Lutein Bioavailability Is Higher from Lutein-Enriched Eggs than from Supplements and Spinach in Men[1,2]

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ABSTRACT Lutein may be protective against diseases such as age-related macular degeneration (ARMD). At present, data regarding bioavailability of lutein from various sources are insufficient. Healthy men (n = 10) participated in an intervention study with a crossover design. After a 2-wk washout period during which they consumed a low-carotenoid diet, the men were administered 1 of 4 lutein doses (lutein supplement, lutein ester supplement, spinach, and lutein-enriched egg) for 9 d. All lutein doses provided 6 mg lutein except for the lutein ester dose, which provided 5.5 mg lutein equivalents. Serum samples were collected from fasting subjects on d –14, 1 (baseline), 2, 3, and 10 and analyzed for changes in lutein concentration. Triacylglycerol-rich lipoproteins (TRL) were separated from postprandial blood samples (0–24 h) after the first lutein dose and analyzed for lutein concentration. Subjects completed all 4 treatments of the study in random order. Results from repeated-measures 1-way ANOVA showed that the baseline and dose-adjusted lutein response in serum was significantly higher after egg consumption than after lutein, lutein ester, and spinach consumption on d 10. There was no significant difference in TRL response. In conclusion, the lutein bioavailability from egg is higher than that from other sources such as lutein, lutein ester supplements, and spinach. The lutein bioavailability from lutein, lutein ester supplements, and spinach did not differ. This finding may have implications for dietary recommendations that may decrease the risk of certain diseases, e.g., ARMD. J. Nutr. 134: 1887–1893, 2004.

KEY WORDS: • *lutein* • *bioavailability* • *supplements* • *spinach* • *egg*

Diets rich in fruits and vegetables have been recommended for preventing diseases (1–3). Among the principal components thought to provide the protection offered by fruits and vegetables are the carotenoids. In particular, lutein, a nonprovitamin A carotenoid, was strongly implicated as being protective against age-related macular degeneration (ARMD)[4] and cataract (4–6). It was reported that the risk of ARMD is inversely proportional to lutein concentrations in the diet, serum, and macula (7–10). The mechanism by which lutein is effective in preventing eye disease is not known, but may involve its role as an antioxidant. In the eye, lutein appears mainly in the photoreceptor axon layer; it has also been found in the rod outer segments and retinal pigment epithelium of the retina (11,12) where it may act as a blue light filter to protect underlying structures from phototoxic damage (6).

Key to the understanding of the determinants of serum and macular concentrations of lutein is its bioavailability from food. Major dietary sources of lutein include green vegetables such as spinach, kale, and broccoli (13). Egg yolks are also a source of lutein, although they contain considerably less than the amount found in spinach (14,15). However, recent reports indicated that egg yolk is a highly bioavailable source of lutein, increasing serum lutein concentrations 110–350 nmol/L for each milligram of lutein ingested (15,16). For comparison, studies using vegetables as the source of lutein reported increases of 20–40 nmol/(L · mg lutein) (17,18) and studies with lutein and lutein ester supplements reported increases of 40 and ~75 nmol/(L · mg lutein), respectively (19,20).

The purpose of this study was to compare the bioavailability of lutein from various sources such as lutein and lutein ester supplements, spinach, and egg using serum and triacylglycerol-rich lipoprotein (TRL) responses in a well-defined, controlled setting. To date, such an evaluation has not been conducted. Because the concentration of lutein in conventional eggs is generally lower than that found in spinach (13,16), this study used eggs from chickens fed a lutein-enriched diet, containing ~5 times the amount of lutein found in conventional eggs.

SUBJECTS AND METHODS

Subjects. Healthy men (n = 10; 26–75 y old) were recruited from the New England area and screened for hematologic variables, serum cholesterol, and triacylglycerol (TG), serum albumin concen-

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[4] Abbreviations used: ANCOVA, analysis of covariance; ARMD, age-related macular degeneration; AUC, area under the concentration-vs.-time curve; FABP, fatty acid binding protein; LC, long-chain; TG, triacylglycerol; TRL, triacylglycerol-rich lipoproteins.

