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Research Article

Consumption of 12 Eggs per Week for 1 Year Raises Serum Zeaxanthin but Doesn't Alter other Carotenoids, Retinolor Tocopherol Concentrations in Humans with Macular Degeneration

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Abstract

Lutein and zeaxanthin are the two major carotenoids that accumulate in the macula of the eye and may help protect it from damaging UV light thus helping to prevent or treat Age-Related Macular Degeneration (AMD). The major dietary sources of these two carotenoids are green leafy and yellow vegetables, and egg yolks. However, when humans consume foods or supplements higher in certain carotenoids, they alter the absorption of other carotenoids and possibly fat soluble vitamins. The current study examined whether the increase consumption of lutein and zeaxanthin in the form of eggs would alter the status of other carotenoids and fat soluble vitamins. Forty-five adults with early AMD were fed either 12 eggs per week (Intervention group (n=27)) or no eggs (Control group (n=18)) for 1 year. They also were refrained from consuming supplements that contained lutein and/or zeaxanthin. Serum carotenoids, vitamin A and vitamin E were measured at baseline and 12 months. Results showed greater increases in serum lutein and zeaxanthin concentrations from baseline to 12 months in the Intervention (53% and 93%, respectively) compared to Control(5% *vs*. -3%, respectively), however, only serum zeaxanthin increased significantly from baseline to 12 months in the Intervention group. Serum carotenes, beta-cryptoxanthin, lycopene, retinol, and tocopherol concentrations did not change in either group from baseline to 12 months. In conclusion, the increased consumption of lutein and zeaxanthin as twelve eggs per week for 1 year did not alter other serum carotenoids, vitamin A or E status in older adults with early AMD.

Keywords: Carotenoids; Vitamin A; Vitamin E; Age-related macular degeneration

Abbreviations

ABCG5: ATP Binding Cassette G5; AMD: Age-related Macular Degeneration; ANOVA: Analysis of Variance; BHT: Butylated Hydroxy Toluene; BMI: Body Mass Index; DDR: 7-Day Diet Record; HPLC: High Performance Liquid Chromatography; IRB: Institutional Review Board; MTBE: Methyl T-Butyl Ether; SEM: Standard Error of the Mean

Introduction

All-trans lutein and all-trans zeaxanthin are among the primary carotenoids found in human plasma. Their benefits with regards to Age-related Macular Degeneration (AMD) have been well-characterized [1,2]. However, there are other carotenoids and vitamins that are also worthy of recognition for having potential health benefits. Most notably, these include all-trans β-cryptoxanthin, all-trans α-carotene, all-trans β-carotene, and all-trans lycopene [3-5]. The dietary benefits derived from these compounds have generally been attributed to the pro-vitamin A activity of all-trans β-carotene and other pro-vitamin A carotenoids [4-7]. However, aside from the pro-vitamin a functions, there are other carotenoids that have been slowly gaining researchers' attention for their potential to deter certain human diseases [4,5,8-11]. For example, carotenoids derived from food intake have been purported to decrease the negative effects of cardiovascular disease, prostate cancer, and AMD, regardless of the gender [4,5,12]. Certainly, serum carotenoids have been proven to contribute beneficial influences on deferring the development of diseases associated with aging and ameliorating some of the symptoms [13-16].

Over the years, a number of research endeavors have led to the realization that, in the general population, carotenoids ability to deterring disease development relies heavily on the fact that they are capable of behaving as antioxidants. It has also been established that the over-consumption of carotenoids can be very detrimental to some specific subpopulations, such as smokers [17]. The deficiency of serum carotenoids have been directly correlated with the onset and exacerbation of many chronic conditions within the existing literature [12,18]. Therefore, the overall benefits of carotenoids must be weighed against the potentially damaging results associated with varying levels, depending on the specific populations. This is a necessary step in avoiding exchanging benefits in one area of health

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at the expense of another [19,20].

