

Original Research



Comparative bioavailability of β -carotene from raw carrots and fresh carrot juice in humans: a crossover study

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Conflict of Interest

The authors declare no potential conflicts of interests.

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ABSTRACT

BACKGROUND/OBJECTIVES: Carrots are a major source of beta-carotene, but comparative studies on different consumption methods are limited. This study compared the rates of β -carotene absorption from fresh carrots versus fresh carrot juice.

SUBJECTS/METHODS: For β -carotene absorption, a separate randomized controlled crossover trial was conducted with 16 healthy adults. The participants consumed 25 mg of β -carotene from raw carrots or fresh carrot juice. Blood samples were collected at the baseline (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h post-consumption.

RESULTS: The carrot juice group exhibited 2.33 times higher peak plasma concentrations 1.5 h post-consumption than those in the raw carrot group. The area under the curve for β -carotene absorption was 2.09 times greater in the carrot juice group than in the raw carrot group. The plasma α -carotene levels increased in both groups, but no significant differences were found. Similarly, no significant changes in the plasma levels of other fat-soluble vitamins were observed. In addition, no significant differences in antioxidant capacity (oxygen radical absorbance capacity and total radical-trapping antioxidant potential) were found between the 2 groups.

CONCLUSION: Consuming fresh carrot juice, without added sugars, may enhance the bioavailability of β -carotene compared to raw produce.

Keywords: Beta-carotene; bioavailability; *Daucus carota*; absorption

INTRODUCTION

β -Carotene is a potent antioxidant known for its role in reducing oxidative stress and preventing various degenerative diseases, including certain cancers. Carrots, being the primary dietary source of β -carotene, have been studied extensively for their health benefits. β -Carotene is converted into vitamin A in the body, which is essential for vision, immune function, and skin health. Therefore, an adequate β -carotene intake is crucial for maintaining overall health [1-4].

Various strategies to enhance the bioavailability of β -carotene have been investigated. One effective method is the consumption of carrot juice rather than raw carrots. The process

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of juicing breaks down the cell walls of the carrots, making β -carotene more accessible for absorption [1,5]. In addition, the fermentation of carrot juice using probiotic strains, such as *Lactobacillus gasseri*, increases the carotenoid and fiber contents, further enhancing its nutritional profile [6,7]. Cooking methods, such as stir-frying, have also been shown to improve the bioavailability of β -carotene compared to raw carrots. For example, the bioavailability of β -carotene from stir-fried carrots was approximately 75%, compared to only 11% from raw carrots [8]. This was attributed to the fat-soluble nature of β -carotene, which is better absorbed in the presence of dietary fats.

Several animal and clinical studies have explored the changes in plasma β -carotene concentrations following carrot juice consumption. A study involving smokers showed that supplementation with carrot juice significantly decreased lymphocyte DNA damage and increased the plasma antioxidant levels [1]. Similarly, a study of breast cancer survivors showed that daily intake of fresh carrot juice led to a significant increase in the plasma carotenoid levels, which are associated with a reduced risk of cancer recurrence [5,9]. Another study on overweight breast cancer survivors showed that regular carrot juice intake effectively increased the plasma total carotenoids and reduced the oxidative stress markers despite not significantly impacting inflammatory markers [7]. These findings highlight the potential of carrot juice for enhancing β -carotene bioavailability and exerting protective health effects.

Therefore, this study compared the plasma β -carotene concentrations after the single consumption of raw carrots and freshly extracted carrot juice. This study aimed to determine the more effective option for β -carotene supplementation by examining the changes in the plasma β -carotene concentration over time after consuming these 2 forms.

SUBJECTS AND METHODS

Materials and preparation of raw carrots and carrot juice

The carrots (Beta Rich) were purchased from an online marketplace. Prior to use, all carrots were washed with tap water and cut into stick shapes with a thickness of 1.5–2 cm. Carrot juice was prepared by juicing the washed carrots using a low-speed blender juicer (H410; Hurom Co., Ltd., Seoul, Korea). For clinical trials, the carrots and carrot juice were freshly prepared on the day of the test, while for the *in vitro* experiments, the carrots and carrot juice were freeze-dried using a freeze dryer and stored at -20°C until analysis. All organic solvents and other chemicals were of analytical grade or complied with the standards required for the cell culture experiments or high-performance liquid chromatography (HPLC) grade.

