid content was similar in both eggs.

Table 1 Diet composition obtained from 24-h recalls during each treatment

Diet variable	*Treatment groups*						*Global P-value*[2]
	n-3 fatty acid enriched egg		*Organic egg*		*Control*		
	LS mean[1]	*95% CI*	*LS mean*	*95% CI*	*LS mean*	*95% CI*	
KCAL	1724	(1485, 2002)	1838	(1581, 2138)	1713	(1473, 1992)	0.613
KJ	7213	(6212, 8375)	7692	(6615, 8944)	7167	(6164, 8334)	0.612
Carbohydrate (g)	258	(218, 305)	274	(231, 325)	242	(204, 287)	0.394
Protein (g)	60	(51, 72)	61	(51, 73)	56	(47, 66)	0.558
Total fat (g)	51	(42, 63)	51	(42, 63)	63	(51, 77)	0.158
Cholesterol (mg)	166[a]	(96, 288)	129[a]	(74, 225)	27[b]	(15, 48)	0.0001
Fiber (g)	24	(19, 30)	29	(23, 36)	26	(20, 32)	0.256
β-Carotene (μg)	2287[a]	(1409, 3712)	2479[a]	(1515, 4054)	1062[b]	(649, 1737)	0.012
Lutein + zeaxanthin (μg)	1412[a]	(1044, 1910)	1254[a]	(921, 1706)	592[b]	(435, 806)	0.0001

Abbreviation: CI, confidence interval.
[1]Least squares means, after back transformation, from 2-way ANOVA with fixed effects for treatment and subject on log-transformed data. Diet composition data were obtained from Nutrition Data System Research Software (NDS-R; Nutrition Coordinating Center, Division of Epidemiology2004 by Regents of the University of Minnesota), and the analytic file was generated using the NDS-R 2007 database for updated lutein and zeaxanthin data.
[2]Values in the same row with different superscript letters are significantly different (Tukey–Kramer procedure). Cholesterol superscript is $P<0.0001$ for n-3 fatty acid enriched egg and organic egg; for β-carotene enriched vs control is $P=0.0365$ and organic vs control is $P=0.0202$; for lutein-zeaxanthin enriched vs control $P=0.0002$ and organic vs control is $P=0.0017$.
Values in the same row with different superscript letters are significantly different.

Table 2 Carotenoid[a] and n-3 fatty acid[b] composition of the treatment eggs[a]

	n-3 fatty acid egg	*Organic egg*
Carotenoids (μg/yolk)		
β-Carotene	24.0 (1.42)	32.5 (2.93)
Lutein + zeaxanthin	550 (55.7)	552 (27.3)
n-3 fatty acids (g/yolk)[a]		
α-Linolenic acid	1.01	0.15
Eicosapentaenoic acid	0.04	0.01
Docosahexaenoic acid	1.5	0.11

[a]Analysis was performed according to the methods described by Guiliano *et al.* (1993), Rock *et al.* (1997) and Bone *et al.* (2003) ($n=3$ egg yolks). Values are means (± s.d.).
[b]Analyses was performed by Covance Inc, Madison, WI, USA ($n=3$ egg yolks). Values are means.

Serum carotenoids

The study baseline values for serum carotenoids, means (95% confidence interval) were 234.8 (169.6, 325.1) nmol/l for serum β-carotene, 196.3 (165.5, 232.8) nmol/l for serum lutein and 26.5 (20.5, 34.4) nmol/l for serum zeaxanthin.

Compared with the control, there was a significant increase ($P=0.004$) in serum lutein after both the organic egg and n-3 fatty acid enriched egg treatments (Table 3), however there was no difference between the two egg treatments for serum lutein. Serum β-carotene increased in both the egg treatments compared with the control and only approached statistical significance ($P=0.069$). The serum zeaxanthin was also higher for the egg treatments compared with the control, but did not reach statistical significance ($P=0.358$). Age, BMI or dietary cholesterol did not influence the effects of the dietary treatments on serum carotenoids.

Discussion

Consumption of n-3 fatty acid enriched eggs significantly increased serum lutein in healthy adults consuming a predominantly plant-based diet. An intake of 6 eggs per week was sufficient to increase serum lutein by 18% in the n-3 fatty acid enriched egg group compared with the control group. Similar observations were made by Surai *et al.*, (2000) who showed that serum lutein levels significantly increased after ingestion of one DHA-enriched egg per day for 8 weeks in healthy adults, consuming an omnivore diet. However, the DHA-enriched eggs used in their study contained about 1.91 mg lutein/egg yolk, whereas the n-3 fatty acid enriched eggs used in our study contained only 0.55 mg lutein/egg yolk. Also, our study is the first to look at serum lutein levels after the intake of n-3 fatty acid eggs in LOV who eat a predominantly plant-based diet.

