〈Brief Note〉

Effects of continuous vegetable juice intake on blood β-carotene levels

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Summary Interindividual variability in the absorption of dietary carotenoids exists, with "low responders" unable to efficiently absorb carotene. This study aimed to clarify the effect of prolonged vegetable juice intake on blood β-carotene levels in healthy individuals. Forty-two research participants in a double-blinded study were equally randomized into 2 groups: (1) a vegetable juice group or (2) a placebo group who received a heavily diluted vegetable juice containing artificial flavor to simulate the test vegetable juice. The participants consumed the drinks in the morning for 12 weeks. Individual blood sampling was conducted at 5 pm once every 4 weeks for 12 weeks to measure the β-carotene levels. In the vegetable juice group, the β-carotene levels increased significantly. In all 3 suspected carotenoid low responders, the β-carotene levels increased to within the normal range. On the other hands, in placebo group, the levels almost unchanged. Our findings indicate that daily intake of vegetable juice is sufficient to fortify β-carotene levels in carotenoid low responders.

Key words: Vegetable juice, Routine intake, β-Carotene

1. Introduction

Intake of green and yellow vegetables in Japan remains lower than that recommended by the Japanese Ministry of Health, Labor, and Welfare[1]. Green and yellow vegetables are rich in carotenoids, such as β-Carotene, and vitamin C, minerals, and dietary fiber. Moreover, in Japanese, about 56% the vitamin-A in diet comes from green and yellow vegetables. Carotenoids are lipid microconstituents found mainly in foods of plant origin. They can be divided into provitamin A and non-provitamin A carotenoids. The main provitamin A carotenoids are

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β-carotene, α-carotene, and β-cryptoxanthin. These carotenoids possess potential vitamin A activity, as a source of retinol. There is no recommended dietary allowances (RDA) for β-carotene specifically, but Biesalski et al[2] reported that assumed optional plasma concentrations of β-carotene is >0.4 μmol/L (>21.4 μg/dL) and the corresponding daily intake is about 2-4 mg. Rich in vitamins and carotenoids, such as β-carotene and vitamin C, these vegetables have been reported to have diverse benefits for human health when taken in the juice rather than the raw form. Fan et al[3] reported the antioxidant effect of vegetable juice, whilst Yamaguchi et al[4] noted the effect of β-cryptoxanthin-reinforced juice on bone formation. Hironaka et al[5] reported that plant sterol-enriched vegetable juice affects serum cholesterol concentration, and we have previously reported improvements effected by vegetable juice intake on depression and anxiety[6]. Additionally, Williams et al[7] reported that vegetable juice reduced systemic inflammation, whilst Kobayashi et al[8] discovered that vegetable/fruit juice fortified with carrot puree shortened mean colonic transit times.

Until recently, it was assumed that carotenoids, being fat soluble, simply followed lipids from the lipid droplets present in the gastrointestinal lumen during digestion to the intracellular lipid droplets where they are stored. More precisely, it was assumed that they were first transferred from the food matrix, where they were embedded, in the fat phase of meal, and then transferred to mixed micelles during triglyceride lipolysis by pancreatic lipase, before being absorbed by passive diffusion, incorporated into chylomicrons and distributed to tissues together with triglycerides and cholesterol. However, the reality is more complex because several proteins are involved, or are suspected of being involved, at different stages in this pathway. First, it is assumed that a digestive enzyme is involved in the hydrolysis of the carotenoids that are esterified. Second, it has been suggested that as observed for cholesterol, apical membrane proteins of enterocyte are involved in the uptake of carotenoids. Finally, proteins are thought to be involved in the intracellular transport of carotenoids across the water environment, i.e. cytosol, of the cells, etc.

β-Carotene is one of the most important antioxidant carotenoids in the human diet; yet, as shown in most clinical studies dedicated to carotenoids, a huge inter-individual variability exists in absorption and blood and tissue responses. Genetic diversity within carotenoid metabolic proteins could be responsible for this[9] as a genome-wide-association study[10] revealed that polymorphisms of retinol-binding protein and transthyretin affect vitamin A status. In addition, absorption may be affected by protein, fat, and dietary fiber intakes[11]. Furthermore, we discovered that pureed carrot is more efficient than boiled carrot for carotenoid absorption, suggesting that preparation is also important (unpublished data).

However, the effect of vegetable juice intake on carotene levels with respect to inter-individual variability in absorption and juice intake in low responders remains still unclear.

We therefore sought to clarify the effect of vegetable juice on β-carotene levels in healthy individuals by using a placebo-controlled vegetable juice study.

