Effects of egg consumption on carotenoid absorption from co-consumed, raw vegetables1,2

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ABSTRACT

Background: Dietary lipids are one of the most effective stimulators of carotenoid absorption, but very limited data exist on the impact of endogenous food sources of lipids to enhance carotenoid absorption. The co-consumption of whole egg with carotenoid-rich foods may increase overall carotenoid absorption via lipid-rich egg yolk.

Objective: We designed this study to assess the effects of egg consumption on carotenoid absorption from a carotenoid-rich, raw mixed-vegetable salad.

Design: Healthy young men (n = 16) consumed the same salad (all served with 3 g canola oil) with no egg (control), 75 g scrambled whole eggs (1.5 eggs) [low egg (LE)], and 150 g scrambled whole eggs (3 eggs) [high egg (HE)] (a randomized crossover design). Control, LE, and HE meals contained 23 mg, 23.4 mg (0.4 mg from eggs), and 23.8 mg (0.8 mg from eggs) total carotenoids and 10.5 g (7.5 g from eggs), and 18 g (15 g from eggs) total lipids, respectively. Blood was collected hourly for 10 h, and the triacylglycerol-rich lipoprotein (TRL) fraction was isolated. Total and individual carotenoid contents, including lutein, zeaxanthin, α-carotene, β-carotene, and lycopene in TRL were analyzed, and composite areas under the curve (AUCs) were calculated.

Results: The total mean (±SE) carotenoid AUC0–10h in TRL was higher for the HE meal than for LE and control meals (125.7 ± 19.4 a compared with 44.8 ± 9.2 b compared with 14.9 ± 5.2 b mmol/L · 10 h, respectively (values without a common superscript letter differ); P < 0.0001). The TRL AUC0–10h of lutein and zeaxanthin increased 4–5-fold (P < 0.001), and the TRL AUC0–10h of carotenoid not present in eggs, including α-carotene, β-carotene, and lycopene, increased 3–8-fold (P < 0.01) for the HE meal compared with the control meal.

Conclusion: These findings support the claim that co-consumption cooked whole eggs is an effective way to enhance carotenoid absorption from other carotenoid-rich foods such as a raw mixed-vegetable salad. This trial was registered at clinicaltrials.gov as NCT01951313. Am J Clin Nutr 2015;102:75–83.

Keywords: carotenoid absorption, triacylglycerol absorption, eggs, triacylglycerol rich lipoprotein fraction, vegetable salad

INTRODUCTION

The 2010 Dietary Guidelines for Americans recommends the consumption of 4.5 cups fruit and vegetables/d, but the average fruit and vegetable intake of US adults is only 2.6 cups/d (1). This low consumption of fruit and vegetables may result in low intakes of fat-soluble, health-promoting, bioactive compounds such as carotenoids from these foods (2). Dietary carotenoids have beneficial biological properties including antioxidant and anti-inflammatory effects, and research has supported the potential for protective effects of carotenoids against many degenerative diseases, including cardiovascular diseases, age-related macular degeneration, and some types of cancer (3–7).

The absorption of carotenoids from a meal can be affected by several factors, including the food matrix, type of food processing or preparation, interactions with other dietary compounds during digestion and absorption, gut status, which may affect digestion and absorption processes, and nutritional status (2). Of these factors that influence the bio-accessibility and bio-availability of carotenoids, the co-consumption of carotenoid-rich foods with dietary lipids may be one of the most effective stimulators of carotenoid absorption (8). For example, healthy young adults had ~12.5-, ~40-, and ~3-fold increases in postprandial α-carotene, β-carotene, and lycopene absorption, respectively, when they consumed a salad with full-fat salad dressing (28 g) compared with a fat-free salad dressing (9). Also, an acute feeding study designed to examine the impact of both the amount and source of dietary lipids on postprandial absorption of carotenoids from mixed-vegetable salads revealed that the amount of co-consumed lipids was a more critical effector than source (with a varying degree of saturation) on carotenoid absorption (10). However, over the past 10 y, low-fat versions of food products typically co-consumed with fruit and vegetables (e.g., salad dressings) are readily available and limit or omit fat from salads (11). Collectively, either low intake of carotenoids from fruit and vegetables or inefficient bio-availability because of a decreased co-consumption of dietary lipids may reduce the potential for

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2Supplemental Table 1 and Supplemental Figures 1 and 2 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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carotenoids to effectively promote health and prevent disease in humans.