Numerous animal feeding studies and also human studies have suggested a possible interaction between carotenoids during intestinal absorption and metabolism [21,22]. Both short term and long term supplementation trials with β-carotene showed a decrease in serum lutein concentrations [7,23]. Additionally, single or combined dose studies of lutein and β-carotene showed a decrease in β-carotene response when the pre-dominant carotenoid was lutein and vice versa [23,24].

We have previously shown that consumption of 2 and 4 egg yolks/d for 5 weeks significantly increased serum lutein and zeaxanthin concentrations in older adults taking cholesterol lowering statins [25] but did not significantly alter other serum carotenoids, tocopherolsor retinol [26]. The objective of the current study was to investigate the effects of a diet consisting of 12 eggs per week for 1 year on serum carotenoids, tocopherols, and retinol in adults. This knowledge will reveal whether or not the long-term benefit of egg consumption pertaining to all-trans lutein and zeaxanthin may be accompanied by negative side effects associated with the other carotenoids, retinol and tocopherols.

Materials and Methods

Subjects

One hundred and twenty-two subjects, who fulfilled the inclusion criteria, were recruited from an existing patient population at Nashua Eye Associates. Subjects who fulfilled the inclusion criteria needed to receive permission from their primary care physician, and sign the informed consent. Inclusion criteria for the protocol included; newly diagnosed participants with early Dry AMD within the last 2 months, >50 years of age, and were willing to have blood drawn for measurement of blood analytes. Exclusion criteria included; anyone who was unwilling to stop taking oral supplements containing lutein and/or zeaxanthin; taking certain chronic/daily medications, i.e., cholesterol-lowering medications; and were <50 years of age. Once identified, laboratory personnel met with participants to provide them with a brief overview of the study, how to fill out a 7 Day Dietary Record (DDR), fasting (>12 hours) procedures for blood draws, and what was expected of them for the successful completion of the study. Included in this meeting were issues of compliance; especially with the consumption of lutein-free oral supplements, the consumption of 12 eggs per week by the Intervention group, and no egg consumption by the Control group. Participants placed in the Intervention group were instructed to consume no more than 2 eggs per day, 12 eggs per week and not to go >2 days without eating eggs. Research personnel were also available to answer questions from participants on how to incorporate 2 eggs into the typical diet. The use of human subjects for this study was approved by the University of Massachusetts, Lowell, Institutional Review Board (IRB). All subjects were given a cash incentive and a certificate of participation at the end of the study.

Study design and diet

The study design and diet analysis have been previously published [27]. Briefly, the study was divided into two phases**.** Phase 1 involved the execution of a 6-week wash-out run-in period where participants were instructed not to eat any pure egg products. A dietary record (DDR) was also collected from all participants that reflected their nutritional intake over a 7-day period during week 5. This served both as the basis for dietary analysis, as well as a measure of participant compliance. At the end of6 weeks, participants were subjected to the collection of minimum 12-hour fasting blood samples (baseline). Phase 2 consisted of a 12-month period in which all participants were assigned to either the Intervention group (12 eggs per week for 12 months) or the Control group (no eggs for 12 months). Assignment into either group was not completely random unfortunately since some subjects were not willing to consume eggs or were currently avoiding them and, thus, were placed into the Control group. Greater than 12-hour fasting blood samples were collected from all participants at 12 months. Seven day DDRs were collected during the third week of each month for 12 months from all participants. Phone calls to participants were made twice a week to remind them of their respective consumption requirements, and to fast 12 hours prior to blood sample collection. Nutrient analysis was performed using Evaluate, version 1.2 dietary analysis tool (Pearson Education, Benjamin Cummings, 2004).