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tration, fat metabolism, history of bowel resection, cardiovascular, hepatic, gastrointestinal, or renal diseases. Only men were studied because of the effect of the menstrual cycle on the concentration of circulating carotenoids in women (21). Patients with a history of active small bowel disease or resection, atrophic gastritis, insulin-requiring diabetes, alcoholism, pancreatic disease, or bleeding disorders were excluded from the study. Other criteria for exclusion included current or recent (previous 2 mo) use of medications that may affect lipid absorption (i.e., antibiotics) or vitamin supplement, and current or recent (previous 6 mo) use of carotenoid supplements. Smoking was not permitted during the course of the study. The study protocol was approved by the Human Investigative Review Committee of Tufts University and the New England Medical Center. Informed consent was obtained from all subjects.

Diets and lutein dose. All lutein doses were consumed in a test meal. Each test meal was a frittata (an unfolded omelet) and differed only in lutein source (e.g., supplements, spinach, egg). In the supplement treatments, the subjects consumed lutein (Vitamin Power) or lutein ester (Cognis, Nutrition and Health) supplements along with a plain frittata cooked with egg white (All Whites, Papetti Foods). In the spinach treatment of the study, subjects consumed a spinach frittata made from egg whites and frozen, chopped spinach (Sysco Foods). In the egg treatment, subjects were provided with an egg frittata made of high-lutein eggs (courtesy of Kemin Fine Foods). Test meals contained a sufficient amount of fat (55–60% of energy) to ensure optimal lutein absorption. In addition, the meals for the various treatments were designed to contain similar amounts of fat (18.8–20.4 g), carbohydrate (6.7–8.0 g), and protein (22.9–26.4 g) (**Table 1**). The only appreciable difference in nutrient composition was that the egg frittata contained 643.1 mg cholesterol, whereas the spinach and plain frittata provided only 17–18 mg of cholesterol. Additionally, the spinach treatment contained 6.9 g fiber compared with 2.0 g in the other treatments.

All doses were designed to provide 6 mg lutein/d with the exception of the lutein ester dose. The lutein ester supplement contained 10.23 mg lutein ester, which is equivalent to 5.5 mg lutein. For each subject, spinach and eggs were analyzed for carotenoids for a precise measure of the amount of food required to obtain a daily dose of 6 mg lutein. Chopped, frozen spinach was received as one lot to minimize variation in nutrient content, divided into aliquots, and stored at −20°C. Eggs required for the entire study (~360) were received in one lot. The entire number of eggs was mixed in a Hobart mixer for ~3 min with no direct source of light, batched into amounts required for each subject, and stored at −20°C. All frittatas for a treatment were prepared the day before d 1.

Study design. Two weeks before the initiation of the study (d −14), the subjects visited the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University; blood samples were drawn from fasting subjects to determine the basal levels of carotenoids, cholesterol, and TG. At this time, subjects were given instructions to consume a low-carotenoid diet at home to lower blood carotenoid levels. Food diaries were kept by subjects for 3 d out of each of the 2 wk to verify carotenoid consumption. Subjects were housed at the Nutrition Center for the first 3 d of the study during which time they consumed a carotenoid-free diet. On d 1 of the study, the test meal was served in the morning (0800 h) after a blood draw from fasting subjects; postprandial blood was drawn at 0900, 1100, 1300, 1500, 1700, and 2000 h. On the next 2 d (d 2 and 3), 10 mL of blood was drawn after an overnight fast. The subjects were discharged from the Nutrition Center after breakfast on d 3 with a 6-d supply of the lutein dose-meal. Subjects were given instructions on how to consume a low-carotenoid diet during the free-living phase of the study. On d 10, fasting subjects returned to the Nutrition Center for a blood draw. After a 2-wk washout period, the subject returned to the Nutrition Center to repeat the study protocol with 1 of the 3 remaining lutein treatments. Each subject completed all 4 treatments of lutein (lutein supplement, lutein ester supplement, spinach, and egg) in random order. Serum was separated and stored at −80°C until TRL preparation and analysis for carotenoids.