Eggs are already established as a highly bioavailable source of specific carotenoids, lutein, and zeaxanthin (12) that is due, in part, to the presence of known promoters of carotenoid intestinal absorption. Both the lipid content and high amount of phospholipids in egg yolk (13, 14) are known to enhance carotenoid intestinal micellarization and absorption (12, 15, 16). The highly bioavailable nature of carotenoids from eggs suggests that egg-derived factors may be further leveraged to improve the bioavailability of carotenoids found in co-consumed fruit and vegetables. Although promising, very limited data exist on the impact of a co-consumed food source of lipid, such as eggs, to enhance carotenoid absorption. Therefore, the aim of this study was to assess the effects of co-consuming cooked whole eggs with a carotenoid-rich, raw mixed-vegetable salad on postprandial carotenoid absorption. We hypothesized that a salad with 75 g (∼1.5 eggs) or 150 g (∼3 eggs) of scrambled whole egg would progressively increase postprandial carotenoid absorption compared with a salad without egg.

METHODS

Subjects

A total of 17 subjects were recruited from the greater Lafayette, Indiana, region and 16 of 17 subjects completed the study (Figure 1). One subject withdrew because of a personal medical condition unrelated to the study. Sixteen healthy young men (8 Asian, 7 Caucasian, and one African American) participated, and their average (mean ± SE) age and BMI (in kg/m²) were 24 ± 1 y and 23.9 ± 0.6, respectively. Exclusion criteria for this study included weight change >3 kg ≤3 mo prestudy, exercising vigorously during the past 3 mo, intestinal disorders including lipid malabsorption or lactose intolerance, abnormal liver- or kidney-function tests, fasting blood glucose concentration >110 mg/dL, smoking, drinking >2 alcoholic drinks/d, and taking lipid-lowering medications or dietary supplements that affect the plasma cholesterol concentration. The study protocol was approved by the Purdue University Biomedical Institutional Review Board, and all subjects provided written informed consent and received monetary compensation for their participation. This trial was registered at clinicaltrials.gov as NCT01951313.

Study design

Each subject completed 3 trials (a randomized, single-blind, crossover design); the treatment-order random assignment was done with SAS 9.2 software (SAS Institute Inc.) (Figure 2). Although subjects and dietitians were not blinded, investigators were blinded until all subjects finished the protocol and all sample analyses were completed. Seven days before each testing day, subjects arrived at the Purdue clinical research center in the morning after a 12-h fast, and blood was collected to analyze plasma carotenoid concentrations. For the next 7 d of each trial, all subjects consumed a low-carotenoid diet designed to reduce plasma carotenoid concentrations (10). On each of the 3 testing days, subjects returned to the clinical research center after a 12-h overnight period of fasting, and a catheter was placed into an antecubital vein. After a baseline blood sample was collected, subjects consumed a carefully portioned raw mixed-vegetable salad without eggs (control), with 75 g scrambled whole eggs (∼1.5 eggs) [low egg (LE)⁵], and with 150 g scrambled whole eggs (∼3 eggs) [high egg (HE)]. After the test meal was consumed, blood samples were collected hourly for 10 h. A low-carotenoid, low-fat lunch was consumed after blood was collected at hour 5. On completion of the first and second trials, 1-wk periods were scheduled during which subjects consumed their habitual unrestricted diets.

Test meal

All subjects consumed a controlled low-carotenoid diet for 7 d before each testing day to reduce plasma carotenoid concentrations and minimize the contribution of carotenoid in the triacylglycerol-rich lipoprotein (TRL) fraction because the carotenoid content in the TRL fraction is known to be elevated from previously consumed meals (17). During the first 5 d of these controlled diet periods, subjects were counseled to consume a very-low carotenoid diet; meals on these days were designed to contain ≈750 μg carotenoids/d. On days 6 and 7 of the controlled diet periods (the 2 d before each testing day), subjects were provided completely controlled, low-carotenoid meals with <500 μg carotenoids/d. On each of the 3 testing days, subjects consumed a carefully portioned test meal including tomatoes, shredded carrots, baby spinach, lettuce, and Chinese wolfberry as sources of carotenoids (Table 1). All salads were served with 3 g canola oil. Control, LE, and HE salads included 3 g, 10.5 g (7.5 g from eggs), and 18 g (15 g from eggs) total dietary lipids, respectively. Scrambled whole eggs were prepared uniformly from large eggs on the basis of the American Egg Board’s recommendation (18) and portioned appropriately to dose. The low-carotenoid, low-fat lunch consumed at hour 5 provided 638 kcal, 25 g protein, 130 g carbohydrate, 1.95 g fat, and 172 μg carotenoids. Consumption of water throughout the day was allowed ad libitum. All menus were developed by a registered dietitian with Pronutra software (version 3.3; Viocare Inc.), and all foods were prepared, portioned, and provided to subjects by research staff in the Department of Nutrition Science Metabolic Kitchen at Purdue University. All dietary counseling and meals provided before and during trials were the same as in our previous study (10).

