

Accumulation and Excretion of Carotenoids after Regular Ingestion of Carrot Juice with a Lycopene-Free Diet

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The relationship between the body accumulation and excretion of β -carotene and lycopene was elucidated. A lycopene-free diet was fed to seven healthy women's university researchers and students, and the effect of ingesting β -carotene via carrot juice was evaluated. The serum β -carotene level was increased as a result of carrot juice ingestion, while the serum lycopene level was decreased as a result of lycopene restriction. It was assumed that the serum carotenoid level reflected daily life-styles and eating habits. A fecal analysis showed that the β -carotene level was increased as a result of carrot juice ingestion. This result suggests that residual β -carotene in the carrot juice that had not been absorbed in the small intestinal tract was excreted in the feces. The lycopene excretion level was increased under the same conditions. Judging from the fact that the dietary lycopene level had been restricted, it is proposed that carrot juice ingestion was related to the increased level of lycopene in the feces via an interactive effect between the ingestion of β -carotene and the excretion of lycopene.

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INTRODUCTION

It is known that carotenoids are synthesized in plants and microorganisms, and that they constitute approximately 600 derivatives. These compounds are derived from phytoene,¹⁾ as their metabolic starting material, through such reactions as unsaturation, intramolecular cyclization and hydroxylation.²⁾⁻⁴⁾ In addition to their role as provitamin A, several *in vivo* reactions of carotenoids, such as antioxidation, inhibition of cancer cell growth and regulation of cholesterol biosynthesis, have recently been elucidated. Various studies including *in vivo* distribution and the effects on diseases have thus been conducted to further investigate the functions of carotenoids.⁵⁾

Mammals cannot synthesize carotenoids *in vivo*; therefore, carotenoids in mammalian systems are derived from plants and microorganisms ingested as food.⁶⁾ It has been reported that the serum carotenoid levels in humans increased after carrot juice ingestion,⁷⁾⁸⁾ and that the plasma carotenoid levels decreased with a low-carotenoid diet.⁹⁾¹⁰⁾ Thus, it is obvious that the level of carotenoids in the human body is largely affected by diet. Although there have been many reports concerning the absorption and accumulation of carotenoids following ingestion, there

have only been a few presented on the excretion of carotenoids that were metabolized by enterobacteria in the intestinal tract.¹¹⁾¹²⁾ Our previous human study¹³⁾ has reported that 14 days ingestion of an ordinary diet supplemented with carrot juice resulted in an increased lycopene level in the feces; although there was the possibility that this increase was brought on by the intake of such lycopene-containing vegetables as tomatoes, the mechanism for this lycopene increase has not been elucidated.

In order to investigate whether or not an increased level of β -carotene ingestion with a lycopene-free diet would influence the body accumulation and excretion of lycopene, the concentrations of β -carotene and lycopene in the serum and feces before and after ingesting carrot juice were measured in this study.

MATERIALS AND METHODS

Ingestion test

1. Subjects

Seven subjects comprising students, research associates and instructors belonging to Showa Women's University were selected. The gender, age, height, weight and BMI of each subject are shown in Table 1. The subjects had no smoking or drinking habits, and neither smoking nor drinking was permitted during

Table 1. Characteristics of the subjects

Sex	Female
Number	7
Age (years)	24.6±6.1
Weight (kg)	47.4±4.1
Height (cm)	154.4±6.1
BMI	20.0±3.0

Each value is the mean±SD.

Table 2. Nutrient content of the carrot juice

Nutrient	Carrot juice	
	one can (160 g)	100 g
Energy (kcal)	78	49
Protein (g)	1	0.6
Crude fat (g)	trace	trace
Carbohydrate (g)	11	6.9
Calcium (mg)	24	15
Sodium (mg)	29	18
Potassium (mg)	460	288
Dietary fiber (g)	0.3	0.2
β -Carotene (mg)	4.6	2.9
Lycopene (mg)	N.D.	N.D.

N.D.: not detected.

the test.