Reagents

Methanol, hexane, and ether, were purchased from Burdick & Jackson (Morristown, NJ); ethanol was purchased from Sigma-Aldrich; and methyl t-butyl ether (MTBE) was purchased from Omnisolv (Charlotte, SC). Deionized water was prepared using a Milli-Q water purifications system purchased from Millipore Co. (Bedford, MA). All solvents used were HPLC grade. Carotenoid standards including All-trans forms of lutein, α-carotene, β-carotene, and β-cryptoxanthin; as well as, γ-, and α-tocopherols and retinol were purchased from Sigma-Aldrich (St. Louis, MO). Tocol (used as the internal standard) was purchased from Matreya (Pleasant Gap, PA). Zeaxanthinand lycopene were extracted from egg yolks and tomato paste, respectively, for identification purposes on chromatograms only.

Blood collection

Blood samples were collected from each participant at baseline and 12 months. Samples were separated by centrifugation at 1500 x g for 12 min at 4°C, serum was collected and divided into aliquots, and stored at -80°C until analyses could be performed on both baseline and 12 month samples simultaneously.

Chromatographic instrumentation

An Agilent 1100 HPLC System was employed for this study, purchased from Agilent Technologies (Germany). The system composed of a G1311A quaternary pump, a G1315A diode array detector equipped to detect multiple wavelengths, a G1313A auto sampler, a G1322A vacuum degasser, a solvent module, a G1321A fluorescence detector and a computer with Hewlett Packard Chem station software.

Extraction of carotenoids, tocopherols and retinol from human serum

The extraction method consisted of combining 100 µL of serum sample with 200 µL of ethanol, followed by vortex mixing for 30 s to cause protein precipitation. Ten µL of internal standard (Tocol, 1 mg/mL) was then added, accompanied by vortex mixing for 30 s. Two mL of 1:1 hexane: ether with 1% ethanol and 0.1% Butylated hydroxytoluene (BHT) was then added, and the mixture was vortex

Table 1: Demographic characteristic of the study population.

Total recruited	**122**	**M**	**F**	**Age**
Declined	43			
Agreed	61			
Dr. Declined	5			
Dropped out	11			
Finished	45	14	31	75 ± 4
Undecided	18			
Height (in)	Baseline	6 Month		12 Month
Intervention	66.0 ± 0.83 (n=27)	66.2 ± 0.95 (n=27)		65.1 ± 0.80 (n=27)
Control	64.9 ± 0.87 (n=18)	64.7 ± 0.96 (n=18)		64.3 ± 0.92 (n=18)
Weight (lbs)				
Intervention	181.3 ± 10.3 (n=27)	186.4 ± 11.8 (n=27)		181.7 ± 11.1 (n=27)
Control	178.6 ± 13.9 (n=18)	178.5 ± 15.0 (n=18)		183.0 ± 19.2 (n=18)
BMI				
Intervention	29.0 ± 1.27 (n=27)	30.8 ± 2.28 (n=27)		30.0 ± 1.66 (n=27)
Control	29.7 ± 2.07 (n=18)	30.1 ± 2.22 (n=18)		30.9 ± 2.86 (n=18)
Age				
Intervention	73.7 ± 1.55 (n=27)			
Control	77.3 ± 2.09 (n=18)			

BMI = weight (lbs)/height (in)^2 x 704

mixed for 60 s. After a 2-min centrifugation cycle at medium speed, the supernatant was removed with a glass pipette, and evaporated to half of its initial volume. At this point, another 2 mL aliquot of 1:1 hexane: ether with 1% ethanol and 0.1% BHT was added, extracted, and evaporated to dryness under nitrogen gas. The analytes were then reconstituted with 50 µL of methanol, of which 30 µL used for HPLC analysis.

Eggs were purchased during different times of the year and from different supermarkets within the greater Nashua, NH area where our research subjects purchased their eggs. Sixty eggs were randomly selected during the course of the study and analyzed on different days to calculate average concentration of lutein and zeaxanthin in the egg yolks. Lutein and zeaxanthin were prepared from egg yolks using the extraction method and design, previously used in our lab [28] while lycopene was extracted from tomato paste using the following stepwise procedure: (1) 2 mL hexane was added to each sample, (2) vortex 30 s, (3) remove supernatant and evaporated to dryness under nitrogen, (4) add 50 µL methanol, (5) vortex 1 min, and (6) inject 30 µL of sample onto the HPLC.