Sample collection and analysis

Blood sample collection and plasma and TRL-fraction isolation

All blood samples were collected into heparinized tubes and centrifuged (3000 × g for 15 min at 4°C) to obtain plasma. Aliquots of fasting-state plasma samples were flushed with nitrogen gas and stored at −80°C until thawed for carotenoid analysis. On each of the 3 testing days, aliquots of fresh plasma from each blood sample were processed to isolate the TRL fraction as previously reported (10). Briefly, 10 mL plasma was transferred to Ultra Clear centrifuge tubes (Denville Scientific Inc.) and preserved with stabilizers including aprotinin, ⁵Abbreviations used: BHT, butylated hydroxytoluene; HE, high egg; LE, low egg; TRL, triacylglycerol-rich lipoprotein.
phenylmethylsulfonyl fluoride, sodium azide, and EDTA (Sigma Chemical Co.). Preserved samples were overlaid with a 1.006-g/mL density solution and centrifuged (L8-M 70; Beckman Instruments Inc.) in a swinging bucket rotor (SW 41 TI; Beckman Instruments Inc.) at 152,000 × g for 35 min at 20°C. The isolated TRL fraction was pipetted into cryostorage tubes, which were flushed with nitrogen gas and stored at −80°C until thawed for analysis. All isolation processes were conducted in a dark room with red light to minimize photo-oxidative reactions.

**Lipid analysis**

Triacylglycerol and total cholesterol concentrations in TRL fractions were measured in duplicate by using a Cobas MIRAS Plus chemistry analyzer (Roche Analytic Instruments).

**Carotenoid extraction**

The extraction of carotenoids from plasma and TRL fractions was done as described by Goltz et al. (10). A total of 200 μL plasma and 2000 μL TRL fractions were deproteinated with methanol and acetone:petroleum ether [1:2 with 0.1% butylated hydroxytoluene (BHT)], and petroleum ether layers were transferred into 11-mL glass vials. This step was repeated twice more by using petroleum ether (0.1% BHT), and the combined petroleum ether layers were dried under nitrogen gas. These extracts were immediately resolubilized and analyzed as described in Carotenoid Analysis.

On each testing day, duplicate test salads and scrambled whole eggs were each homogenized by using a blender (KitchenAid), and aliquots were put into storage tubes, flushed with nitrogen gas, and stored at −80°C until thawed for analysis. The extraction of carotenoids from salad homogenates was conducted as described by Goltz et al. (10) with minor modifications. A total of 0.5 g homogenate was combined with ~0.25 g sodium bicarbonate and ~0.5 g celite, and carotenoids were extracted with a 1:1 solution of acetone:petroleum ether (0.1% BHT). The salad solvent suspension was vacuum filtered through 2 no. 1 Whatman filter papers (VWR International) into a 125-mL Erlenmeyer flask. Two additional extractions were performed with petroleum ether (0.1% BHT) until no residual color remained in the plant tissue filtrate. Combined acetone and petroleum ether layers were saponified by mixing with 40% KOH in methanol on a magnetic stir plate for 45 min at 30°C. The saponified extract was quantitatively transferred to a separator funnel and washed with distilled water and saturated sodium chloride. The aqueous layer was drained, and the petroleum ether layer was poured through a funnel that contained glass wool and a layer of anhydrous sodium sulfate to remove residual water and collected into a 50-mL volumetric flask. Subsequently, the petroleum ether layer was diluted to a total volume of 50 mL with petroleum ether, and 50-mL aliquots were dried under nitrogen gas and stored at −80°C until analysis.

Extractions of carotenoids in homogenized samples of scrambled whole eggs were done as described by Handelman.
Table 1

<table>
<thead>
<tr>
<th>Energy, kcal</th>
<th>Protein, g</th>
<th>Carbohydrate, g</th>
<th>Lipids, g</th>
<th>Fiber, g</th>
<th>Carotenoids, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>65</td>
<td>3 (18)</td>
<td>12 (74)</td>
<td>0.6 (8)</td>
<td>4</td>
</tr>
<tr>
<td>Oil</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>3.0 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Test salad (vegetables + oil)</td>
<td>92</td>
<td>3 (13)</td>
<td>12 (52)</td>
<td>3.6 (35)</td>
<td>4</td>
</tr>
</tbody>
</table>

1Vegetables included 100 g beefsteak tomatoes, 62 g shredded carrots, 70 g baby spinach, 25 g romaine lettuce, and 5 g Chinese wolfberry.