2. Ingestion plan

The carrot juice used in this study was a commercially available canned brand (Carrot Juice, 160 g; Kagome Co. Ltd.). The β -carotene content was 4.6 mg per can according to the ingredients label shown in Table 2. An HPLC analysis showed that the β -carotene level per can was 4.7 ± 0.1 mg, and that the lycopene level was less than the HPLC detection limit. A lycopene-free diet period of 2 weeks was selected for this carrot juice-loading experiment. The subjects were obliged to take the special lycopene-free diet throughout this period, the first week being designated as the preliminary period. During the second week, each subject had to ingest two cans of carrot juice per day, one can each at breakfast and supper to give a β -carotene intake of 9,200 μ g/day, this period being designated as the loading period. The ingestion of any vegetables or fruit having a red color, such as tomatoes, processed tomato foods (tomato juice, ketchup, tomato puree, pizza sauce, canned tomatoes, etc.), watermelons and pink grapefruit, was forbidden throughout the experimental period. Specially

prepared meals were designated during the period,¹⁴⁾ the nutritional content of the diet per day being 1,830 kcal in total energy, 250 g of carbohydrates, 70 g of proteins, 60 g of lipids, and 800 μ g of β -carotene (508 μ g retinol equivalent), with lycopene at less than the detection limit. Each subject was required to collect and submit blood and feces samples at four prescribed times: as shown in Fig 1, one day before the preliminary period (I), one day before the loading period (II), one day after the loading period (III) and four weeks after the loading period (IV). Blood was collected by medical personnel.

3. Observance of ethical principles

Each subject was fully briefed regarding the purpose of this study, and notified that they could opt out and discontinue participation at any time. Written agreement with each subject was obtained. The protocol for this study was based on the ethical principles for medical research involving human subjects of the World Medical Association Declaration of Helsinki. This study was conducted with the approval of the ethical committee of Showa Women's University (approval number 99-03).

Serum measurement

1. Preparation of serum

Whole blood samples were left to stand for one hour at room temperature after collection, and were centrifuged at 3,000 rpm for 10 min in order to obtain serum from the supernatant. Each serum sample was divided into aliquots, kept in the dark, rapidly frozen at -80°C and stored at -20°C until needed for analysis.

2. Analysis of carotenoids in the serum

Carotenoids were extracted from the serum samples according to the method of Sakamoto *et al.*,¹⁵⁾ using ethanol and hexane. The Shimadzu HPLC system used in this study consisted of an LC-6A pump unit, an SCL-6B system controller, an SPD-6AV UV-Vis spectrophotometric detector, a CR-3A Chromatopac, an RF-535 HPLC fluorescence monitoring unit, and a C-R1A Chromatopac with an Inertsil ODS-2 column (ϕ 6.0×150 mm, GL Sciences). The HPLC analysis was performed with acetonitrile: ethanol (3:2, v/v) at a flow rate of 2 ml/min at 20°C . Visible absorption detection of the carotenoids was performed at 450 nm.

The detected peaks were identified by comparing the retention times with standards. The external standard method was used for quantitative calculation, using xanthophylls, β -carotene, lycopene (Sigma) and β -apo-8'-carotenal (Fluka Biochemika). Serum

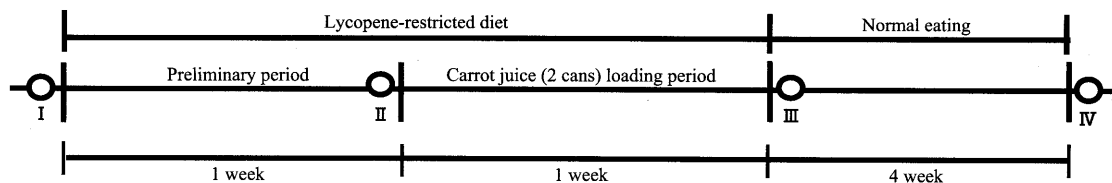


Fig. 1. Experimental design

Each subject was required to collect blood and submit feces samples at set times. Set times: I, one day before the preliminary period; II, one day before the loading period; III, one day after the loading period; IV, 4 weeks after the loading period. Blood was collected by a doctor. Fecal samples were collected in plastic bags, and were immediately placed in laminated bags containing a deoxygenating reagent. The samples were stored under anaerobic refrigerated conditions and submitted for analysis within 24 h of collection.

concentrations were determined by using curves for each standard.