TABLE 1

Composition of a test meal for each treatment (lutein, lutein ester, spinach, and egg) (59)

	Lutein	Lutein ester[1]	Spinach	Egg
Energy, *kJ*	1327	1327	1235	1240
Fat, *g*	20.4	20.4	19.6	18.8
Carbohydrate, *g*	6.8	6.8	6.7	8.0
Protein, *g*	26.4	26.4	23.4	22.9
Fiber, *g*	2.0	2.0	6.9	2.0
Cholesterol, *mg*	18.0	18.0	17.1	643.1
Lutein, *mg*	6.0	5.5	6.0	6.0
Zeaxanthin, *mg*	0.4	0.3	0.2	0.7
β-Carotene, *mg*	ND[2]	ND	2.0	ND

[1] Test meal consumed with supplement.
[2] Not detected.

Triacylglycerol-rich lipoprotein fraction separation. In an attempt to obtain detailed information on intestinal absorption of lutein, TRL was separated from serum and analyzed for lutein content by HPLC. The TRL fractions were used to improve the discrimination of newly absorbed carotenoids from endogenous pools. Cumulative-rate ultracentrifugation was used to isolate TRL from d 1 (0800 h), 0900, 1100, 1300, 1500, 1700, 2000 and d 2 (0800 h) blood samples from fasting subjects by the method of Terpstra et al. (22) with slight modification. In brief, the serum layer with background density of $\rho = 1.250$ kg/L was overlaid with salt solution of $\rho = 1.225$ kg/L, a salt solution of $\rho = 1.100$ kg/L, and distilled water ($\rho = 0.998$ kg/L). The samples were centrifuged at 111,082 × *g* at 20°C for 31 min. After ultracentrifugation, the top 0.5 mL was collected and washed with saline solution for a total volume of 1.5 mL. Samples were frozen at −80°C until carotenoid analysis.

Serum and triacylglycerol-rich lipoprotein extraction for carotenoids. The d 1, 2, 3, and 10 serum samples from fasting subjects were analyzed for carotenoids by HPLC as described previously (18). TRL carotenoids were extracted by the same method with minor modifications. Briefly, 6 mL of chloroform:methanol (2:1, v:v) and 100 μL of echinenone (gift from Hoffmann-La Roche) were added to 1.5 mL of TRL sample as the internal standard. After addition of 0.5 mL saline solution, the mixture was centrifuged (800 × *g* for 15 min) and lower phase was collected and dried under nitrogen. The extraction was repeated with 3 mL of hexane. The dried residue was dissolved in 75 μL ethanol, and 60 μL was injected onto the HPLC system. All procedures were performed under red light.

HPLC analysis for carotenoids. The HPLC system comprised a Waters 600S controller (Millipore), Waters 616 pump, Waters 717 autosampler, Waters 996 photodiode array detector, and C30 carotenoid column (3 μm, 150 × 4.6 mm, YMC). The HPLC mobile phase was methanol:methyl-*tert*-butyl ether:water (83:15:2, by vol, with 1.5% ammonium acetate in water) for solvent A and methanol: methyl-*tert*-butyl ether:water (8:90:2, by vol, with 1% ammonium acetate in water) for solvent B. Quantitation of lutein was based on the extinction coefficient for lutein in ethanol ($E^{1\%}_{450nm} = 2400$). Lutein was quantified by determining the peak area at 445 nm in the HPLC chromatogram calibrated against a known amount of standard. The lutein standard was a gift from Roche Vitamins (now DSM Nutrition). HPLC performance was monitored by assaying samples from the National Institute of Standards and Technology (Gaithersburg, MD). The lower limit of detection for this method is 0.2 pmol for carotenoids. To verify the precision of this analytical procedure, triplicates of stored plasma pool samples (stored at −80°C) were extracted and assayed every few months. The interassay CV for this pool ($n = 25$) was 4%; the intra-assay CV ($n = 9$) was 4%. Recovery of the internal standard averaged 97%.