We also showed a 17% increase in serum lutein as compared with the control with organic eggs, which contained a similar amount of lutein as the n-3 fatty acid enriched eggs. Regular eggs contain about 0.12 mg lutein/egg yolk, which is 78% lower than the lutein content of the eggs used in our study. The eggs used in our study were not purposefully lutein enriched, but the higher content may be because of the nutrient-dense chicken feed. In previous studies, increased serum lutein following egg consumption has been documented in young adults, post-menopausal women and older men (Goodrow *et al.*, 2006; Greene *et al.*, 2006); in normo- and hyper-cholesterolemic men and women (Handelman *et al.*, 1999; Surai *et al.*, 2000; Chung *et al.*, 2004); and after intake of regular, lutein enriched and n-3 fatty acid enriched eggs (Handelman *et al.*, 1999; Surai *et al.*, 2000; Chung *et al.*, 2004; Clark *et al.*, 2006; Goodrow

Table 3 Serum β-carotene, lutein, and zeaxanthin concentrations at the end of each diet treatment

Carotenoid nmol/l	*n-3 fatty acid egg*		*Organic egg*		*Control*		*Global P-value*
	LS mean[1]	*95% CI*	*LS mean*[1]	*95% CI*	*LS mean*[1]	*95% CI*	
β-Carotene	302.6	(193.5, 258.9)	313.3	(200.9, 488.7)	248.9	(159.8, 387.8)	0.069
Lutein	222.9[a]	(191.9, 258.9)	218.7[a]	(188.8, 253.3)	184.7[b]	(159.5, 213.8)	0.004
Zeaxanthin	33.3	(22.9, 48.3)	32.9	(22.7, 47.6)	26.7	(18.4, 38.6)	0.358

Abbreviation: CI, confidence interval.
[1]Least squares mean (95% CI) after back transformation. Values are adjusted for period effect and period baseline.
Values in the same row with different superscript letters are significantly different ($P<0.05$) using paired comparisons, adjusted for multiple comparison by Tukey–Kramer method.
Values in the same row with different superscript letters are significantly different.

et al., 2006; Greene *et al*., 2006; Herron *et al*., 2006) in an omnivore diet. Our study contributes to the existing literature by showing that n-3 fatty acid enriched eggs and organic eggs also increase serum lutein in LOV adults consuming a predominantly plant-based diet. Only one study failed to show an increase in serum lutein after egg consumption; however, they showed an increase in macular pigment optical density, which is another marker for dietary lutein bioavailability (Wenzel *et al*., 2006).

Serum zeaxanthin levels increased by 6.4 and 33% in the n-3 fatty acid enriched egg group and organic egg group, respectively, compared with the no egg control, but this was not statistically significant. This lack of statistical significance may be attributed, in part, to the greater variability in serum zeaxanthin values among subjects, a small number of subjects, and also a fairly lower concentration in our study subjects (mean range 26–35 nmol/l) compared with that observed (Handelman *et al*., 1999; Goodrow *et al*., 2006; Wenzel *et al*., 2006) by others (mean range 40–116 nmol/l). Hypercholesterolemic men and women consuming 1.3 egg yolk/day for 4.5 weeks showed a 142% increase in serum zeaxanthin (Handelman *et al*., 1999), which is equivalent to having 9 eggs/week compared with the 6 eggs/week fed to our subjects. Others (Bone *et al*., 2003; Goodrow *et al*., 2006; Greene *et al*., 2006) have shown increases in serum zeaxanthin levels following egg consumption when the intervention was much longer (12 weeks) than our study's intervention (8 weeks), or increases were observed in much older subjects (mean age 79 years) than our subjects (mean age 38 years).

Dietary lutein and zeaxanthin may have a role in the prevention of dry age-related macular degeneration (Wenzel *et al*., 2006; Nolan *et al*., 2007), which is the leading cause of severe vision loss in the developed countries. Increasing intake of foods rich in lutein and zeaxanthin is known to increase blood concentrations (Handelman *et al*., 1999; Surai *et al*., 2000; van Het Hof *et al*., 2000; Chung *et al*., 2004; Clark *et al*., 2006; Herron *et al*., 2006) and macular pigment concentrations (Wenzel *et al*., 2006; Nolan *et al*., 2007) of these carotenoids. Plant sources such as spinach contain more lutein than eggs, but the bioavailability is much lower (Chung *et al*., 2004), likely because lutein in spinach and other leafy vegetables is located in the chloroplast as a pigment–protein complex; making its release from the matrix more difficult (Tyssandier *et al*., 2003). In the egg, the carotenoids are located in a lipid matrix, making it more easily available.

Both the n-3 fatty acid enriched eggs and organic eggs provide a bioavailable source of lutein as indicated by the increase in serum lutein levels in LOV. Both lutein and DHA are implicated in the prevention of age-related macular disease (Johnson *et al*., 2008). The n-3 fatty acid enriched eggs may also be a viable dietary choice to obtain n-3 fatty acids DHA and eicosapentaenoic acid for LOV and others that avoid fish either because of taste aversions, or concerns regarding heavy metal, organochloride and polybrominated diphenyl ether toxicities (Mozaffarian and Rimm, 2006; Anderson *et al*., 2008).

Conflict of interest

The authors declare no conflict of interest.

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