β -carotene concentration of carrots and carrot juice

β -carotene was extracted from the carrots and their corresponding carrot juices using a modified version of the method described by Kim *et al.* [10]. All samples were freeze-dried and ground into powder. For each sample, 0.5 g of the powdered sample was mixed with 20 mL of ethanol (EtOH) and shaken in a water bath at 75°C for 10 min. Subsequently, 5 mL of 80% potassium hydroxide (KOH) was added, and the mixture was shaken again at 75°C for a further 10 min before being placed on ice for 5 min to cool. After cooling, 5 mL of hexane and an equal volume of distilled water were added, and the mixture was vortexed vigorously for 5 min. The mixture was then centrifuged at 1,977 g for 3 min at 5°C . The upper hexane layer was collected and transferred to a rotary evaporator. The remaining EtOH–water phase was discarded, and the pellet was resuspended in 10 mL of ethanol, vortexed, and shaken again

at 75°C for 10 min. After 5 min of cooling on ice, 2.5 mL of hexane and an equal volume of distilled water were added, and the mixture was vortexed vigorously before centrifugation. The upper hexane layer was collected and combined with the previous extract. This process was repeated once more for a total of 3 extractions. The combined hexane layers were concentrated using a rotary evaporator under reduced pressure at 40°C until thoroughly dried. The dried extract was dissolved in 2 mL of ethanol (85:15, v/v), vortexed for 2 min, and filtered through a 0.45 µm syringe filter for β-carotene analysis.

Subjects and study design

This study was a randomized, controlled, crossover trial with 2 groups: a raw carrot group and a raw carrot juice group. Sixteen healthy adults, male and female, aged 20 to 30 yrs, were recruited. None of the participants were smokers. The participants were instructed to completely abstain from consuming processed or raw carrots, as well as any foods or supplements containing β-carotene, for one week before the experiment as a washout period. The participants' physical characteristics were assessed by measuring their height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), blood pressure, and blood glucose levels. The nutrient intake was assessed using the 24-h recall method, and the CAN program 6.0 (The Korean Nutrition Society, Seoul, Korea) was used to evaluate the nutrient intake data. The 16 participants were assigned randomly to the carrot (carrots 320 g + canola oil 50 mL; β-carotene 25 mg) or carrot juice (530 g + canola oil 50 mL; β-carotene 25 mg) groups. During the experimental period, the participants were provided with standardized meals, as shown in **Table 1**, which did not contain β-carotene. Blood samples were collected from each participant before consumption (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after consumption. The collected blood samples were used to analyze the bioavailability of beta-carotene and assess the antioxidant capacity and the ability to reduce DNA damage. This study was approved by the Institutional Review Board (IRB) of Kyungnam University (approval number: KUIRB 1040460-A-2024-005), and informed consent was obtained from the participants who volunteered for the study.

Table 1. Nutritional composition of standardized meals provided to the participants during the experimental period

Meal types	Menu
Afternoon snacks	Steamed white rice cake
Lunch	Cooked rice Soft tofu stew Braised pork and konjac Braised potato Braised cucumbers Pickled radish
Dinner	Cooked rice Dried pollack soup Royal Tteokbokki Grilled ham Stir-fried anchovies Pickled radish
Evening snacks	Grilled chicken Sprite
Nutrients	Amount
Energy	
Calorie (kcal)	2,492.4
Carbohydrate (g)	430.3
Protein (g)	97.2
Fats (g)	36.1
Fat-soluble vitamins	
β-Carotene (µg)	0.0

Plasma fat-soluble vitamins concentration

Extraction of fat-soluble vitamins in plasma

The fat-soluble vitamins (retinol, γ -tocopherol, α -tocopherol, α -carotene, β -carotene, and coenzyme Q10) were extracted from plasma using a modified version of the method reported by Lee *et al.* [11]. Subsequently, 100 μ L of tocol, 500 μ L of EtOH, and 3 mL of hexane were added to 100 μ L of plasma, followed by vortexing for 2 min. The mixture was then centrifuged at 1,977 g for 5 min at 5°C, and 2 mL of the upper hexane layer was collected and evaporated to dryness under nitrogen. The dried residue was dissolved in 150 μ L of a methanol: methylene chloride (85:15, v/v) solution by vortexing for 2 min.