Food analysis. Carotenoids were extracted from lutein and lutein ester supplements and analyzed as mentioned above. In brief, one capsule of supplement was dissolved in 3 mL hexane and ethanol added to total 250 mL. From this solution, 0.5 mL was taken and ethanol added to total 50 mL; 50 μL of this mixture was injected into the HPLC system. The lutein and lutein ester supplements contained

6 and 5.5 mg lutein equivalent/capsule, respectively. In addition, the lutein and lutein ester supplements provided 0.4 and 0.3 mg zeaxanthin, respectively. Spinach and eggs were analyzed periodically throughout the study duration (~1 y) (23). Spinach contained 6.89 ± 0.08 mg lutein, 0.22 ± 0.01 mg zeaxanthin, and 2.34 ± 0.06 mg β-carotene per 100g wet wt (n = 7). Eggs contained 2.67 ± 0.11 mg lutein, 0.31 ± 0.02 mg zeaxanthin/100 g wet weight (n = 10). This is ~5 times the amount found in conventional eggs (16). The lutein content of eggs included the 8% contribution from lutein esters.

Data analysis. Results are expressed as geometric means ± SEM. The lutein response in TRL fraction was quantified as the area under the concentration-vs.-time curve (AUC) using KaleidaGraph (Synergy Software). Data were verified for normality (Shapiro-Wilk test); when necessary, they were log transformed before statistical analysis. A paired t test was performed to study the increase in serum lutein concentration from baseline. To study the differences in mean lutein response, cholesterol, and TG among lutein sources, a repeated-measures 1-way ANOVA was performed, followed by the Tukey-Kramer test for multiple comparisons. To determine whether the higher lutein response in egg treatment was due to the high cholesterol content in eggs, 2-way ANOVA and an analysis of covariance (ANCOVA) were performed. Pearson correlation coefficients were calculated to test the correlation between subject characteristics and lutein response. For statistical analyses except for paired t test, baseline and dose-adjusted lutein values were used. A P-value < 0.05 was considered significant. Statistical analyses were performed using SAS version 8 (24).

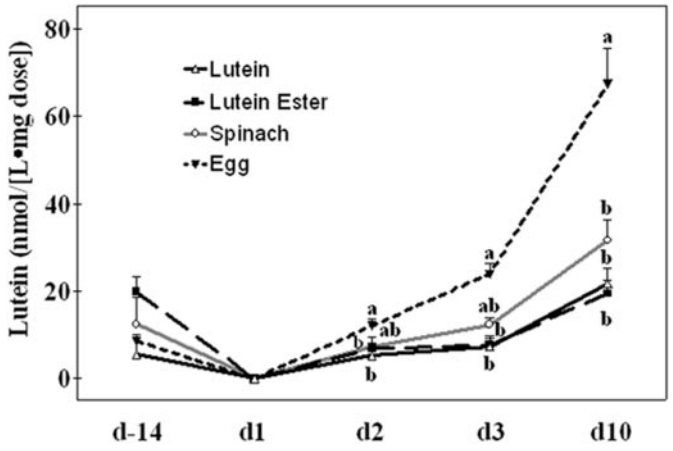

FIGURE 1 Changes in serum lutein concentration after ingestion of 6 mg/d lutein from lutein, lutein ester, spinach, and egg (5.5 mg in lutein ester treatment) in healthy adult men. Values are geometric means ± SEM, n = 10. All values are subtracted from the baseline value of each treatment and adjusted for dose. Data were analyzed by repeated-measures one-way ANOVA after log transformation and the Tukey-Kramer test. Means at a time without a common letter differ, P < 0.05.

RESULTS

Subject characteristics. The mean age of subjects was 52 ± 6 y (range 26–75 y). Of the 10 men, 5 were within a normal weight range (BMI: 21.7–24.7 kg/m^2) and 5 were overweight or obese (BMI: 25.3–35.6). According to the National Cholesterol Education Program guidelines, 6 men had normal cholesterol concentrations (3.80–4.97 mmol/L) and 4 were borderline-high (5.20–6.16 mmol/L) (25). One man had a high TG concentration (25.52 mmol/L) and 2 were borderline-high (19.53 mmol/L each). All of the men finished all 4 treatments of the study, but 2 men in the spinach treatment and 1 in the egg treatment were not able to provide a sufficient number of blood samples for TRL separation.

