Effects of Mayonnaise on Postprandial Serum Lutein/Zeaxanthin and β -Carotene Concentrations in Humans

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(Received April 28, 2009)

Summary To clarify the effects of different physical forms of oil on postprandial serum lutein/zeaxanthin and β -carotene concentrations, we performed a vegetable meal loading test. Eighteen healthy subjects participated in the test, which consisted of broccoli as a control (CON) meal, broccoli with oil (OIL), and broccoli with mayonnaise (MS), consumed in random order. After collection of fasting blood samples, subjects consumed one of the three test meals. Fasting and postprandial changes in serum carotenoids were assessed 2, 4, and 6 h after ingestion of each test meal. Serum lutein/zeaxanthin and β -carotene concentrations were measured. Although no significant change was noted after the CON meal, the serum lutein/zeaxanthin concentration was higher at 4 h after consumption of the OIL meal, and at 2, 4 and 6 h after consumption of the MS meal, as compared with the fasting state. Serum β -carotene concentrations did not change after ingestion of either the CON or the OIL meal but were elevated 2, 4, and 6 h after MS ingestion as compared with the fasting state. The incremental areas under the curves (IAUCs) of serum lutein/zeaxanthin and β -carotene concentrations were higher after the MS meal than after the CON meal. IAUCs after the OIL meal exhibited no statistically significant differences from the CON and MS meals. We suggest that mayonnaise contributes to increase serum lutein/zeaxanthin and β -carotene concentrations when consumed with vegetables rich in these carotenoids.

Key Words mayonnaise, lutein, zeaxanthin, β -carotene, vegetable

Decreased macular pigment density is reportedly associated with age-related deterioration of visual function, e.g. age-related macular degeneration (AMD) (1). AMD is a major cause of blindness in elderly people in developed countries (2–4). AMD is a disease caused by injury at the center of the retina, which decreases the patient's central vision. This impaired visual condition decreases quality of life in the elderly.

Lutein and zeaxanthin (a stereoisomer of lutein) are the dominant carotenoids in the retina and macula (5). Lutein and zeaxanthin, but not provitamin A, are xanthophylls. Xanthophylls act as antioxidants and shield ocular structures from blue light, which causes retinal injury leading to AMD (6, 7). Previously, numerous studies showed that lutein has the potential to delay the development of AMD (8–13). Lutein and zeaxanthin have been described as protecting photoreceptors from apoptosis induced by oxidative stress in vitro (8). In an animal study, lutein supplementation led to suppression of choroidal neovascularization development associated with inflammatory processes and subsequent upregulation of inflammation molecules (9). The results of observational (10) and intervention (11) studies also indicated that dietary lutein may have a protective effect

against AMD. Johnson et al. demonstrated that consumption of spinach and corn, which contain lutein, in the daily diet for 15 wk raised the serum lutein concentration (12). In addition, Delcourt et al. showed that high plasma lutein and zeaxanthin concentrations were associated with a reduced risk of AMD (13). Continuous ingestion of vegetables rich in lutein might contribute to an increased serum lutein concentration, thereby lowering the risk of AMD.

In 2006, the Food and Drug Administration concluded that there was no credible evidence to support a health claim for lutein or zeaxanthin intake in relation to the risk of AMD (14). However, the National Eye Institute started a new randomized clinical trial called the Age-Related Eye Disease Study 2, designed to evaluate the effects of nutrients including lutein on the development of AMD (15).

To potentially reduce the risk of AMD as suggested in the above-mentioned studies, it may be beneficial for the elderly to increase lutein consumption. Fats and oils reportedly increase absorption of the lutein contained in vegetables (16). However, fat and oil intake in the elderly is reportedly lower than that in younger populations (17). Therefore, effective methods are required to increase the intake of lutein from lutein-containing foods in the elderly population.

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Carotenoids are dissolved in oil in the stomach and subsequently absorbed in the intestine in the form of an oil-in-water (O/W) emulsion. Mayonnaise, a popular type of dietary O/W emulsion, is a potentially efficient food for enhancing carotenoid absorption.

