Mayonnaise Contributes to Increasing Postprandial Serum β-Carotene Concentration through the Emulsifying Property of Egg Yolk in Rats and Humans

Sayaka TAKEDA¹, Mamoru KIMURA¹, Ranko MARUSHIMA¹, Ayako TAKEUCHI¹, Kazue TAKZAWA¹, Yuji OGINO¹, Yasunobu MASUDA¹, Masaaki KUNOU¹, Mineo HASEGAWA¹,² and Chizuko MARUYAMA¹

¹Research & Development Division, Kewpie Corporation, 5–13–1, Sumiyoshi-cho, Fuchu, Tokyo 180–0034, Japan
²National Institute of Agrobiological Sciences, 2–1–2 Kannondai, Tsukuba, Ibaraki 305–8602, Japan

Department of Food and Nutrition, Japan Women’s University, 2–8–1, Mejirodai, Bunkyo-ku, Tokyo 112–8681, Japan

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Summary We performed in vitro, animal, and human studies to clarify the effect of mayonnaise on β-carotene intake and its mechanism. In an artificial gastric juice model, we examined the transfer of β-carotene from grated carrot to mayonnaise or vegetable oil. Mayonnaise was more easily dispersed in artificial gastric juice than vegetable oil. The β-carotene concentration was greater in mayonnaise than vegetable oil. In rats, the postprandial serum β-carotene concentration in the mayonnaise group (β-carotene with mayonnaise) was higher than that in the control (β-carotene only) and vegetable oil (β-carotene with vegetable oil) groups. Continuous feeding of dietary β-carotene (14 d), employing mayonnaise or egg yolk, resulted in an increased accumulation of β-carotene in the liver. In a human study, diets were provided in the form of (1) carrot as a control (CON), (2) carrot juice (JU), (3) carrot with oil (OIL) and (4) carrot with mayonnaise (MS). Following collection of fasting blood samples, nine adult males consumed one of the four diets in random order. Fasting and postprandial changes in serum β-carotene were assessed at 2, 3, 4, 6 and 8 h following ingestion of each diet. The incremental areas under the curves of serum β-carotene concentration were higher following MS than following both CON and JU. In conclusion, we suggest that mayonnaise contributes to raising the serum β-carotene concentration when consumed with carrots rich in β-carotene, and that its mechanism is related to the emulsifying property of the egg yolk contained in mayonnaise.

Key Words mayonnaise, emulsification, egg yolk, β-carotene, carrot

A provitamin A β-carotene, which is abundant in green and yellow vegetables, is converted into retinol in the body as required. Retinol is essential for growth and differentiation of several types of cells and tissues. Recently, antioxidant, antitumor, and immunological effects of β-carotene were reported; thus β-carotene is expected to promote human health (1–3). In Japan, the intake of vegetables high in β-carotene is not adequate. Therefore, it is beneficial to find more effective ways to consume vegetables so that sufficient β-carotene is absorbed for healthy growth in children and maintenance of health.

It has been reported that β-carotene in cooked or processed vegetables is absorbed more efficiently than that in raw vegetables (4–6). Moreover, as β-carotene is a lipophilic nutrient, its absorption following vegetable consumption was shown to increase more significantly when associated with the concurrent ingestion of fat (7, 8). In a previous report, the presence of mayonnaise contributed to increased β-carotene absorption (9).

Mayonnaise comprises egg, vegetable oil and vinegar. Eggs are rich in protein, cholesterol, phospholipids, vitamins and minerals. Therefore, we believe that mayonnaise is a nutritious food, and it is efficacious in situations of malnutrition and/or retinol deficiency.

To clarify the effect of mayonnaise on β-carotene absorption and its mechanism, we performed an in vitro study to confirm the solubility of β-carotene as a lipophilic nutrient using artificial gastric juice. Following consumption of β-carotene with oil or mayonnaise, β-carotene concentrations in serum and liver were compared in an animal study. We also compared the postprandial serum β-carotene concentrations in adult males to reveal the most efficient means of consuming carrot, i.e., raw, juice, with oil or with mayonnaise, for enhancing β-carotene absorption.