Lutein response in serum. The serum lutein concentrations decreased from d −14 to d 1, demonstrating the subjects' adherence to a low-carotenoid diet during the prestudy period. By d 2, lutein concentrations were significantly higher than d 1 baseline values for each treatment (**Table 2**). The increase from baseline was higher in those consuming eggs (16.0 ± 1.4 nmol/[L·mg dose]) than in those administered lutein or lutein esters on d 2 (P < 0.05) (**Fig. 1**). The difference among treatments was greatest on d 10 (P < 0.001). The increase for egg consumption [67.3 ± 8.2 nmol/(L · mg dose)] was significantly greater (P < 0.05) than for spinach consumption [31.7 ± 4.6 nmol/(L · mg dose)]; compared with lutein [21.7 ± 3.5 nmol/(L·mg dose)] and lutein ester consumption [19.5 ± 3.1 nmol/(L · mg dose)], the difference was even greater (P < 0.001, respectively). Unlike the other treatments, there was a significant increase in serum cholesterol concentrations for egg consumption (4.74 ± 0.21 on d 1, 4.79 ± 0.25 on d 2, 5.14 ± 0.23 on d 3, and 5.78 ± 0.31 mmol/L on d 10, P < 0.05). However, subsequent analysis of the interaction between serum cholesterol and dose types using 2-way ANOVA with 1 repeated factor revealed that there was no interaction between those 2 variables. Results from the ANCOVA showed that the difference among treatments persisted even after adjustment for cholesterol (P < 0.05 on d 2, P < 0.001 on d 3, P < 0.01 on d 10). The difference in mean lutein response among lutein treatments remained significant after adjustment for serum TG concentration also (P < 0.05 on d 2, P < 0.01 on d 3, P

TABLE 2

Absolute serum lutein concentrations without adjustment for baseline and dose before and after ingestion of 6 mg/d lutein contained in a lutein supplement, a lutein ester supplement, spinach, or egg in healthy men[1,2]

d	Lutein	Lutein ester[3]	Spinach	Egg
		nmol/L		
−14	191.7 ± 35.1	239.7 ± 31.2***	228.4 ± 45.3***	195.0 ± 19.9***
1	158.2 ± 23.2	130.6 ± 16.2	135.8 ± 18.3	124.8 ± 16.1
2	189.9 ± 23.6**	168.4 ± 22.8**	179.3 ± 23.1***	197.6 ± 20.2***
3	202.3 ± 22.5**	172.8 ± 16.9**	210.2 ± 23.8***	268.5 ± 23.0***
10	288.1 ± 31.5***	237.6 ± 22.3***	326.1 ± 43.5***	528.5 ± 56.3***

1 Values are geometric means ± SEM, n = 10.
2 Asterisks indicate difference from d 1 means (paired t test on log-transformed data): * P < 0.05, ** P < 0.01, *** P < 0.001.
3 The lutein ester treatment provided 5.5 mg lutein equivalents.

< 0.001 on d 10). No lutein esters were detected in serum after any of the treatments.

Lutein response in triacylglycerol-rich lipoprotein fraction. The lutein response with egg consumption was 11.1 ± 5.2 nmol/[(L · mg dose) · h] (range: 1.8–51.0, $n = 9$). Lutein AUC after spinach, lutein, and lutein ester consumption were 6.3 ± 1.6 nmol/[(L · mg dose) · h] (range: 2.1–16.8, $n = 8$), 6.2 ± 1.5 nmol/[(L · mg dose) · h] (range: 1.0–14.5, $n = 10$), and 4.8 ± 1.2 nmol/[(L · mg dose) · h] (range: 0.6–13.0, $n = 10$), respectively. One subject had a high lutein response in the TRL for all treatments, especially egg. The higher mean AUC for egg consumption was due largely to values from that subject. After excluding those data, the mean AUC of lutein, lutein ester, spinach, and egg treatments were, 5.3 ± 1.3, 4.6 ± 1.4, 4.8 ± 0.8 and 6.1 ± 1.5 nmol/[(L · mg dose) · h], respectively. The TRL lutein response did not differ with or without values from that subject. Although the lutein response in serum and TRL fraction had a similar trend, with egg consumption yielding the highest response, there was no correlation between the lutein response from the TRL fraction and serum response at d 2 or 10 for any of the treatments. No lutein ester was detected in the TRL after any of the treatments.