To clarify whether or not mayonnaise increases lutein absorption, we examined the effect of mayonnaise on postprandial serum lutein concentration.

MATERIALS AND METHODS

Test meals. Green vegetables such as broccoli and spinach are good sources of lutein (18, 19), and broccoli is customarily consumed with mayonnaise more frequently than spinach in Japan, according to output from Shoku-MAP® (a system and a registered trademark of NTT Data Lifescape Marketing Corporation, Tokyo, Japan). Therefore, we used broccoli as the lutein source for the test meals in this study. The test meals consisted of 75 g of boiled broccoli as the control (CON) meal, 75 g of boiled broccoli with 14 g of oil as the OIL meal, and 75 g of boiled broccoli with 15 g of mayonnaise as the MS meal.

Broccoli was boiled for 4 min, then drained in a colander, cut into florets, and processed into a paste using a food processor (Panasonic Corporation, Osaka, Japan). Each portion of the broccoli paste (75 g) was wrapped in plastic and frozen until its use in the experiment. Wrapped broccoli was thawed using a microwave oven (500 W, Panasonic Corporation) for 3 min just before the experiment.

Oil and egg-yolk-type mayonnaise was prepared at the R & D Division of Q.P. Corporation (Tokyo, Japan). The experimental vegetable oil was composed of 85% canola oil and 15% soy oil. The mayonnaise was composed of 70% experimental vegetable oil, 14% egg yolk, 13% vinegar, and 3% other ingredients such as salt, spices, etc.

One serving of oil (14 g) or mayonnaise (15 g) was used as the dressing for the OIL and MS meals, respectively, just before consumption.

The nutrient contents of the test meals, as determined by the Japan Food Research Laboratories (Tokyo, Japan), are shown in Table 1. Lutein and β -carotene contents did not differ among the three test meals. The fat contents of the OIL and MS meals were 14.5 and 11.5 g, respectively.

Subjects. This study was conducted in compliance with the spirit of the Helsinki Declaration of 1964 (revised in 2008) after obtaining approval from the Institutional Review Board of Japan Women's University. Twenty-four subjects, 23–47 y of age, judged to be healthy based on regular medical check-ups, were recruited to participate in this randomized crossover intervention study. Written informed consent was obtained from all participants. Five men were excluded because of high serum triglyceride (TG) concentrations (>150 mg/dL) in the fasting state, as demonstrated during the experiment. One man was also excluded as he was currently taking a prescribed medication. Results from the remaining 10 men and 8 women were

Table 1. Nutrient contents of the test meals.

	CON	OIL	MS
Weight			
Broccoli (g)	75	75	75
Vegetable oil ¹ (g)	0	14	0
Mayonnaise ² (g)	0	0	15
Nutrients			
Energy (kcal)	23	152	125
Protein (g)	2.6	2.6	2.9
Fat (g)	0.5	14.5	11.5
Carbohydrate (g)	3.7	3.7	3.9
Sodium (mg)	2	2	108
β -Carotene (μ g)	535	535	535
Lutein (mg)	1.04	1.04	1.07
Zeaxanthin (mg)	0	0	0.01
Cholesterol (mg)	0	0	24

CON, control meal; OIL, broccoli with vegetable oil meal; MS, broccoli with mayonnaise meal.

¹Fatty acid composition of experimental vegetable oil: 5.0% palmitic acid, 0.2% palmitoleic acid, 0.1% heptadecenoic acid, 2.1% stearic acid, 55.2% oleic acid, 25.0% linoleic acid, 9.4% α -linolenic acid, 0.6% arachidic acid, 1.1% icosenoic acid, 0.4% behenic acid, 0.1% lignoceric acid, 0.2% tetracosenoic acid, and 0.6% others.

²Mayonnaise: 70% experimental vegetable oil, 14% egg yolk, 13% vinegar, and 3% other ingredients (salt, spices, etc.).

included in our analyses.