HPLC analysis of serum carotenoids, tocopherols, and retinol

A diode array detector was used to quantify carotenoids at 450 nm wavelength and retinol, at 325 nm. A fluorescence detector was used to quantify Tocol and alpha- and gamma-tocopherol, with an excitation wavelength of 290 nm and emission wavelength of 330 nm. A 3µm ProntoSIL reversed-phase 250 mm x 4.6 mm C_{30} column (Mac-Mod Analytical, Inc., Chadds Ford, PA) was used with the following mobile phase gradient: (A) 100% methanol and (B) 100% MTBE initiated at 95% A and 5% B then decreased to 95% A at 10 min, 83% A at 13 min, 70 % A at 20 min, 65% A at 29 min, 45% A at 34 min, 38% A at 40 min, 30% A at 45 min and then maintained for 20 min and returned to original conditions (95% A) at 77 min, finishing after 3 min, for resolution within a total of 80 min. The flow rate was set at 0.5 mL/min; and the column temperature maintained at 22°C. The mobile phase was carefully developed with polarity compatibility consideration of phases and anticipated analytes. A total of 9 major analytes, consisting of 6trans-forms of carotenoids (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene), 2 isomers of tocopherol (α-tocopherol and γ-tocopherol), and retinol were quantified and identified by comparing retention times with those of the corresponding external standards and inspecting diode array three-dimensional plot scans (run from 250-500 nm wavelength) for wavelength maxima. Zeaxanthin and lycopene were identified by their spectra and chromatographic characteristics as examined in identified food sources.

Quantification of analytes was carried out by incorporating the internal standard (1 µM Tocol) with an external standard mixture (0.1 µM of each carotenoid; 10 µM of each tocopherol, and 1 µM of retinol) and calculated based on peak area ratio of each analyte to internal standard. Zeaxanthin was calculated as if it was lutein and lycopene was calculated as if it was beta-carotene.

Statistical analyses

Statistical analyses were facilitated through the use of SigmaStat software (Jandel Scientific, San Rafael, CA). Turkey's significant difference test was implemented when a statistical significance was realized at a standard alpha level of 0.05 through the execution of analysis of variance (ANOVA). A Paired T-Test was used to determine significant changes between baseline and at 12 months for serum carotenoids, tocopherols and retinol levels measurements within subjects. A simple T-Test was executed for the determination

Table 2: Macronutrient intake as measured by the seven day diet records (7 DDR).

	Baseline		6 Month		12 Month	
Nutrient	Intervention (n=27)	Control (n=18)	Intervention (n=27)	Control (n=18)	Intervention (n=27)	Control (n=18)
Energy (Kcal)	1827.0 ± 99.6	1537.4 ± 136.5	1507.9 ± 79.5	1248.0 ± 99.2	1532.8 ± 195	1270.0 ± 75.0
Protein (g/day)	75.0 ± 4.03	68.9 ± 6.61	70.9 ± 3.53*	54.0 ± 4.09*	69.1 ± 4.64*	54.5 ± 3.77*
Carbohydrate (g/day)	217.7 ± 18.8A	187.1 ± 15.4	154.0 ± 8.98B	168.6 ± 12.9	166.9 ± 13.1B	176.2 ± 16.8
Fiber (g/day)	15.5 ± 2.71	15.4 ± 1.91	11.5 ± 0.99	14.1 ± 1.39	12.9 ± 1.10	15.0 ± 1.77
Total Fat (g/day)	68.5 ± 4.68	56.8 ± 6.23	66.0 ± 6.51*	45.8 ± 4.42*	65.6 ± 4.63*	41.8 ± 2.92*
Saturated Fat (g/day)	20.5 ± 1.14	18.6 ± 2.56	19.6 ± 1.40*	13.7 ± 1.57*	20.0 ± 1.49*	12.5 ± 1.28*
Cholesterol (mg/day)	178.2 ± 13.7A	165.8 ± 19.0	500.8 ± 25.2*B	128.8 ± 18.1*	473.5 ± 25.4*B	106.3 ± 11.5*