MATERIALS AND METHODS

In vitro study. Mayonnaise was prepared by the Kewpie Corporation (Tokyo, Japan) and contained less than 1 μg/100 g β-carotene. Vegetable oil and pepsin were purchased from the Nissin Oillio Group, Ltd.
(Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. Mayonnaise (10 g, containing 7.5 g of vegetable oil) or 7.5 g vegetable oil with 10 g of grated carrot was stirred in 80 mL of artificial gastric juice (pH 2.2, 0.1% pepsin (10,000 units/mg) using a stirrer (100 rpm: Three-One Motor Heidon Type 600G; Shinto Scientific Co., Ltd., Tokyo, Japan) at 37˚C. Stirring times were 5, 10, 30 and 60 min. Two stirred samples were prepared for each test. After stirring, the vegetable oil and mayonnaise were separated from the stirred sample by centrifugation (1,000 g for 10 min). Water was added to the separated vegetable oil and mayonnaise, and impurities were removed by centrifugation (1,000 g for 10 min). The mayonnaise was frozen (−40˚C) and thawed (37˚C) to break down the emulsion. Then, the oil was separated from the mayonnaise by centrifugation (1,000 g for 10 min). Prepared samples were stored at −40˚C pending analysis.

Animal study for oral administration. Mayonnaise, which was prepared by the Kewpie Corporation, comprised 70% canola oil, 15% egg yolk, 13% vinegar, 1.8% salt and 0.2% spices. β-carotene and -cornstarch were purchased from Wako Pure Chemical Industries, Ltd. and the Oriental Yeast Co., Ltd. (Tokyo, Japan), respectively. β-carotene (2 mg/g) was dispersed in 5% β-cornstarch solution that had been prepared as a gel by heating. Male Wistar rats (8 wk; Japan SLC, Inc., Shizuoka, Japan) were bred in a temperature- (23˚C±1˚C) and humidity-controlled (50%±2%) room with a 12-h light cycle (light from 8:00 to 20:00) and allowed to acclimate for 7 d. After 12-h overnight fasting, 2 g/kg body weight (BW) of the β-carotene solution was orally administered to each rat. Subsequently, 4 g/kg BW of water (CON diet), 3 g/kg BW of canola oil (OIL diet; Nissin Oillio Group, Ltd.) and 4 g/kg BW of mayonnaise (MS diet) were administered. The amount of canola oil was equivalent to that of oil in mayonnaise. Following oral administration, blood samples were collected from the abdominal cava under general anaesthesia after 2, 4, 6 and 8 h. Serum was separated by centrifugation (1,000 × g for 30 min) at 4˚C and stored at −40˚C pending analysis.

Animal study for continuous feeding of dietary β-carotene. The mayonnaise and canola oil used were identical to those used for oral administration. Egg yolk lecithin (PL-30) was prepared by the Kewpie Corporation. Table 1 shows the composition of the test diets. β-carotene was dissolved in corn oil. Each diet contained 7.2 μg/g β-carotene, which is the same amount as that of the retinol activity equivalents for the AIN-93G formula (10). MS and OIL diets contained 5.0% of mayonnaise and 3.75% of canola oil, respectively. YO diet contained 3.5% of canola oil and 0.25% of egg yolk. The oil content in all diets was equal. Sodium cholate (0.25%) was blended with all test diets to increase fat absorption. Twenty-four male Wistar rats (4 wk; Japan SLC, Inc.) were acclimated for 7 d and were then split into four equal groups. Pair-fed rats were fed one of the test diets for 14 d. Following fasting for 12 h, blood and liver samples were collected under pentobarbital sodium anaesthesia. Blood was treated as mentioned above, while the liver samples were stored at −40˚C pending analysis.