All experiments were performed at a constant temperature in a humidified chamber with low lighting at a temperature of 20°C and a humidity of 55%.

Feces measurement

1. Collection of feces

Fecal samples were collected in plastic bags, and were immediately placed in laminated bags containing a deoxygenating reagent. Each sample was stored under anaerobic refrigerated conditions and submitted within 24 hours of collection. The total weight and pH value were measured, aliquots (5 g) were taken for water content and carotenoid measurements, each being rapidly frozen in the dark at -80°C under nitrogen and stored at -20°C until needed for analysis.

2. Analysis of carotenoids in the feces

A frozen fecal sample was thawed at room temperature. Carotenoids were extracted by using acetone:methanol (1:1, v/v) and then hexane, as described previously.¹³⁾ The extracted solid material was stored at -20°C under nitrogen until needed for an HPLC analysis under the same analytical conditions as those described for the abovementioned serum analysis.

3. Fecal moisture (%)

The fecal moisture content (%) was measured by using the vacuum drying method.¹⁶⁾

Statistical analysis

Each data value is presented as the mean \pm standard deviation. A significance test for each measurement was performed by a paired *t*-test, and statistical significance was set at $p < 0.05$. The statistical software package used in this study was StatView Ver. 5.0 (SAS Institute).

RESULTS

Analysis of carotenoids in the serum

Changes in the serum carotenoid levels over the sampling period are shown in Table 3.

There was no change in the β -carotene level during the preliminary period, but the level was doubled from one day before to one day after the loading period by the ingestion of carrot juice. The level 4 weeks after the loading period had decreased to the same level as that of the before loading period. The lycopene level was significantly lowered (from 82.85 $\mu\text{g}/\text{dl}$ to 56.23 $\mu\text{g}/\text{dl}$) during the 2-week lycopene-free diet period, but had recovered to the same level as that before the loading period after 4 weeks.

Analysis of carotenoids in the feces

There was a great difference in fecal weight according to the individual. However, there was no difference in the individual's fecal weight between each collecting day. Accordingly, the weight, water content, and pH value of an individual's fecal samples did not vary throughout the test period (Table 4).

The results of the carotenoid analysis are shown in Table 5. The level of β -carotene was significantly lowered (from 27.66 $\mu\text{g}/\text{g}$ to 8.71 $\mu\text{g}/\text{g}$) in samples from before the preliminary period to before the loading period, while the level one day after the loading period had started was 73.84 $\mu\text{g}/\text{g}$, which was significantly higher than that of the preceding samples. The level of lycopene obtained after the preliminary period was significantly reduced to approximately one-fifth of that on the day before this period had started. However, the lycopene level after the juice ingestion period was 10.14 $\mu\text{g}/\text{g}$, which was significantly higher than the 3.43 $\mu\text{g}/\text{g}$ observed on the day after the lycopene-free diet period. The level 4 weeks after the juice ingestion period ended did not significantly

Table 3. Serum carotenoid level at each collection time

Carotenoid ($\mu\text{g}/\text{dl}$)	I	II	III	IV
β -Carotene	53.07 \pm 9.38 ^a	52.10 \pm 11.75 ^a	97.76 \pm 22.14 ^b	58.14 \pm 13.93 ^a
Lycopene	82.85 \pm 44.50 ^a	70.50 \pm 24.28 ^{a,b}	56.23 \pm 12.56 ^b	82.02 \pm 20.31 ^a

Each value is the mean \pm SD. Collection times: I, one day before the preliminary period; II, one day before the loading period; III, one day after the loading period; IV, 4 weeks after the loading period. ^{a,b}Values bearing different letters are significantly different at $p<0.05$.