*Statistically significant different at p< 0.05 between Intervention and Control at 6 and 12 months.
Values in row not sharing an upper case letter are significantly different at p< 0.05 between time points for Intervention.
Values are Mean ± SEM

Table 3: Serum carotenoid concentrations (µmol/L) after 12 months of treatment.

Carotenoid	Treatment	Baseline (95% CL)	12 Months (95% CL)	Mean % Change (95% CL)
Lutein	Control (n = 18)	0.354 ± 0.072 (0.097 – 0.624)	0.372 ± 0.078 (0.064 – 0.741)	11.91 (-75.32 – 54.87)
	Intervention (n = 27)	0.272 ± 0.045 (0.080 – 0.732)	0.417 ± 0.088 (0.113 – 0.821)	81.38 (-60.61 – 190.91)
Zeaxanthin	Control (n = 18)	0.076 ± 0.014 (0.020 – 0.191)	0.074 ± 0.010 (0.023 – 0.215)	1.85 (-73.14 – 110.27)
	Intervention (n = 27)	0.061 ± 0.017* (0.014 – 0.221)	0.118 ± 0.021 (0.029 – 0.349)	90.23 (-20.73 – 200.46)
α –carotene	Control (n = 18)	0.218 ± 0.020 (0.128 – 0.251)	0.256 ± 0.032 (0.160 – 0.283)	27.73 (3.96 – 51.51)
	Intervention (n = 27)	0.224 ± 0.035 (0.152 – 0.292)	0.248 ± 0.035 (0.175 – 0.307)	11.19 (-8.60 – 30.97)
β –carotene	Control (n = 18)	0.586 ± 0.277 (0.325 – 0.573)	0.618 ± 0.219 (0.273 – 0.938)	2.67 (-23.58 – 29.91)
	Intervention (n = 27)	0.522 ± 0.148 (0.329 – 0.574)	0.482 ± 0.163 (0.267 – 0.490)	-3.20 (-25.04 – 18.64)
β -cryptoxanthin	Control (n = 18)	0.074 ± 0.011 (0.042 – 0.097)	0.083 ± 0.012 (0.045 – 0.101)	11.22 (-16.19 – 38.62)
	Intervention (n = 27)	0.065 ± 0.019 (0.053 – 0.076)	0.064 ± 0.019 (0.041 – 0.076)	3.85 (-18.95 – 26.65)
Lycopene	Control (n = 18)	0.128 ± 0.018 (0.062 – 0.154)	0.130 ± 0.041 (0.046 – 0.182)	5.19 (-18.95 – 23.86)
	Intervention (n = 27)	0.141 ± 0.036 (0.079 – 0.194)	0.139 ± 0.047 (0.054 – 0.206)	-6.57 (-21.81 – 8.67)

Values for Baseline and 12 Months are Mean ± SEM.
Mean % change is the average of percent differences for each individual within treatment groups at Baseline and 12 Months and not the percent difference between the Mean Baseline and Mean 12 Month values.
* Values are significantly different between Baseline and 12 months for at p< 0.05.

of significant changes between the Intervention and Control groups at baseline and at 12 months. All values were expressed as mean + the standard error of the mean (SEM).

Results

Subject characteristics and diet

Of the 122 subjects interviewed for the study; 61 declined or were undecided about joining the study, 5 were declined by their primary care physician, and 11 dropped out or moved away during the 1 year treatment period, leaving 45 participants to complete the study (n=27 Intervention, n=18 Control), 14 males and 31 females (average age 75 years old) (Table 1). None of the subjects were removed from the study by their primary care physicians due to negative changes on serum lipids during the 12 months. There were no changes in height, weight or body mass index (BMI) in subjects during the course of the 12-month intervention (Table 1).