In the present study, animal tests were conducted in accordance with the guidelines set for the care and use of laboratory animals by the Ministry of the Environment, Government of Japan.

β-carotene concentration measurements for in vitro and animal studies. The oil was separated from each in vitro sample and was dissolved in acetonitrile/tetrahydrofuran (12:5, v/v). β-carotene was extracted from the serum samples as per the procedure of Riso et al. (1). Ethanol (1.0 mL) containing 0.15% (w/v) of 2,6-t-butyl-4-methylphenol (5.0 μg/mL of echinenone was added as an internal standard) and 500 μL of distilled water was added to 500 μL of serum and mixed for 30 s. Then, 2.0 mL of hexane/ethyl acetate (9:1, v/v) was added to the solution. After mixing for 1 min, the upper phase was separated by centrifugation (1,000 × g for 10 min) at 4˚C. The same operation was then
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Human study. 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. Nine adult males, who were judged to be healthy based on regular medical check-ups, were recruited to participate in this randomised, crossover intervention study. The males were 24–44 y of age (31.3 ± 2.0 y) and their average body mass index (BMI) was 23.7 ± 2.9 kg/m². Written informed consent was obtained from all participants. The diets consisted of 100 g of grated carrot as the control diet (CON), 50 g of commercial carrot juice as JU diet, 100 g of carrot with 14 g of vegetable oil as OIL diet and 100 g of carrot with 15 g of mayonnaise as MS diet. The oil and egg yolk-type mayonnaise were prepared at the R & D Division of the Kewpie Corporation. The experimental vegetable oil comprised 85% canola oil and 15% soy oil. The mayonnaise comprised 70% experimental vegetable oil, 14% egg yolk, 13% vinegar and 3% other ingredients such as salt and spices. One spoonful of oil (14 g) or mayonnaise (15 g) was used as the dressing for OIL and MS diets, respectively, just prior to consumption. The nutrient contents of the test diets are shown in Table 2.

Table 2. Nutrient contents of the test diets.

|        | CON | JU | OIL | MS  |
|--------|-----|----|-----|-----|
| Weight |     |    |     |     |
| Carrot (g) | 100 | 0  | 100 | 100 |
| Carrot juice (g) | 0  | 50 | 0  | 0   |
| Vegetable oil¹ (g) | 0  | 0  | 14 | 0   |
| Mayonnaise² (g) | 0  | 0  | 0  | 15  |
| Energy (kcal) | 37 | 16 | 166 | 140 |
| Protein (g) | 0.6 | 0.4 | 0.6 | 1.0 |
| Fat (g) | 0.1 | 0.2 | 14.1 | 11.3 |
| Carbohydrate (g) | 9.0 | 3.1 | 9.0 | 9.0 |
| Sodium (mg) | 25 | 21 | 25 | 130 |
| Cholesterol (mg) | 0  | 0  | 26 |      |
| α-Carotene (µg) | 1,950 | 1,350 | 1,950 | 1,950 |
| β-Carotene (µg) | 2,650 | 2,500 | 2,650 | 2,650 |

¹ Fatty acid composition of experimental vegetable oil was as follows: 5.9% of palmitic acid, 0.2% of palmitoleic acid, 2.3% of stearic acid, 56.2% of oleic acid, 24.7% of linoleic acid, 7.0% of α-linolenic acid, 0.5% of arachidic acid, 1.1% of icosenoic acid, 0.3% of behenic acid, 0.1% of docosenoic acid, and 1.7% of others.
² Mayonnaise was composed of 70% experimental vegetable oil, 14% egg yolk, 13% vinegar, and 3% other ingredients such as salt and spices.

CON, control diet; JU, carrot juice diet; OIL, carrot with vegetable oil diet; MS, carrot with mayonnaise diet.