Correlation of lutein response with other variables. Numerous researchers reported an inverse relation between BMI (kg/m^2) and serum carotenoid concentration (26,27) and carotenoid response after supplementation (28). To test the inverse relation between BMI and serum carotenoid response in our subject, we divided the subjects into 2 groups according to their BMI and compared the serum lutein response of normal-weight subjects (BMI: 23.64 ± 0.58, $n = 5$) and overweight subjects (BMI: 28.84 ± 1.87, $n = 5$). The result from a Student's t test showed that the response tended to be lower in the overweight group consuming spinach ($P = 0.08$ on d 3 and $P < 0.05$ on d 10). However, there was no correlation between BMI and d 10 serum lutein response for any of the treatments using the Pearson correlation coefficients. A significant negative relation was observed between age and d 10 serum lutein response ($r = -0.65$, $P < 0.05$) for lutein ester consumption. There was a significant relation between baseline lutein concentration and serum lutein response for spinach consumption ($r = 0.88$, $P < 0.001$, d10). A significant correlation was observed between the TRL lutein response and the serum TG concentration at baseline when the men consumed spinach and egg ($P < 0.05$ and $P < 0.01$, respectively). However, after exclusion of the data from 1 subject with high serum TG, the difference persisted only for egg consumption ($r = 0.81$, $P < 0.05$, d 10).

DISCUSSION

This is the first study that evaluated lutein bioavailability from lutein, lutein ester supplements, spinach, and egg in a well-defined, controlled study. Our laboratory and other researchers reported high interindividual variability in carotenoid bioavailability (17,29,30). To minimize the effect of this variability, we adapted a crossover design instead of a randomized comparison study across subjects. The major findings of this study are as follows: *1*) serum lutein response is highest after egg consumption compared with supplements and spinach; *2*) lutein response from spinach is comparable to that from lutein supplements; and *3*) serum lutein responses from lutein and lutein ester supplements do not differ.

Lutein bioavailability from eggs. A significantly higher serum lutein response was found for egg consumption compared with the other treatments in this study (Fig. 1). In egg yolks, lutein is located in the digestible lipid matrix, which is composed of cholesterol, TG, and phospholipids (31). The cholesterol content of the egg yolk may enhance the bioavailability of lutein from egg yolks. It was demonstrated that consumption of a test meal containing high cholesterol (280 and 700 mg) causes a higher postprandial lipid response in plasma and various lipoprotein fractions, e.g., higher TG response in TRL, than when a low cholesterol test meal was consumed (0 and 140 mg) (32). In our study, a higher TG response was expected with egg consumption, in which dietary cholesterol was high (643 mg cholesterol vs. 17–18 mg for the other treatments). A high TRL and serum TG response may result in higher lutein response. This is in line with the work of Henderson et al. (33) in which a greater serum β-carotene response was correlated with a greater TG response to a meal. Also, Borel et al. (29) reported a correlation between chylomicron β-carotene response and chylomicron TG response. However, in this study, we did not observe a higher TG response after egg consumption. Also, the lutein response after egg consumption remained higher than those of other treatments even after adjustment for serum cholesterol or TG.