Table 4. Fecal measurements at each collection time

	I	II	III	IV
Fecal weight (g)	100.1 \pm 70.4	111.8 \pm 58.0	103.4 \pm 66.2	113.1 \pm 89.5
Fecal moisture (%)	70.0 \pm 13.2	68.7 \pm 9.4	67.4 \pm 5.8	68.2 \pm 9.7
Fecal pH	7.1 \pm 0.3	7.5 \pm 0.1	7.6 \pm 0.1	7.3 \pm 0.3

Each value is the mean \pm SD. Collection times: I, one day before the preliminary period; II, one day before the loading period; III, one day after the loading period; IV, 4 weeks after the loading period. No significant difference was apparent between each collection time.

Table 5. Fecal carotenoid level at each collection time

Carotenoid ($\mu\text{g}/\text{g}$)	I	II	III	IV
β -Carotene	27.66 \pm 20.66 ^a	8.71 \pm 3.95 ^b	73.84 \pm 46.23 ^c	10.38 \pm 7.58 ^b
Lycopene	16.58 \pm 13.06 ^a	3.43 \pm 3.77 ^b	10.14 \pm 3.40 ^a	13.43 \pm 8.37 ^a

Each value is the mean \pm SD. Collection times: I, one day before the preliminary period; II, one day before the loading period; III, one day after the loading period; IV, 4 weeks after the loading period. ^{a,b,c} Values bearing different letters are significantly different at $p<0.05$.

differ from the level on the day before the test period started.

DISCUSSION

There have been several contradictory reports regarding the interaction between β -carotene and lycopene. An incorporation test using colon cancer carcinoma-derived Caco-2 cells has indicated that their absorption levels decreased when combined.¹⁷⁾ In contrast, it has also been reported that β -carotene increased the absorption level of lycopene,¹⁸⁾ and that lycopene had no obvious influence on the absorption of β -carotene.¹⁹⁾ The mechanisms for the absorption and accumulation of β -carotene and lycopene are not yet understood. Therefore, in order to elucidate the relationship between the body accumulation and excretion of β -carotene and lycopene resulting from β -carotene ingestion, a carrot juice ingestion test was conducted in conjunction with a lycopene-free diet in order to minimize the influence of diet-derived lycopene.

The serum analysis showed almost no difference

between the serum β -carotene level prior to the test period and that after the preliminary diet period; thus, the β -carotene level is considered not to have been affected by restricting the lycopene ingestion. The level was significantly higher after the carrot juice ingestion period (by approximately 1.9 times) when compared with that before juice ingestion, which is consistent with previous reports by Kim *et al.*⁷⁾ and Sakamoto *et al.*⁸⁾ It is thus indicated that the accumulation of β -carotene was increased by the ingestion of carrot juice.

The increased level of β -carotene was largely due to the fact that the ingested carrot was in the form of juice. In the process of juice production, β -carotene, which is located in such organelles as chloroplasts and chromoplasts, is exposed to the liquid phase by the mechanical destruction of vegetable tissues. Thus, the incorporation of β -carotene into complex micelles in the small intestine after ingestion becomes easier, and its absorption into mucosal epithelial cells of the small intestine is improved. It is also possible that ingested lipids and proteins helped the absorption of β -

carotene, as the carrot juice was consumed with meals.²⁰⁾⁻²²⁾

The level of β -carotene decreased to the starting level within 4 weeks after completing juice ingestion; it was thus assumed that the initial serum β -carotene level reflected the everyday lifestyle and eating habit. The serum lycopene level was decreased as a result of the lycopene restriction, this being consistent with reports by Rock *et al.*⁹⁾ and Burri *et al.*¹⁰⁾ This indicates that the decreased ingestion of lycopene influenced its accumulation. Although the amount of β -carotene ingested from carrot juice was increased, no effect on the serum lycopene level was apparent.