Experimental design. For supper before each experimental day, the subjects were given purchased dishes consisting of boiled rice, low-fat curry without carrots, and yogurt. The diets provided 684 and 852 kcal, 25.6 and 28.1 g of protein, 6.6 and 6.9 g of fat, and 127.4 and 164.5 g of carbohydrate for female and male subjects, respectively. After an overnight fast of at least 12 h, blood was collected and the subjects consumed one of the test meals. Blood samples were collected 2, 4, and 6 h after test meal ingestion. The subjects were instructed to remain as sedentary as possible during the experiment. All subjects were asked to eat the CON, OIL and MS meals in random order. The washout period between the tests was at least 1 wk. Subjects consumed their usual diets during the washout period. The subjects were instructed to avoid strenuous activity during the test period.

Biochemical measurements. Serum was immediately separated from whole blood by centrifugation ($1,200 \times g$ for 10 min) at room temperature, and stored at -20°C until analysis. Biochemical measurements were conducted at the laboratory of Nikken SEIL Co., Ltd. (Shizuoka, Japan). Total cholesterol, high-density lipoprotein cholesterol, TG, non-esterified fatty acid, phospholipid (PL), and ketone bodies were measured enzymatically. Low-density lipoprotein cholesterol was determined using the Friedewald equation (20). Serum aspartate aminotransferase, alanine aminotransferase, and γ -glutamyltranspeptidase concentrations were measured using the method of the Japan Society of Clin-

Table 2. Biological parameters and serum carotenoids and retinol concentrations at fasting state.

	CON	OIL	MS
Biological parameters			
TC (mg/dL)	191±5	194±7	190±6
HDL-C (mg/dL)	70±4	70±5	71±4
TG (mg/dL)	78±8	82±8	78±6
NEFA (mEq/L)	0.49±0.04	0.51±0.07	0.59±0.06
PL (mg/dL)	202±6	210±9	205±7
Ketone body (mmol/L)	49±10	66±19	68±17
LDL-C (mg/dL)	105±4	108±6	104±5
AST (IU/L)	23±1	23±1	24±2
ALT (IU/L)	20±3	21±2	22±3
γ-GTP (IU/L)	19±3	21±3	20±3
Serum carotenoids and retinol			
β-Carotene (μg/dL)	44.9±8.8	45.8±10.1	41.2±8.4
β-Cryptoxanthin (μg/dL)	49.2±12.3	45.5±9.5	42.4±9.4
Lutein/zeaxanthin (μg/dL)	87.1±7.5	83.4±6.8	73.6±4.9
Lycopene (μg/dL)	17.8±1.7	17.6±1.1	14.8±1.5
Retinol (μg/dL)	58.4±4.0	58.5±3.1	57.0±2.7

Values are means±SE, *n*=18.

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; NEFA, non-esterified fatty acid; PL, phospholipid; LDL-C, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase.

Fig. 1. Postprandial changes in serum lutein/zeaxanthin and β -carotene concentrations. $\circ$, CON meal; $\blacktriangle$, OIL meal; $\blacksquare$, MS meal. Means±SE. **p*<0.05 vs. fasting state by Dunnett's test. No significant differences in serum lutein/zeaxanthin and β -carotene concentrations were observed at 2, 4 or 6 h postprandially among the three test meals. *n*=18.

ical Chemistry (21).

Serum carotenoid and retinol concentration measurements. Serum was stored at -20°C in lightproof tubes until analysis. Serum carotenoids (β -carotene, β -cryptoxanthin, lutein/zeaxanthin, and lycopene) and retinol concentrations were measured by high-performance liquid chromatography according to the procedure of Sowell et al. (22) at the laboratory of Nikken SEIL Co., Ltd. Carotenoids and retinol were separated on a Wako-sil-II 5C18 column (4.6×250 mm; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and eluted using a linear mobile-phase gradient from 100% solvent A containing acetonitrile/dichloromethane/methanol (60/30/10, v/v/v) to 100% solvent B containing acetonitrile/dichloromethane/methanol (85/5/10, v/v/v) over

30 min. The flow rate was 1.0 mL/min. Since serum lutein and zeaxanthin concentrations were not separable by the methods we utilized, they were measured as a combined value designated lutein/zeaxanthin.