Statistical analysis. Results are expressed as means ±SE. Tukey’s test was applied for comparisons among the groups in both animal and human studies. If there were significant differences, the time courses of changes in serum β-carotene, TG and PL from the fasting state onward were assessed with repeated-measures ANOVA and were analysed with Dunnett’s test. Based on trapezoidal rules, the incremental areas under the curves (IAUCs) of serum β-carotene concentrations were calculated as the increase in response above baseline minus any drop below the baseline. 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

In vitro study

As shown in Fig. 1, mayonnaise was dispersed in artificial gastric juice following stirring. However, the mixture of vegetable oil and artificial gastric juice was separated into oil and water phases following stirring. The

repeated. The upper combined phase was considered to represent the β-carotene extract. The solvent was then removed by nitrogen gas. The residue was redissolved in acetonitrile/tetrahydrofuran (12 : 5, v/v). Liver tissue (0.5 g) was homogenised in saline solution (1 mL). Pyrogallol ethanol (5%, 15 mL) and potassium hydroxide (60%, 1.0 mL) were added to the liver homogenate. Following saponification (70˚C, 30 min) and cooling, 10 mL of 1% sodium chloride and 15 mL of hexane/ethyl acetate (9 : 1, v/v) were added. β-carotene was extracted from the liver with the same method used for serum. The solvent was removed by nitrogen gas and was re-dissolved in ethanol. High-performance liquid chromatography (HPLC) (Hitachi UV Detector L-7400 (453 nm); Hitachi High-Technologies Corporation, Tokyo, Japan) was used for measurement of β-carotene concentration. β-carotene for the in vitro and animal studies (serum) was separated in a packed ODS column (4×150 mm, #3056; Hitachi High-Technologies Corporation) at a flow rate of 1.0 mL/min. β-carotene for the animal study, i.e. liver, was separated on a packed ODS column (4×120 mm, WH-C18A; Hitachi High-Technologies Corporation) with a flow rate of 0.5 mL/min. The mobile phase was accomplished by acetonitrile/tetrahydrofuran (12 : 5, v/v).

Carotene contents were similar among the diets. Prior to each experimental day, the subjects fasted of a minimum of 12 h, the subjects consumed one of the diets. Blood samples were collected at 0, 2, 3, 4, 6 and 8 h following diet ingestion. The washout period between the tests was at least 7 d, during which time all the participants consumed their usual meals. The participants were instructed to avoid strenuous activity during the test period. The collected serum was immediately separated by centrifugation (1,200 ×g for 10 min) at room temperature, and stored at −20˚C pending analysis. Blood chemical analysis was conducted at the laboratory of SRL, Inc. (Tokyo, Japan). Total cholesterol, triglyceride (TG) and phospholipids (PL) were measured enzymatically. High-density lipoprotein cholesterol was measured by the direct method (12). β-carotene and retinol were measured by HPLC (13, 14). Retinol-binding protein was measured by nephelometry (15).
\( \beta \)-carotene concentration in each sample was higher when stirred with mayonnaise than with vegetable oil (Fig. 2).

**Postprandial changes in serum \( \beta \)-carotene concentration in rats**

Postprandial serum \( \beta \)-carotene concentration following consumption of the MS diet was higher at 6 h \((p<0.01)\) and 8 h \((p<0.01)\) than following consumption of the CON and OIL diets (Fig. 3).

**\( \beta \)-carotene concentration in rat liver following 14 d on test diet**

No significant differences were observed in the whole body and liver weight or in serum \( \beta \)-carotene concentration among the four test diets. In the liver, \( \beta \)-carotene concentration following consumption of the YO \((p<0.05)\) and MS \((p<0.05)\) diets was higher than the CON diet (Table 3).

**Postprandial changes in serum \( \beta \)-carotene concentrations in male adults**

There were no significant differences in fasting serum biochemical parameters or \( \beta \)-carotene-related parameters among the four groups (Table 4).

Retinol and retinol-binding protein did not change during the experimental period in any group (data not shown).