The fecal analysis showed that the β -carotene level one week after the preliminary period was significantly less than that one day before the same period. Since there was a little β -carotene included in the lycopene-free diet, it is assumed that β -carotene was fully absorbed during the period of lycopene-free diet consumption. The average β -carotene content in the lycopene-free diet was 790 $\mu\text{g}/\text{day}$, which is lower than the suggested amount of carotenoid ingestion for a healthy person (about 1,500 $\mu\text{g}/\text{day}$, Schabath *et al.*²³⁾; 2,100 $\mu\text{g}/\text{day}$, Kinoshita *et al.*²⁴⁾; carotenoid equivalent of 3,500 $\mu\text{g}/\text{day}$, Japan National Nutrition Survey²⁵⁾). Therefore, it is assumed that the ingested β -carotene had been completely absorbed to maintain the serum level, thus resulting in a reduced excretion level. Carrot juice ingestion supplied β -carotene at approximately, 9,200 $\mu\text{g}/\text{day}$, which was about eleven times greater than that in the lycopene-free diet period. Since increased serum β -carotene and β -carotene excretion levels were observed, it is assumed that residual β -carotene in the carrot juice that had not been absorbed in the small intestine was excreted in the feces.

Comparing the lycopene level in the feces at the start with that during the last days of the preliminary period, the latter was significantly lower. We assumed at first that the excretion level of lycopene would be reduced, even after the carrot juice ingestion period, as the ingested lycopene was nearly zero. However, following carrot juice loading, this excretion level recovered and reached 3-fold the earlier level. It has been suggested in a previous study that the increased level of lycopene in the feces after the carrot juice ingestion period would have been due to food-derived lycopene, as there was no dietary restriction. Because of the dietary restriction of the lycopene-free diet in this present study, it is suggested that carrot juice ingestion was related to the increased level of lycopene

in the feces. Considering the facts that the carrot juice contained no lycopene, that we prepared the meals for the subjects, and that there were no reports of the subjects ingesting any food items containing lycopene, it is likely that the excreted lycopene was not derived from the carrot juice or food.

The results suggested interaction between the increased ingestion of β -carotene and the excreted lycopene. We have two hypotheses to explain these results:

1. Although β -carotene and lycopene have similar structures, the former possesses provitamin activity and the latter does not. Therefore, β -carotene may be preferentially utilized upon absorption and the body may excrete the accumulated lycopene into the feces.

2. The synthesis pathway for lycopene from β -carotene has not been identified. However, there are various reactions in the human body, such as redox reactions and reactions that involve enterobacteria, and it is thus possible that lycopene was synthesized from β -carotene through a ring-opening reaction. If lycopene can be synthesized in the stomach or small intestine, it will be absorbed from the small intestine, and the serum lycopene level may be increased. However, in this study, the serum lycopene level was lowered when β -carotene was administered. It is therefore possible that lycopene may have been synthesized in the colon.

Further investigation by animal and *in vitro* experiments is required to elucidate how β -carotene and lycopene interact *in vivo*. This study provides important preliminary data on the relationships between the ingestion, accumulation and excretion of carotenoids, and on the effects of ingested carotenoids on carotenoid accumulation.

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リコペンを制限した食事条件下でのニンジンジュース連続飲用による
カロテノイドの体内蓄積と排泄

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本報は、 β -カロテン投与による β -カロテンとリコペンの体内蓄積および排泄の関係を明確にするため、食事由来リコペンの影響を避け、リコペンを含まない食事条件においてニンジンジュース飲用試験を行った。血清 β -カロテンは、ニンジンジュース飲用で有意に増加した。一方血清リコペンはリコペン制限により減少傾向を示した。これらより血清カロテノイドレベルは、日常の食生活や食習慣が大きく影響していることが示唆された。ニンジンジュース飲用で β -カロテンの糞便中への排泄量が増加した。この結果はジュース中に含まれる β -カロテンのうち小腸より吸収されなかった残渣が、糞便中に排泄されたことを示唆している。リコペン排泄量は、ニンジンジュース飲用で増加した。本試験はリコペンを制限して実施していることから、ニンジンジュース飲用は、糞便中リコペンレベルの増加に関与すると推測され、また、摂取 β -カロテンと排泄リコペン量の増加には相互作用の存在が推測された。

キーワード: β -カロテン, リコペン, 蓄積, 排泄, リコペンフリー食。