2. Methods

Research participants

A total of 42 volunteers (8 men and 34 women, aged 18–56 years; average age, 32.8 ± 11.3 years), who were judged as healthy with no abnormal liver, kidney and lipid-digestion-absorption functions as assessed by standard biochemical data were recruited for this study. At entry, all participants provided written informed consent to participate in this study. They were then randomly divided into 2 groups. The data were collected from September through November 2015 and analyzed in December 2015.

The study protocol was approved by the ethics committee of the Institute of Medicine, University of Tsukuba (no. 836). All methods were carried out according to the relevant guidelines and regulations.

Interventions

The intervention involved 2 kinds of vegetable juice: “Ichinichibun no Yasai,” which is rich in carrot and tomato concentrate (ITO En, Tokyo, Japan) and a placebo that contained one-twentieth the vegetable amount of “Ichinichibun no Yasai” and contained added flavors to simulate the test vegetable juice taste. The participants were sorted into the 2 groups of this double-blinded study and instructed to consume their assigned drink (200 mL/day) at every breakfast for 12 weeks. The nutrient contents of the juices are summarized in Table 1. Plain vegetable juice (200 mL) contains 10 mg of β-carotene per daily dosage. Ordinary supplements contain 7.9 mg per day for β-carotene. It has been proposed that carotenoids and retinoids are agents that may prevent lung cancer and cardiovascular disease. Omenn et al[13] reported after an average of 4 years of supplementation, the combination of 30 mg of β-carotene and 25,000 IU of retinol had no benefit and may have had an adverse effect on the incidence of lung cancer (a relative risk of lung cancer of 1.28). Thus, this study seemed to have no potential side effects.

Measurements of nutrients in blood

Peripheral venous blood samples were collected into tubes containing a serum separator gel at around 5 pm once a week for 4 weeks. β-carotene was determined using high-performance liquid chromatography (LSI Medience, Japan). The reference intervals for β-carotene is 6.6-105.2 μg/dL.

Statistical analysis

Among 42 applicants, 3 participants cancelled before the first blood collection, and 2 participants cancelled later on in the study. Data from the 39 individuals who participated in the first blood collection and psychological testing were analyzed. The

Table 1. Nutrient contents (per 200 mL) of the vegetable juices used in this study

Contents	**Plain vegetable juice**	**Placebo**
Energy	75 kcal	90 kcal
Protein	1.9 g	0.2 g
Lipid	0.2 g	0.2 g
Carbohydrate	14.6 g	21.7 g
Fiber	3.3 g	1.9 g
Na	103.2 mg	6.5 mg
Ca	142.3 mg	110.9 mg
Fe	0.5 mg	0 mg
Mg	58.6 mg	32.5 mg
K	648.1 mg	52.9 mg
Zn	0.3 mg	0.04 mg
Vitamin A	1100 μg	35 μg
β carotene*	**10,274 μg**	431 μg
Vitamin E	2.3 mg	0.2 mg
Folic acid	48 μg	0.5μg
Vitamin C	136.3 mg	132.3 mg
Sucrose	6.5 g	0.2 g
Lycopene	13.8 mg	0.8 mg
NaCl	262 mg	16.4 mg

*Bielski et al2 reported that the recommended daily intake of β-carotene from foodstuffs is 2-4 mg to optimize the plasma levels.

results were expressed as means ± standard deviations (SDs). The *t* test was used to compare the mean values of the differences between the baseline and 12-week scores in the vegetable juice and placebo groups. Analysis of variance (ANOVA) and Dunnett test were used to compare score changes within the groups. Statflex (version 6) software was used for all the statistical analyses.

3. Results

Baseline data

As shown in Table 2, the 2 groups did not differ significantly in terms of the mean values of age, and β-carotene. The β-carotene levels were widely distributed (3–145 μg/dL). Four participants were extremely low in β-carotene level.

Changes in blood β-carotene

As shown in Figure 1, the mean β-carotene levels were significantly and time-dependently ($p < 0.01$) increased in the vegetable juice group, whilst the placebo group saw a slight decrease. In the vegetable juice group, the mean β-carotene levels rose over 12 weeks, from 30 to 128 μg/dL (5-fold higher), and the mean increase in the β-carotene levels was 98 μg/dL. In the placebo group, the mean β-carotene levels decreased over 12 weeks, from 42 to 39 μg/

Table 2. Baseline characteristics of vegetable juice group and placebo group

	Vegetable juice group	Placebo group	Difference	p value
Participants	19	20		
Sex, M/F	4 / 15	4 / 16		
Age (yrs)	32.5 ± 12.0	33.8 ± 11.7	-1.3 ± 11.8	0.73
	(18 - 52)	(20 - 55)		
β-Carotene	30.3 ± 18.0	41.7 ± 33.5	-11.4 ± 27.1	0.2
(μg/dL)	(3.0 - 49.5)	(8.4 - 145.1)		