Quantitative analysis of fat-soluble vitamins

The resulting solution was transferred to a HPLC vial for fat-soluble vitamin analysis. The fat-soluble vitamin content was quantified using liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). The analysis was performed using a Thermo Scientific Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Bremen, Germany), with 100 μ L of the fat-soluble vitamin extract injected. Separation was conducted on a Watchers 120 ODS-AP column (150 \times 4.6 mm, 5 μ m), and detection was set to 270 nm (coenzyme Q10), 295 nm (γ - and α -tocopherol), 325 nm (retinol), 450 nm (lycopene and α - and β -carotene) using a UV detector. The column temperature was maintained at 5°C, with a mobile phase of methanol: methylene chloride (85:15, v/v) at a flow rate of 1.0 mL/min. Quantification was conducted using retinol, γ -tocopherol, α -tocopherol, α -carotene, β -carotene, and coenzyme Q10 standards (Sigma-Aldrich Co., St. Louis, MO, USA).

Plasma antioxidant activity

The oxygen radical absorbance capacity (ORAC) The antioxidant activity of plasma, in terms of the change in fluorescence decay caused by the generation and elimination of oxidative radicals, was measured using an ORAC assay [12]. Peroxyl radicals were generated using 5 mM 2,2'-azobis(2-methylpropionamidine) dihydrochloride. The fluorescent standard solution was prepared with 40 nM fluorescein using the method reported by Ou *et al.* [13]. Fluorescence measurements were taken using a FLUOstar microplate reader (BMG Labtech, Ortenberg, Germany) with an excitation and emission wavelength of 485 and 535 nm, respectively. The results are expressed as the micromolar Trolox equivalents (μ M TE) per gram of the sample by comparing the area under the curve protected using 1 μ M Trolox, a water-soluble derivative of vitamin E (6-hydroxy-2,5,7,8-tetramethylchroman-2-carbonyl acid).

The total radical-trapping antioxidant potential (TRAP) of plasma was measured using the inhibition assay method described by Rice-Evans and Miller [14]. This method measures the degree of absorbance inhibition of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation, which is formed by an interaction of ferryl myoglobin radical species, generated by activating ABTS (150 mM) and metmyoglobin (25 mM) with H₂O₂ (75 mM). Absorbance inhibition is proportional to the antioxidant capacity present in the sample (0.84% plasma). The samples were incubated at 30°C for 8 min, and the change in absorbance was measured at 740 nm using an ultraviolet-visible (UV-VIS) spectrophotometry (UV/VIS) spectrophotometer. The total antioxidant capacity of the plasma sample was calculated using a Trolox calibration curve and is expressed as the Trolox equivalent antioxidant capacity (mM).

Statistical analysis

All data were entered into an MS Excel database system and analyzed using SPSS (version 23.0) for Windows (IBM Corp., Armonk, NY, USA). The percentages and mean values \pm SE deviation or SE of the mean were calculated for each variable. The differences over time within the raw carrot or raw carrot juice groups were evaluated using analysis of variance with Duncan's multiple range test, with significance determined at $P < 0.05$. In addition, the differences between the raw carrot and raw carrot juice groups were assessed using a Student's *t*-test, with statistical significance set at $P < 0.05$.

RESULTS

β -carotene concentration in carrots and carrot juice

Table 2 lists the β -carotene concentrations in raw carrots and carrot juice. The β -carotene concentration in carrots was significantly higher than in carrot juice (7.88 ± 0.24 vs. 4.78 ± 0.24 mg/100 g). Based on this analysis, the participants consumed 320 g of carrots and 530 g of carrot juice to ensure the intake of 25 mg of β -carotene in both groups.

Characteristics of the subjects

Table 3 lists the demographic and baseline characteristics of the study participants. Both groups, those consuming carrots and those consuming carrot juice, consisted of 16 participants each, with six males and 10 females in both groups. The average age of the participants was 22.75 ± 0.48 and 22.38 ± 0.42 yrs in the carrot and carrot juice groups, respectively. The height, body weight, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), WC, and HC were similar in both groups. Based on the dietary and nutritional information assessed prior to the clinical trial, the participants' average daily caloric intake was $2,535.8 \pm 177.9$ kcal, with carbohydrate, protein, and fat consumption within the normal range. Furthermore, the intake of β -carotene was 0 mg per day (data not shown).

Table 2. β -Carotene concentration in raw carrots and carrot juice

Variables	β -Carotene (mg/100 g)
Carrots	7.88 ± 0.24
Carrot juice	4.78 ± 0.24

Values are presented as mean \pm SE ($n = 4$).