A higher response after egg consumption may also be due to the fatty acid composition of eggs. Compared with the other test meals, the egg meal was low in long-chain PUFA (LC-PUFA, 2.3 vs. 7.8 g). Hu et al. (34) reported higher β-carotene bioavailability after consumption of β-carotene with beef tallow (containing 81 mg cholesterol), i.e., saturated fatty acids compared with sunflower oil (containing 6 mg cholesterol), i.e., LC-PUFA. They proposed that higher β-carotene bioavailability from beef tallow consumption might be related to fatty acid binding protein (FABP). Hollander and Ruble (35) suggested that FABP, which is necessary for the intracellular transport of fatty acids, might be involved in β-carotene transport within the cell. LC-PUFAs have a higher affinity for FABP than SFA (36) and thus more effectively compete with β-carotene for FABP-mediated intracellular transport. This same mechanism may contribute to the higher lutein response after egg consumption. That is, in a test meal with a relatively small amount of LC-PUFA, such a competition would not exist.

Lutein bioavailability from spinach vs. supplements. It is assumed that during the absorption process, carotenoids have to be released from the intact food matrix and be incorporated into dietary emulsion lipid droplets and subsequently into mixed micelles. In dark green leafy vegetables such as spinach, lutein is located in the chloroplast where it occurs as a component of photosynthetic pigment-protein complexes (37). Due to difficulty of release from the vegetable matrix, lutein bioavailability from vegetables is expected to be lower than from supplements. However, we did not find lower bioavailability from spinach compared with supplements. One explanation for the similar responses among spinach and supplements may be that although the food matrix would compromise bioavailability, other components contained in spinach could offset this by providing protection against the oxidative metabolism of lutein. Examples of such components in spinach include β-carotene, vitamin C, and folate. However, this notion does not agree with previous results. van het Hof et al. (19) reported that the lutein response from cooked mixed vegetables was ~67% of that from a lutein supplement after a 4-wk intervention period. In another study, Castenmiller et al. (38) reported that the relative lutein bioavailability of whole-leaf spinach and minced spinach compared with that of supplement was 45–55%. One explanation for the difference between our results and those of others may be that we optimized our test meals for lutein absorption. Our meals

contained 55–60% energy from fat vs. ~30% energy from fat in these other studies. However, it should be noted that studies evaluating serum lutein response to varying doses of dietary fat have not been conducted. A difference in the cooking method may make a difference in the relative bioavailability of lutein after spinach consumption vs. supplements. In our study, spinach was cooked at 175°C for 20 min with oil instead of blanching it in water for 90 s as others have done (38). It was reported that in an in vitro digestion system, β-carotene was more accessible when the vegetables were cooked in the presence of oil than when they were cooked without oil (39).

Lutein vs. lutein ester bioavailability. Esterified fat-soluble nutrients such as vitamins A and E are absorbed in the small intestine as free alcohol (40). It was suggested that ester hydrolysis by lipases is indispensable before absorption. Xanthophylls, depending on the food source, occur in free form and as fatty acid derivatives. Esterified xanthophyll is found at low levels in many fruits and vegetables. For example, β-cryptoxanthin esters are found in fruits such as apricot, orange, and papaya, and lutein ester, the predominant form in marigold petals, is also found in avocado, zucchini, and pumpkin (41). There are reports of lutein ester in human tissues (42,43). However, only the free form appears in serum after the intake of xanthophyll esters under physiologic conditions (44,45). As in the case for vitamin A and E, a hydrolysis reaction seems to be a prerequisite for xanthophyll ester absorption.

It was initially believed that lutein esters were less bioavailable than the free form because of their characteristics (20). Xanthophylls, including lutein, have hydroxyl group(s) that makes them significantly more hydrophilic than carotenes. Because of this hydrophilic property, a substantial proportion of free lutein is expected to be situated on the surface of dietary emulsion lipid droplets in the intestinal lumen. On the other hand, hydrophobic carotenes such as β-carotene and lycopene are more likely to be situated in the hydrophobic core of the lipid droplets (46). In relation to β-carotene, lutein is more readily absorbed (19,47), possibly due to the relative ease of transfer from dietary emulsion lipid droplets to micelles (48). To date, no reliable data exist on the hydrophobicity of lutein esters, but lutein esters contain fatty acids, mainly palmitate, esterified to the hydroxyl groups. These large hydrophobic molecules are more likely to be situated in the core of lipid droplets. Furthermore, lutein esters must go through hydrolysis by lipases in the intestinal lumen or in the enterocytes before absorption.