The baseline dietary data is calculated from the DDR collected during week 5 of Phase 1 and rather than show dietary data for each month throughout the twelve-month treatment period (no differences were observed for dietary data within the Intervention or Control group between month 1 and month 12), only the six-month and twelve-month dietary data are presented in (Table 2). As expected, dietary cholesterol consumption was significantly greater in the Intervention group compared to the Control group at both the six- (288%; p< 0.05) and twelve- (345%; p< 0.05) month time point, as well as within the Intervention group when comparing baseline to six- (181%; p< 0.05) and baseline to twelve- (166%; p< 0.05) months (Table 2). Dietary protein, total fat, and saturated fat intake was significantly higher in the Intervention group compared to the Control group at six- (31%, 44%, and 43% respectively; p< 0.05) and twelve- (27%, 57%, and 60% respectively; p< 0.05) months (Table 2). In addition, the Intervention group consumed significantly less dietary carbohydrates at six- (-29%; p< 0.05) and twelve- (-23%; p< 0.05) months compared to baseline (Table 2).

Table 4: Serum tocopherol and retinol concentrations (μmol/L) after 12 months of treatment.

Vitamins	Group	Baseline (95% CL)	12 months (95% CL)	Mean % Change (95% CL)
α -tocopherols	Control (n = 18)	87.42 ± 11.7 (63.21 – 110.81)	87.81 ± 9.84 (66.37 – 107.20)	3.85 (-12.78 – 8.98)
	Intervention (n = 27)	77.37 ± 5.89 (66.84 – 88.54)	78.76 ± 7.26 (63.84 – 93.47)	2.40 (-4.08 – 4.51)
γ-tocopherol	Control (n = 18)	8.24 ± 1.83 (5.34 – 10.01)	8.21 ± 1.79 (6.14 – 10.66)	-0.01 (-2.29 – 2.27)
	Intervention (n = 27)	11.95 ± 1.28 (8.91 – 14.23)	12.06 ± 1.31 (9.38 – l5.97)	-1.29 (-3.24 – 4.50)
Retinol	Control (n = 18)	1.19 ± 0.08 (0.98 – 1.39)	1.18 ± 0.11 (0.95 – 1.40)	-0.004 (-12.74 – 14.35)
	Intervention (n = 27)	1.03 ± 0.08 (0.87 – 0.91)	1.11 ± 0.10 (0.91 – 1.30)	9.15 (-1.45 – 19.75)

Values for Baseline and 12 Months are Mean ± SEM.

Mean % change is the average of percent differences for each individual within treatments at Baseline and 12 Months and not the percent difference between the Mean Baseline and Mean 12 Month values.

Serum carotenoid, retinol and tocopherol concentrations

Serum zeaxanthin concentrations increased significantly within the Intervention group (93%) after twelve months of treatment when compared to baseline, while no change for serum zeaxanthin was observed within the Control group (-3%) (Table 3). Although, there was no significant difference between the Intervention and Control groups at the end of the twelve-month treatment period for serum zeaxanthin concentrations, the Intervention group had 59% higher serum zeaxanthin concentrations compared to the Control, even though they had 20% lower levels at baseline (Table 3). A notable, but not significant increase in serum lutein concentrations was also observed within the Intervention group compared to no change within the Control group after twelve months compared to baseline (53% *vs.* 5%, respectively) (Table 3).The Intervention group had 12% higher serum lutein concentrations compared to the Control group after twelve months, but started at baseline with 23% lower concentrations (Table 3) but neither difference was significant. Serum α-carotene concentrations increased slightly within the Intervention group (11%) and within the Control group (17%) after twelve months compared to baseline, but not significantly and were similar between groups at baseline and twelve months (Table 3). Serum β-carotene decreased slightly in the Intervention group (-8%) after twelve months compared to baseline while the Control increased slightly (5%) after twelve months compared to baseline (Table 3). Neither change was significant. When comparing across groups, the Control group had slightly higher serum β-carotene concentrations compared to the Intervention group after twelve months (28%) but the Control group started at baseline with 12% higher concentrations at baseline, so these differences between groups were not significant (Table 3). Serum β-cryptoxanthin concentrations did not change within the Intervention group after 12 months compared to baseline (-2%) but did rise slightly within the Control group at 12 months compared to baseline (12%) but not significantly. Also, serum β-cryptoxanthin concentrations were slightly higher in the Control group compared to the Intervention group after 12 months (30%) and at baseline (14%) but not significantly (Table 3). Serum lycopene concentrations did not change in either the Intervention or Control group after twelve months compared to baseline (-1% and 2%, respectively) and were slightly higher, but not significantly, at baseline and after twelve months in the Intervention compared to the Control group (10% and 7%, respectively) (Table 3).