Statistical analysis. Results are expressed as means±SE. Fisher's PLSD test was applied for comparisons among the three test meals. Time courses of serum carotenoids, TG and PL from the fasting state onward were assessed with repeated-measures ANOVA and analyzed with Dunnett's test, if there were significant differences. The incremental areas under the curves (IAUCs) of serum lutein/zeaxanthin and β -carotene concentrations were calculated as the increase in response above baseline minus any drop below the baseline, based on trapezoidal rules. Covariance analy-

Fig. 2. IAUCs of serum lutein/zeaxanthin and β -carotene concentrations. White bar, CON meal; gray bar, OIL meal; black bar, MS meal. Means $\pm$ SE. Covariance analysis was applied for IAUCs comparisons among the three test meals, and serum concentrations in the fasting state served as covariates. Means with different letters are significantly different ($p < 0.05$). $n = 18$.

Fig. 3. Postprandial changes in serum TG and PL concentrations. ○, CON meal; ▲, OIL meal; ■, MS meal. Means $\pm$ SE. * $p < 0.05$ vs. fasting state by Dunnett's test. There were no significant differences in serum TG or PL concentrations at 2, 4, and 6 h postprandially among the three test meals. $n = 18$.

sis was applied for IAUCs comparisons among the three test meals, and serum concentrations in the fasting state served as covariates. P values less than 0.05 were considered significant. All statistical analyses were performed using the software package Dr. SPSS II for Windows (SPSS Inc., Tokyo, Japan).

RESULTS

Characteristics of subjects

The mean age of the 18 subjects was 31.3 ± 1.6 y, and their average body mass index was 22.6 ± 0.8 kg/m². There were no significant differences in fasting serum biochemical parameters, carotenoids or retinol concentrations among the three groups (Table 2).

Postprandial changes in serum lutein/zeaxanthin and β -carotene concentrations

As shown in Fig. 1, there was no significant postprandial change in the serum lutein/zeaxanthin concentration after CON meal consumption as compared

with the fasting state. However, concentrations were higher at 4 h after the OIL meal ($p < 0.01$), and 2 h ($p < 0.05$), 4 h ($p < 0.01$) and 6 h ($p < 0.01$) after the MS meal, as compared with the fasting state. The serum β -carotene concentration did not change after consumption of the CON and OIL meals as compared with the fasting state. After consumption of the MS meal, β -carotene concentrations were higher at 2 h ($p < 0.05$), 4 h ($p < 0.01$), and 6 h ($p < 0.01$) than in the fasting state.

Compared with the CON meal, IAUCs of serum lutein/zeaxanthin and β -carotene concentrations were not different after OIL meal consumption, but were higher ($p < 0.05$) after MS meal consumption (Fig. 2). These differences among the three test meals were essentially the same in men and women.

Postprandial changes in serum TG and PL concentrations

The serum TG concentration decreased after consumption of the CON meal as compared with the fasting state ($p < 0.01$). However, after both the OIL and the MS

Fig. 4. IAUCs of serum TG and PL concentrations. White bar, CON meal; gray bar, OIL meal; black bar, MS meal. Covariance analysis was applied for IAUC comparisons among the three test meals, and serum concentrations in the fasting state served as covariates. Means $\pm$ SE. Means with different letters are significantly different ($p < 0.05$). $n = 18$.

meal, TG concentrations were increased at 2 h, and then decreased to levels lower than the fasting state at 6 h. Postprandial changes were observed in the serum PL concentration, which increased and was higher at 4 h after OIL meal consumption than in the fasting state ($p < 0.05$). After the MS meal, the serum PL concentration was elevated at 4 h ($p < 0.05$) and 6 h ($p < 0.01$) as compared with the fasting state (Fig. 3). IAUCs of serum TG and PL concentrations were higher after the OIL meal than after the CON meal. However, after the MS meal there were no significant differences as compared with the CON meal (Fig. 4).