Bowen et al. (20) reported higher bioavailability from lutein ester formulation compared with the free form although this difference was not significant. However, in their study, the lutein and lutein ester supplements had different formulations (crystalline vs. powder, respectively). This difference in formulation can lead to different dissolution in emulsion lipid droplets/micelles and eventually to different bioavailability. In the present study, lutein and lutein ester supplements had the same formulation (crystalline in oil) and there were no significant differences in lutein bioavailability measured by serum and TRL responses (Fig. 1). Therefore, the results of our study confirm the speculation that humans have a very efficient hydrolysis system for xanthophyll esters and that ester hydrolysis is not the limiting step for lutein ester absorption (49). In the present study, there was a negative relation between age and d 10 serum lutein response ($r = -0.65$, $P < 0.05$) after consumption of the lutein ester. This result suggests a possible decline in lutein ester hydrolysis capacity in older subjects. This is an area that warrants further study.

Because enterocytes cannot secrete a substantial amount of chylomicron in the absence of dietary fat, dietary fat intake is crucial in carotenoid absorption. For β-carotene, there is no increase in absorption when fat intake is >3–5 g (50). Information on an adequate fat amount for xanthophyll absorption is scarce. Roodenburg et al. (51) found that the lutein response was higher when lutein esters were consumed with a high-fat spread (36 g fat provided with the test meal) than with a low-fat spread (3 g). Bowen et al. (20) observed that the AUC difference between lutein and lutein ester treatments (lutein ester AUC – lutein AUC) was higher in subjects with a fat intake ≤ 11 g. In contrast, subjects consuming ≥19 g fat had a lower AUC difference. This result implies that lutein absorption from the supplement was more favorable at a fat intake ≥ 19 g, rather than in the range ≤ 11 g. In our study, ~18 g fat was provided with the lutein doses. This amount appears to minimize the absorption difference between lutein and lutein ester.

Correlation between lutein response and subject characteristics. There were several reports concerning high interindividual variability in serum responses to an oral dose of carotenoid. Similar to previous findings, we also found a large variation in the response of lutein among our subjects. To further evaluate factors that may be involved in the serum and TRL responses, we analyzed the data with respect to various subject characteristics. There were no consistent correlations between the lutein response and subject characteristics including age, baseline serum cholesterol, and TG, baseline serum lutein value, and BMI. This is in agreement with a recent study examining lutein bioavailability from a supplement (52).

No correlation was observed between serum lutein response and that of TRL. This may be due to a transfer of lutein among lipoproteins in circulation. Surface polar lipid components of the TRL and other lipoproteins such as cholesterol, phospholipids, and α-tocopherol exchange lipoprotein particles in the circulation (53–56). Lutein, which possesses polar functional groups, is expected to undergo the same process after entering the bloodstream. In fact, the transfer of lutein, but not of β-carotene, among lipoproteins was confirmed in biological emulsions (46) and human lipoprotein samples (57). Due to this transfer, the TRL lutein concentration may underrepresent the true extent of lutein absorption (58). The fact that there was a correlation between TRL β-carotene response and serum β-carotene response ($r = 0.87$, $P = 0.01$, d 2) after spinach consumption (the only treatment in which β-carotene was consumed) suggests that the lack of correlation between TRL and serum lutein responses was due largely to the transfer of lutein among lipoprotein particles.

In conclusion, this is the first report that compares lutein bioavailability from common dietary sources (egg, spinach, supplements). It should be noted that the eggs used in this study contained ~5 times the amount of lutein contained in conventional eggs. Comparison of the bioavailability of lutein from eggs and spinach necessitated the use of these eggs. Although conventional eggs are not commonly considered to be a rich source of lutein because of the relatively low concentrations, the bioavailability from this source may be high. Future studies are required to examine the bioavailability of lutein from these more commonly available eggs. The mechanism by which egg increases lutein bioavailability is not known, but is presumably due to other components in egg. Furthermore, the bioavailability of lutein from supplements appears to be comparable to that of the spinach, the common food source. This finding may have implications for dietary recommendations that will decrease the risk of certain diseases, e.g., ARMD.

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