As shown in Fig. 4(a), there was no significant change in postprandial serum \( \beta \)-carotene concentration change following consumption of the CON diet when compared with the fasting state. There were no differences following consumption of the JU diet when compared with the CON diet. At 4 h \((p<0.05)\), the serum carotene concentration was higher after con-
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Table 3. Effect of test diets intake over 12 wk in rats.

|                | CON   | OIL   | YO    | MS    |
|----------------|-------|-------|-------|-------|
| Body weight (g) | 146.0 | 150.0 | 151.5 | 146.5 |
| Liver weight (g) | 6.3   | 6.8   | 6.8   | 6.6   |
| Serum β-carotene concentration (µg/L) | 7.9 | 6.5 | 7.7 | 9.4 |
| Liver β-carotene concentration (µg/g) | 2.8 | 3.4 | 4.1 | 4.1 |

Values are means±SE. CON, control diet; OIL, CON+vegetable oil; YO, CON+vegetable oil+egg yolk; MS, CON+mayonnaise. No significant differences were observed for body and liver weight, and serum β-carotene concentration among four test meals. Means with different letters are significantly different (p<0.05) by Tukey’s test. n=6.

Table 4. Serum biological data for fasting state in adult males.

|                | CON   | JU    | OIL   | MS    |
|----------------|-------|-------|-------|-------|
| β-Carotene (µg/dL) | 21.9 | 28.1  | 27.7  | 26.1  |
| Retinol (µg/dL)   | 52.4 | 51.7  | 51.4  | 53.1  |
| Retinol binding protein (µg/dL) | 3.8 | 3.9  | 3.9   | 3.9   |
| TG (mg/dL)       | 77±7 | 94±14 | 99±15 | 85±8  |
| PL (mg/dL)       | 205±7| 216±5 | 218±6 | 215±7 |
| HDL-C (mg/dL)    | 62±4 | 63±3  | 64±5  | 64±4  |
| TC (mg/dL)       | 187±9| 191±8 | 190±8 | 194±8 |

Values are means±SE. TG, triglyceride; PL, phospholipid; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. No significant differences were observed among four test diets. n=9.

Fig. 4. Effect of mayonnaise on serum β-carotene concentration in adult males. (a) Postprandial changes: values indicate the variables against the fasting state. ○, CON meal; ●, JU diet; ▲, OIL diet; ■, MS diet. Means±SE; *p<0.05 vs. fasting state by Dunnett’s test; means, which are represented by different letters, are significantly different (p<0.05) among the four test diets at 4 h and 8 h, according to Tukey’s test. n=9. (b) Incremental area under the curves (IAUCs) of serum β-carotene concentration in adult males: means±SE; means with different letters are significantly different (p<0.05) by Tukey’s test. n=9.

Consuming the OIL diet than after consuming the CON diet. At 4 h (p<0.01) and 8 h (p<0.01), the serum β-carotene concentration was significantly increased after consuming the MS diet than that after consuming the CON diet. The IAUCs of serum β-carotene concentration did not differ following consumption of the JU and OIL diets when compared with the CON diet. However, the IAUCs for β-carotene following consumption of the MS diet were higher than those following the CON and JU diets (p<0.05). There were no significant differences between the β-carotene IAUCs for the MS and OIL diets (Fig. 4(b)).

Postprandial changes in serum TG and PL concentrations in male adults

As shown in Fig. 5, the serum TG concentration decreased following consumption of the JU diet when compared with the fasting state (p<0.01). Following consumption of both the OIL and MS diets, TG concentration was higher than that following the JU diet at 3 h, and then decreased to a level lower than that of the
The postprandial PL concentration was higher at 4 h following the OIL diet \((p<0.05)\) than that following the CON diet. The postprandial PL concentration at 4 h following consumption of the MS diet did not differ from that following consumption of the OIL diet. Moreover, the serum PL concentration was higher at 6 h following the OIL diet than in the fasting state. Following consumption of the MS and OIL diets, the peak serum TG concentration was seen at 3 h while the serum PL concentration peaked from 4 to 6 h.