DISCUSSION

Our findings suggest that oil or mayonnaise intake with broccoli might contribute to raising the postprandial serum lutein/zeaxanthin concentration. Our results are similar to those reported by Unlu et al., who found that lutein absorption from salad increased with the addition of 24 g of avocado oil (16). In our study, the OIL and MS meals contained 14.5 and 11.5 g of fat, respectively, lower than the values in their study. Although the amount of fat in the MS meal was smaller than that in the OIL meal, the MS meal raised the postprandial serum lutein/zeaxanthin concentration as effectively as the OIL meal. However, as the reported ratio of lutein to zeaxanthin in human serum is approximately 3:1 (23), the changes in the serum lutein/zeaxanthin concentration measured in this study would presumably reflect lutein intake, since broccoli does not contain zeaxanthin.

Not only the lutein/zeaxanthin concentration, but also the β -carotene concentration increased after ingestion of broccoli with mayonnaise, as compared with CON meal consumption. Emulsification has been suggested to be effective in improving absorption of lipophilic components (24, 25). Our results suggest that carotenoids are more easily incorporated into an O/W emulsion of mayonnaise because of its amphiphilic property.

The major ingredient difference between oil and mayonnaise is the presence of egg yolk, which has an approximately 30% PL content, in the latter (26). In vitro, the amounts of zeaxanthin and β -carotene increased in PL-stabilized TG droplets as compared to the pure TG droplets (27). In an animal repeated dose experiment, plasma and liver lutein levels in rats fed mixed micelles containing PLs were higher than in those not fed PLs (28). Thus, the PLs in mayonnaise might play a key role in the absorption of carotenoids since PLs were among the components of micelles (29).

From the intestinal absorption aspect, uptake of carotenoids by the intestine may be influenced by the state of relative sufficiency (29). Day to day variations in serum lipids and carotenoids, as observed in previous studies (30–32), should be considered among the factors which affect postprandial concentrations. In this study, there were no significant differences in serum lutein/zeaxanthin or β -carotene concentrations among the three test meals. Moreover, lower serum concentrations of lutein/zeaxanthin and β -carotene were reportedly associated with male gender, and dietary carotenoid intake may differ between men and women (33). In our study, IAUCs of serum lutein/zeaxanthin and β -carotene concentrations did not differ between men and women when the serum concentration in the fasting state was taken into consideration.

Moreover, IAUCs of serum TG and PL concentrations showed no significant differences after consumption of the MS and CON meals, despite the IAUCs of serum lutein/zeaxanthin and β -carotene concentrations being higher after the MS meal than after the CON meal. These results suggest that mayonnaise increases serum lutein/zeaxanthin and β -carotene concentrations without postprandial hyperlipidemia.

β -Carotene contributes to an increased antioxidant capacity of blood (34). High fat (28 g) intake was reported to increase β -carotene absorption from vegetables (35). Our results suggest small amount (11.5 g of fat) of mayonnaise to be more effective in increasing the

postprandial serum β -carotene concentration, which is suspected to be beneficial for preventing lipid peroxidation.

We conducted this study to identify effective methods of increasing lutein concentrations in the elderly. Digestion and absorption of carotenoids are considered to be influenced by aging with impairments of gastrointestinal transit, lipase and bile salt secretion (36). Since our subjects were not elderly, further studies are needed to clarify effects on the absorption of lutein in the elderly population.

In conclusion, mayonnaise is suggested to contribute to increasing serum lutein/zeaxanthin and β -carotene concentrations when consumed with a vegetable rich in these carotenoids. To clarify the most effective method of obtaining lutein from lutein-rich vegetables with mayonnaise, further studies are required on the optimal consumption volume in the elderly.

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