2Values in parentheses are total energy content, %.

Compliance with 7-d pretesting controlled diets

Average reductions in individual and total plasma carotenoid concentrations after the 7-d controlled low-carotenoid–diet periods ranged from 22% to 33% (P < 0.0001), consistent with the dietary compliance leading into the testing days (Table 2).

Total carotenoid contents in the test salad and scrambled whole eggs

During the study period, total carotenoid content in test salads and in scrambled whole eggs was 23.0 ± 0.5 mg/serving (range: 18.7–27.3; CV: 0.11) (Figure 3A) and was 5.0 ± 0.3 μg/g of scrambled whole eggs (range: 3.1–7.4; CV: 0.26) (Figure 3B), respectively. Therefore, 75 and 150 g scrambled whole eggs provided 0.4 and 0.8 mg total carotenoids, respectively, to the test meal. Thus, 100%, 98.3%, and 96.6% of total carotenoids in control, LE, and HE test meals, respectively, were from the salad, and 0%, 1.7%, and 3.4% of total carotenoids, respectively, were from scrambled whole eggs. Individual carotenoid contents from salad and scrambled whole eggs were also measured (Supplemental Table 1).

Triacylglycerol and total cholesterol contents in TRL fraction

From hours 2 to 6, the HE consumption presented a higher baseline corrected triacylglycerol content in the TRL fraction than did LE and control consumption (Figure 4A), and the composite...
triacylglycerol AUC_{0–10h} in the TRL fraction was higher for the HE consumption than for LE and control consumption (79.8 ± 20.1 compared with 21.1 ± 4.9 compared with 12.7 ± 4.2 mg/dL · 10 h; P < 0.001) (Figure 4B). The TRL fraction total cholesterol content was not different in trials during the 10 h of testing (except for hour 4) and the total cholesterol AUC_{0–10h} in TRL was not different in trials (Supplemental Figure 1).

Changes in total and individual carotenoid contents in TRL fraction

TRL total and individual carotenoid responses supported the impact of egg consumption on carotenoid absorption. Until hour 3, the HE meal presented a significantly higher total carotenoid content in TRL than did the control meal, and at hour 4, dose-dependent progressive responses were observed. From hours 5 to 10, the HE meal still presented a higher total carotenoid content in TRL than did LE and control meals (Figure 5A). Lutein and zeaxanthin TRL response were also influenced by co-consumed scrambled whole eggs. As with total carotenoid, dose-dependent progressive responses were observed at hour 4, and the HE meal still presented a higher lutein content in TRL than did LE and control meals from hours 5 to 10. The HE meal also presented a higher zeaxanthin response than did the control meal from hours 1 to 10. Similar responses were observed for carotenoids beyond those shown in eggs such as α-carotene, β-carotene, and

![Figure 3](https://academic.oup.com/ajcn/article-abstract/102/1/75/4564351)

**Figure 3** Reproducibility of total CAT contents in the test salad (A) and scrambled whole eggs (B) during the testing phase of the study. CAT, carotenoid.
lycopene. The HE meal presented higher α-carotene, β-carotene, and lycopene contents in TRL than did the control meal during 10 h (Supplemental Figure 2).

The total carotenoid AUC\(_{0-10h}\) in TRL was also higher for the HE meal than for LE and control meals (125.7 ± 19.4 compared with 44.8 ± 9.2 compared with 14.9 ± 5.2 nmol/L · 10 h; \(P < 0.0001\)). Moreover, lutein, zeaxanthin, α-carotene, β-carotene, and lycopene AUC\(_{0-10h}\) in TRL were higher for the HE meal than for LE and control meals (Figure 5B).