Table 3. Subjects characteristics

Characteristics	Carrots ($n = 16$)	Carrot juice ($n = 16$)
Sex		
Male ($n, \%$)	6 (37.5)	6 (37.5)
Female ($n, \%$)	10 (62.5)	10 (62.5)
Age (yrs)	22.75 ± 0.48	22.38 ± 0.42
Hight (kg)	168.04 ± 2.03	168.39 ± 1.91
Body weight (kg)	74.62 ± 5.51	72.88 ± 5.00
BMI (kg/m^2)	26.33 ± 1.82	25.59 ± 1.56
SBP (mmHg)	123.06 ± 2.89	124.06 ± 3.49
DBP (mmHg)	78.38 ± 1.98	78.94 ± 1.44
WC (cm)	89.25 ± 4.57	85.69 ± 4.11
HC (cm)	103.13 ± 2.59	103.00 ± 2.52

Values are presented as number (%) or mean \pm SE.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; HC, hip circumference.

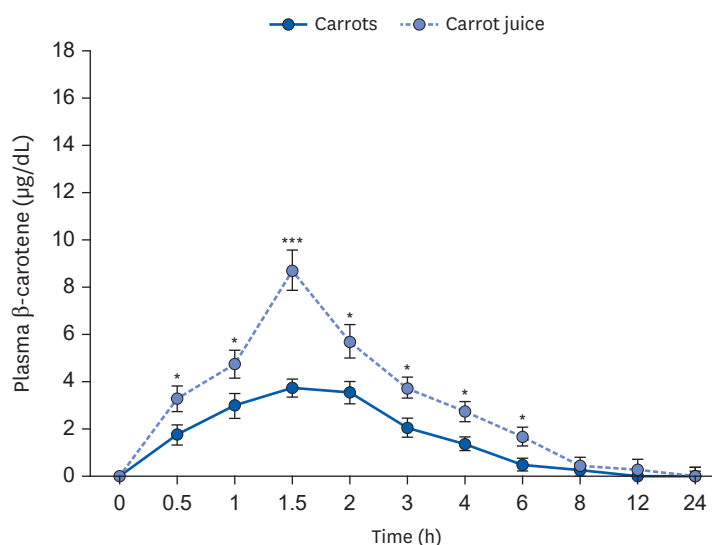


Fig. 1. Plasma β -carotene concentration after carrots and carrot juice intake. Values are presented as mean \pm SE. * $P < 0.05$, *** $P < 0.001$ significantly different between groups (Student's t -test).

Plasma fat-soluble vitamin concentration

Figs. 1 and 2 show the plasma fat-soluble vitamin measurement results. **Fig. 1** provides a visual representation of the mean \pm SE for both groups, indicating the overall trends in the plasma β -carotene concentrations. Significant differences in β -carotene absorption between the raw carrot and carrot juice groups were observed from 0.5 to 6 h post-consumption, with the plasma β -carotene levels peaking at 1.5 h in both groups. The raw carrot group reached $3.74 \pm 0.39 \mu\text{g/mL}$, while the carrot juice group peaked at $8.72 \pm 2.72 \mu\text{g/mL}$, as shown in **Fig. 1**. The peak plasma concentration in the juice group was 2.33 times higher than in the raw carrot group. By 8 h post-consumption, the plasma β -carotene levels returned to near the baseline in both groups.

Furthermore, the area under the curve (AUC) for β -carotene absorption was 2.09 times greater in the juice group than in the raw carrot group, suggesting enhanced bioavailability from carrot juice (raw carrots: $15.58 \pm 2.36 \mu\text{g/mL}$, carrot juice: $32.56 \pm 4.39 \mu\text{g/mL}$, data not shown). The plasma α -carotene levels increased in both groups after consumption, but significant differences were observed within each group. Similarly, no significant changes in the plasma concentrations were observed for retinol, γ -tocopherol, α -tocopherol, lycopene, or coenzyme Q10 following the consumption of raw carrots or carrot juice (**Fig. 2**). Hence, carrot juice significantly improves the bioavailability of β -carotene compared to raw carrots, likely due to the breakdown of the cell walls and the improved release of β -carotene during the juicing process.

Plasma antioxidant activity

Table 4 lists the changes in plasma antioxidant activity, measured using the ORAC and TRAP assays, following the intake of carrots and carrot juice. The ORAC values peaked at eight and 12 h in both groups (1.03 ± 0.09 or $1.15 \pm 0.09 \mu\text{M TE}$ for carrots and 1.27 ± 0.14 or $1.14 \pm 0.04 \mu\text{M TE}$ for carrot juice). On the other hand, the TRAP values did not show a similar trend, with fluctuations observed in both groups.