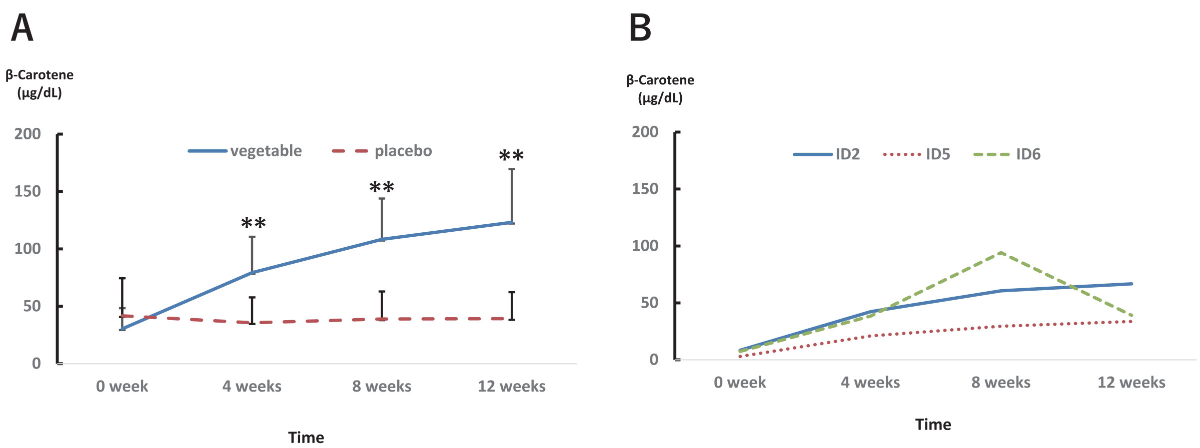

Fig. 1 Effects of juice intake on blood β-carotene concentrations (μg/dL).
(A): The mean values of β-carotene levels in the vegetable juice group (n=18) and the placebo group (n=19). (B): The values of the suspected carotenoid low responders (ID2, 5,6). The data are expressed as means ± standard deviations (SDs). **: p<0.01

dL. The levels of 3 low responders are also shown in Figure 1. The levels were time-dependently increased, but the increases were much below the mean levels.

During this study 2 participants cancelled spontaneously without any side effects, thus, Table 3

Table 3. Changes in β-carotene levels in the vegetable juice group and the placebo group

ID	sex	age	β carotene μg/dL							
Vegetable juice			0 week	4 weeks	8 weeks	12 weeks	Change	Change %	Mean	CV%
1	Female	34	42.0	103.7	95.3	153.5	111.5	365	98.6	46.3
＊2	Female	48	**8.4**	42.3	60.6	66.7	58.3	794	44.5	58.9
3	Female	41	17.6	78.4	102.3	120.4	102.8	684	79.7	56.3
4	Female	24	24.6	68.3	114.8	128.9	104.3	524	84.2	56.3
＊5	Male	24	**<3.0**	21	29.5	33.7	30.7	1123	21.8	62.4
＊6	Female	20	**7.4**	38.4	94.1	39.1	31.7	528	44.8	80.6
7	Female	27	34.8	cancelled	-					
8	Male	25	51.4	110.4	117.7	127.5	76.1	248	101.8	33.7
9	Male	22	42.1	112.9	171.8	151.2	109.1	359	119.5	47.8
10	Female	23	73.9	137.9	153.1	171.3	97.4	232	134.1	31.6
11	Female	52	42.8	97	146.1	193.6	150.8	452	119.9	54.0
12	Female	24	24.2	80.4	125	127.8	103.6	528	89.4	54.4
13	Female	51	33.6	96.2	139	161.6	128.0	481	107.6	52.3
14	Female	23	42.3	113.1	146.8	156.5	114.2	370	114.7	45.1
15	Female	47	25.2	96.2	88.9	102.5	77.3	407	78.2	45.7
16	Female	24	16.1	54.4	107.2	188.5	172.4	1171	91.6	81.5
17	Male	18	15.2	56.6	95.1	104.1	88.9	685	67.8	60.0
18	Female	45	49.5	103.1	148.9	166.9	117.4	337	117.1	44.8
19	Female	45	21.4	61.1	87.2	110.8	89.4	518	70.1	54.6
Mean		32.5	30.3	79.3	108.3	128.0	98.0	526	88.1	53.7
Placebo										
20	Female	32	52.7	52	59.9	72.6	19.9	137	59.3	16.1
21	Female	55	24.6	17.9	24	33.3	8.7	135	25.0	25.4
22	Female	55	44.8	33.6	37.1	43.2	-1.6	96	39.7	13.2
23	Female	40	19.7	16	29.9	25.2	5.5	128	22.7	26.9
24	Female	24	17.7	15	15.9	23.4	5.7	132	18.0	21.0
25	Female	23	50.3	40.3	40.6	41.2	-9.1	82	43.1	11.2
26	Male	34	42.6	33.2	47.2	35.5	-7.1	82	39.6	16.3
27	Male	26	38.2	31.8	23.6	21.0	-17.2	55	28.7	27.4
＊28	Male	40	***8.4**	13.2	14.3	19.5	11.1	232	13.9	32.9
29	Female	20	24.4	35.4	23.2	47.0	22.6	193	32.5	34.2
30	Female	21	24.3	28.1	42.3	46.2	21.9	190	35.2	30.2
31	Male	52	46.6	25.7	34.7	28.5	-18.1	61	33.9	27.4
32	Female	29	35.4	45.8	37	33.1	-2.3	94	37.8	14.7
33	Female	34	21.5	18.3	17.7	21.9	0.4	102	19.9	10.9
34	Female	29	23.7	33.4	57.8	35.0	11.3	148	37.5	38.5
35	Female	30	52.1	38.1	cancelled					
36	Female	26	30.1	34.9	26.6	24.9	-5.2	83	29.1	15.2
＊37	Female	27	***14.3**	12.4	16.8	13.0	-1.3	91	14.1	13.8
38	Female	25	145.1	85.9	95.7	75.3	-69.8	52	100.5	30.7
39	Female	54	116.6	100.7	95.4	106.1	-10.5	91	104.7	8.7
Mean		33.8	41.7	35.6	38.9	39.3	-1.9	115	38.7	21.8