DISCUSSION

It is well known that dark-green leafy vegetables and eggs are good sources of carotenoids, in particular, lutein and zeaxanthin (19). Although dark-green leafy vegetables contain more lutein and zeaxanthin than do eggs (19), eggs are highly bioavailable presumably because of the presence of the lipid matrix, which is composed of cholesterol, triacylglycerol, and phospholipids in egg yolk (20). One large whole egg (∼50 g) generally contains 4.8 g total lipids, which are mostly present in egg yolk, with 1.8 g MUFAs, 1.0 g PUFAs, and 1.6 g SFAs (21). Research that assessed the bio-availability of lutein from lutein supplements, spinach, and eggs documented a higher lutein bio-availability with egg consumption than with other treatments (22). The high bio-availability of carotenoid from eggs suggests that egg-derived factors may be leveraged to improve the bioavailability of other carotenoids in co-consumed fruit and vegetables. The current study used a carotenoid-rich raw mixed-vegetable salad to assess the impact of an endogenous food source of lipids to enhance carotenoid absorption. The eggs consumed with the LE and HE salads provided ∼0.4 and ∼0.8 mg total carotenoids (1.7% and 3.4% of total carotenoids consumed in these trials). Thus, 100.0%, 98.3%, and 96.6% of total carotenoids consumed from control, LE, and HE test meals were from the salad. The findings from the current study support that co-consuming 150 g (3 eggs) cooked whole eggs is an effective way to enhance the absorption of carotenoids from the salad that are not found in eggs.
Dietary lipids are an important determinant of carotenoid absorption (8, 23) by enhancing carotenoid intestinal micellization and the incorporation of carotenoid into a chylomicron. The extent of micellarization of carotenoid increases when the amount of soybean oil added to a salad puree and the amount of oil are increased (24). Human clinical trials also supported that the amount (9, 10) and type (10, 25) of dietary lipids influence carotenoid absorption. The triacylglycerol content in TRL fractions generally represents the response of the ingestion of dietary lipids (26, 27). We showed that the change in the triacylglycerol content in TRL fractions was greater after ingestion of the HE meal than after ingestion of LE and control meals, which indicated that the endogenous lipids in eggs were digested and absorbed. It was reported that increasing amounts of dietary cholesterol also impact postprandial lipoprotein responses in humans (28), and HE consumption provided 560 mg dietary cholesterol (21). The total cholesterol content in TRL fractions presented similar responses as the total carotenoid content in TRL fractions during 10 h of testing, but we did not observe a statistical difference of the total cholesterol AUC0–10h in TRL fractions in trials.

FIGURE 5 Mean (±SE) normalized baseline-corrected total CAT content in the TRL fraction during the 10-h testing day (A) and the composite total and individual CAT AUC0–10h in the TRL fraction (B). Different superscript letters indicate statistical differences in CON, LE, and HE conditions (P < 0.0001). CAT, carotenoid; CON, control; HE, high egg; LE, low egg; TRL, triacylglycerol-rich lipoprotein.

Consistent with the triacylglycerol response, HE consumption increased total and individual carotenoid absorption compared with LE and control consumption, including 2- and 5–6-fold increases in the TRL AUC0–10h of lutein and zeaxanthin, respectively, and 2–3 and 4–9-fold increases in the TRL AUC0–10h of carotenoids beyond those in eggs, including α-carotene, β-carotene, and lycopene. These results are consistent with previous clinical studies that showed greater increases in individual carotenoid absorption when 20 g dietary lipids (10) and 28 g salad dressing (9) were consumed. The consumption of a high-fat mixed meal (29) and a meal containing beef tallow (25) increased β-carotene absorption, and another lipid-rich food source including avocado also increased carotenoid absorption from a vegetable, whereby 150 g avocado (24 g lipids) increased lutein, α-carotene, and β-carotene absorption (30). In addition, differences have been observed in the micellarization of carotenoids (in vitro) between meals coformulated with meat (ham, chicken, or beef) and vegetable oils (31), but in all cases, the inclusion of dietary lipids in any form appeared to consistently potentiate absorption. Collectively, this group of studies, including the current study, consistently shows that the consumption of lipid-rich...
foods is an effective strategy to enhance carotenoid absorption. Little information is available regarding the optimal amount of dietary lipids to maximally enhance carotenoid absorption, but our study suggests that 18 g dietary lipids from eggs were sufficient to increase the absorption of all individual carotenoids from raw mixed-vegetable salads.

Except for lutein, no differences of total and individual carotenoid absorption were observed between control and LE consumption in the current study. This observation contrasts with research that reported the co-consumption of 75 g avocado consumption in the current study. Rotenoid absorption were observed between control and LE raw mixed-vegetable salads.

Dietary lipids to maximally enhance carotenoid absorption, but hancers from in vitro (24, 35) and in vivo (10) studies.

SFAs are considered relatively poor carotenoid-absorption enhancers from in vitro (24, 35) and in vivo (10) studies. In conclusion, the absorption of carotenoids contained in carotenoid-rich foods such as raw-vegetable salads can be effectively enhanced by co-consuming cooked whole eggs. These results support that egg, which is a nutrient-rich food that contains essential amino acids, unsaturated fatty acids, and B vitamins (21), may be used to increase the nutritive value of vegetables, which are underconsumed by the majority of people living in the United States (1, 45).

The authors’ responsibilities were as follows—JEK, MGF, and WWC: designed the research and analyzed data; JEK and SLG: conducted the research; and investigators remaining fully blinded for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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