Serum retinol concentrations increased slightly, but not significantly, within the Intervention group (8%) and did not change within the Control group (-1%) although serum retinol concentrations were slightly higher in the Control group compared to the Intervention group at both baseline (16%) and after twelve months (6%) (Table 4). Mean while, no changes occurred for serum α-tocopherol concentrations within both the Intervention group and Control group after twelve months compared to baseline (2% and 0%, respectively) but serum α-tocopherol concentrations were slightly higher, not significantly, in the Control group at both baseline and twelve months compared to the Intervention group (13% and 11%, respectively) (Table 4). Serum γ-tocopherol concentrations did not change within either the Intervention group or Control group after twelve months compared to baseline (1% and 0%, respectively) but the Intervention group did have slightly higher, but not significantly, serum γ-tocopherol concentrations compared to the Control group at both baseline and after twelve months (45% and 47%, respectively) (Table 4).

Discussion

This study demonstrated that increased dietary intake of lutein and zeaxanthin, through the consumption of 12 whole eggs per week for 1 year, does not substantially impact the absorption of tocopherol, retinol, and other major carotenoids. The findings of this study are consistent with our previous short-duration study [26], where the consumption of 2 or 4 eggs per day for 5 weeks was shown to increase serum lutein and zeaxanthin, while posing no significant impact on other serum carotenoids, or serum vitamin A and vitamin E concentrations. Analyses of the lutein and zeaxanthin content of the egg yolks randomly purchased were 256+24 and 228+21 μg/yolk, respectively on average. However, since these eggs were store-bought from grocery markets around the greater Nashua, NH area (same stores as our study population), the concentration of lutein and zeaxanthin in the eggs consumed by the subjects may not necessarily be equated to what we measured in these randomly purchased eggs throughout the duration of the study. The eggs used in the study once extracted as previously stated, were run on the HPLC as if they were subject serum samples to ascertain carotenoid, vitamin A and vitamin E concentrations. Only all-trans lutein and all-trans zeaxanthin was observed to be found in the egg yolks we examined as far as carotenoids are concerned. Although, the USDA database reports small amounts of α-carotene (6 μg/yolk), β-carotene (15 μg/yolk) and β-cryptoxanthin (15 μg/yolk) in egg yolks [29]. These levels are relatively minor compared to the amount of zeaxanthin and

lutein in egg yolks. Even though eggs may contribute a significant amount of vitamin A and vitamin E [30], no changes were observed in serum retinol or tocopherol concentrations during the 1 year. This is not surprising given that serum retinol and possibly tocopherol concentrations are under tight homeostatic control [31].