**DISCUSSION**

Our findings suggest that the combination of mayonnaise and carrot may be an effective way to raise the postprandial serum \(\beta\)-carotene concentration in humans. This agrees with our previous study, showing that mayonnaise may be beneficial for increasing the absorption of \(\beta\)-carotene and lutein in broccoli \((9)\).

\(\beta\)-carotene in food is solubilised in the stomach via formation of chyme with digested food. Following solubilisation, \(\beta\)-carotene is absorbed in the upper part of the small intestine. In the present study, we compared the level of dissolution between mayonnaise and vegetable oil using an artificial gastric juice model. We found that mayonnaise eluted \(\beta\)-carotene more efficiently from grated carrot than vegetable oil. In an O/W emulsion like mayonnaise, hydrophilic groups are found externally while lipophilic groups are found internally. Therefore, dispersal of mayonnaise occurs easily in the water phase, and thus, dispersion is uniform in artificial gastric juice.

Based on the high \(\beta\)-carotene concentration of postprandial serum in rats, the MS diet appears to enhance \(\beta\)-carotene absorption more efficiently than the OIL diet. In addition, continuous intake of dietary \(\beta\)-carotene with the mayonnaise or egg yolk diet resulted in accumulation of \(\beta\)-carotene in the liver. However, we did not examine the concentration of retinol in serum and liver in the animal study as it was surmised that mayonnaise and egg yolk would contribute to an increase in \(\beta\)-carotene absorption.

A key ingredient of mayonnaise, egg yolk contains approximately 7% lecithin \((16)\), which includes phosphatidylcholine. Lyso phosphatidylcholine was shown to increase the absorption of \(\beta\)-carotene using a Caco-2 cell model \((17)\). High \(\beta\)-carotene concentrations in the serum and liver were reported following lyso phosphatidylcholine intake in rats \((18)\). Following ingestion, phosphatidylcholine is converted to lyso phosphatidylcholine by phospholipase \(A_2\). Lyso phosphatidylcholine is then absorbed in the small intestine. Therefore, we speculate that lecithin in the egg yolk in mayonnaise contributes to enhanced \(\beta\)-carotene absorption and accumulation in the liver through emulsification. Further studies are required to clarify the effect of egg yolk lecithin on \(\beta\)-carotene absorption.

Postprandial serum \(\beta\)-carotene concentrations in rats were higher after consumption of the MS diet than after the OIL diet. However, no significant difference was observed between these concentrations in human subjects following consumption of these two diets. One of the reasons for the difference between the animal and human studies may be the smaller volume of ingested mayonnaise \((15 \text{ g} = 0.2 \text{ g/kg BW})\) by the human participants than by rats \((2.0 \text{ g/kg BW})\). Furthermore, the lower fat content of the MS diet \((11.2 \text{ g})\) compared to the OIL diet \((14.0 \text{ g})\) may reduce the effect of mayonnaise. The effects of large-volume or long-term mayonnaise intake with vegetables rich in \(\beta\)-carotene await future investigations.

Consuming extracted juice may ease nutrient intake from vegetables. However, no increase in \(\beta\)-carotene absorption was observed with either the JU or CON diets in the present study \((Fig. 4(b))\). Therefore, we suggest that only juice without fat may not effectively enhance \(\beta\)-carotene intake.

Generally, no symptoms of excessive \(\beta\)-carotene are known, because it is transformed into retinol when necessary. However, a high intake of a \(\beta\)-carotene supplement was found to be harmful in some clinical trials \((19–22)\). Therefore, \(\beta\)-carotene should be consumed...
habitually with meals.

All participants in the present study were adult males. The health function of \( \beta \)-carotene can be expected in various subjects. Thus, further studies are needed in various age groups, females and under different health conditions to clarify the effects of mayonnaise on \( \beta \)-carotene absorption and its safety.

In conclusion, mayonnaise enhances \( \beta \)-carotene absorption from vegetables in rats and adult human males. We suggest that the underlying mechanism involves the emulsifying property of egg yolk in mayonnaise.

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