2 participants retired at 4 weeks. * Suspected carotenoid low responder.

shows the changes from baseline to 12 weeks for the remaining 37 total participants within the 2 groups. There were 5 suspected carotenoid low responders as evidenced by low levels or low absorption rates. In the vegetable juice group, all 3 low responders (ID 2,5,6) saw rises in β-carotene levels to above 30 μg/dL, but in the placebo group, the levels of the 2 low responders (ID 28, 37) remained almost unchanged.

The changes in β-carotene levels varied from 30.7 to 172.4 μg/dL in the vegetable juice group. Those changes were extremely low among the low responders. The amount of change% in β-carotene levels varied from 232 to 1171% in the vegetable juice group. Those changes were relatively high in the low responders.

We also examined the intra-individual variations of the changes. The mean coefficient of variation (CV)% of β-carotene levels in vegetable juice group was 53.7% (33.7~81.5%). The mean CV% of β-carotene levels in the placebo group was 21.7% (8.7~34.2%). In the vegetable juice group, all 18 participants had increased β-carotene levels.

4. Discussion

Despite the juices being taken in equal amounts, the changes in blood β-carotene levels were widely distributed in the participants. The suspected low responders did see a rise in β-carotene levels, but, given the lower levels of increase than those of the other participants, this was taken as evidence of poorer absorption of carotenoids.

According to Borel's hypothesis,[9] "high responder" are those subjects who absorb carotenoid well because most proteins involved in carotenoid metabolism are efficient, while "low responder" are those who absorb carotenoids with bad because most of the proteins involved in carotenoid metabolisms are inefficient. We previously reported[12] that participants with a low level of β-carotene showed low level increase of β-carotene, apparently due to genetic variants of the proteins. We also showed[12] that ingestion of plain vegetable juice dramatically increased the levels of β-carotene and that the increase varied among individuals and correlated with the pre-supplementation β-carotene levels.

Thus, in this study we refer to participants with low-level β-carotene as 'carotenoids low responders'.

Variants in human genes encoding for the proteins involved in carotenoid metabolism may affect carotenoid blood levels as the ratio between the highest responder and the lowest responder (with respect to carotenoid levels) was discovered to be about 20:1[4]. Additionally, the highest blood β-carotene level was about 50 times higher than the lowest level.

In the vegetable juice group, all 18 participants had increased carotenoid levels after only 4 weeks, suggesting that vegetable juice intake efficiently fortifies the blood with β-carotene. As reported by Maiani et al[14] on the positive and negative effects of food processing, storage, and cooking that affect carotenoid content and bioavailability, vegetable juice may be the most useful for carotenoid absorption.

Our findings indicate that daily intake of vegetable juice is sufficient to fortify β-carotene levels in carotenoid low responders. However, a pronounced interindividual variability exists among those who drink vegetable juice daily, especially regarding carotenoid absorption efficiency. Continuous vegetable juice intake is useful to fortify blood β-carotene levels even in carotenoid low responders.

Conflicts of interest

The authors have no conflicts of interest.

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