It is a critical finding that increased consumption of dietary lutein and zeaxanthin, of approximately1 mg/d for 1 year in the current study did not decrease serum concentrations of other carotenoids, tocopherols and retinol. This finding provides evidence that higher dietary doses of lutein and zeaxanthin consumed in the form of egg yolks does not interfere with the absorption of the other carotenoids in the diet. Our findings are in accordance with the study by Tyssandier, et al. [32], which showed that a 3-week plasma carotenoid response is not diminished after consumption of carotenoids from different vegetable sources. Lutein consumed as a supplement, however, has been shown to decrease serum β-carotene concentration, unlike lutein consumed from foods [33]. The findings produced mixed results to a marginal extent, in that serum α-carotene did not change in the intervention group, but increased in the control group. This could raise the possibility that egg yolk consumption may actively prevent elevations in serum α-carotene concentrations rather than simply producing reductions. Serum concentrations of β-carotene, on the other hand, did decrease in both groups, which negate the possibility of it being an effect of the intervention. Therefore, though some small effects were observed, most of the outcomes were not statistically significant. This is not to diminish the clinical importance of the variations resulting, suggesting the need for future studies that employ larger sample sizes for more definitive conclusions.

In light of the aforementioned information, together with prior studies, it is clear that consumption of egg yolks may serve as a non-pernicious dietary mean of increasing serum lutein and zeaxanthin-a finding that is consistent with previous studies [34,35]. Additionally, consuming egg yolks appears to be a viable method of obtaining the benefits associated with increased lutein and zeaxanthin, while maintaining the existing fraction, future absorption, and normal functioning of other carotenoids, which is consistent with findings published by Blesso, et al [9]. However, as examined in a previous study, there were concerns over the thought that a substantial dietary intake of eggs may negatively influence serum concentrations of lipids, lipoprotein, cholesterol, and C-reactive protein to such an extent that may increase the risk for cardiovascular disease (CVD) development [36-38]. Previous studies examined the impact of dietary cholesterol on a sample of individuals that were recognized as "hyper-responders" individuals that presented with an exaggerated response to the diet [34,35]. The resulting LDL-C and HDL-C was, in fact, not a function of increased egg consumption and the subsequent plasma carotenoid increase was actually a beneficial source of nutrition for this subgroup, as well as the normal population [35].

There are potential confounding factors, which may influence the absorption of carotenoids that were beyond the design of this study to capture. For instance, in an earlier study by Herron, et al. [39] determined that a polymorphism in the ATP binding cassette G5 (ABCG5) favored lutein absorption from an egg-rich diet in otherwise healthy patients, versus in patients without the polymorphism. In a different study, Kostic, et al. [23] reported that co-administration of lutein and β -carotene resulted in an interaction where lutein absorption was reduced to 54-61% of its value when administered alone. The authors noted the complex metabolic pathways by which carotenoids are released into the plasma, including two mechanisms for β-carotene's release including early release via chylomicrons in the bloodstream followed hours later by slower release from the liver cells as a component of very low density lipoprotein (VLDL-C). Given this example of a complex metabolic pathway following absorption into the bloodstream from dietary ingestion, it is important in future studies to incorporate a more sophisticated array of endpoints (including, for instance, VLDL-C) to capture the full observable extent of multi-phasic metabolism across carotenoid spectra.

In the same study by Kostic, et al. [23] it was observed that lutein inhibits the bioconversion of β-carotene to pro-vitamin a (retinol) in the intestinal mucosa. Given the infeasibility of measuring carotenoids in the intestinal mucosa, the current study instead focused on their plasma levels. However, it is important to consider the differences between the two environments, with respects to the way that metabolism takes place.

Furthermore, some limitations should also be noted within the context of the present study. First and foremost, the study heavily relied on the subjects adhering to the predetermined guidelines, in terms of egg consumption and the lack. Thus, the potential exists that the marginal effect may be underestimated and will present as a more distinct variation in a sample where compliance may be more strictly adhered to for greater accuracy. Therefore future studies should employ more subjects to resolve this matter.

Conclusion

This study seems to show that by sustaining a diet sufficient in eggs, the benefits associated with increased serum lutein and zeaxanthin may be obtained, and that the consumption of up to twelve eggs per week, at least for one year, will have no significant impact on the maintenance of other carotenoids or vitamin A or E concentrations in serum.

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