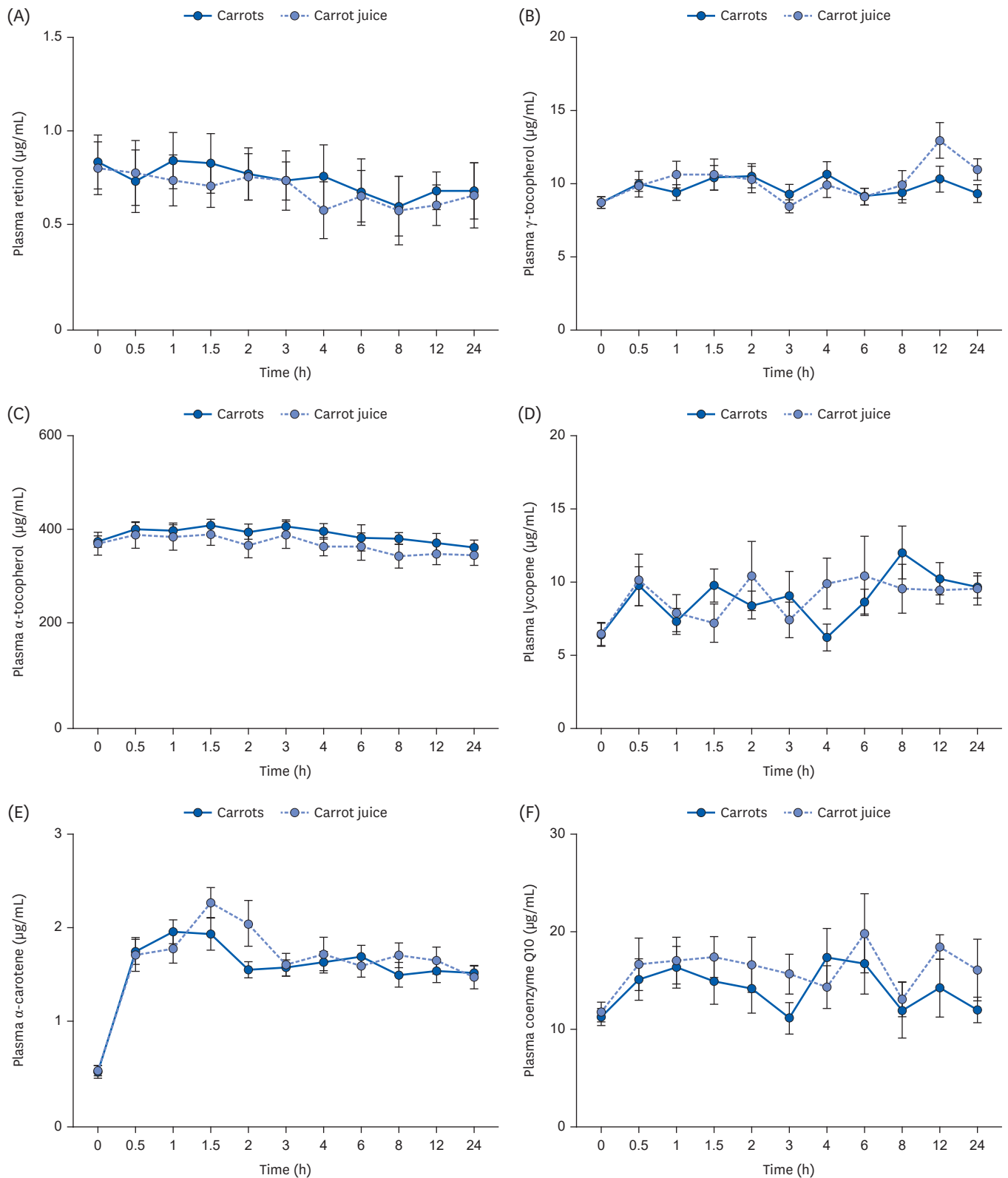


Fig. 2. Plasma retinol, γ -tocopherol, α -tocopherol, lycopene, α -carotene, and coenzyme Q10 concentrations after carrots and carrot juice intake. (A) Retinol, (B) γ -tocopherol, (C) α -tocopherol (D) lycopene, (E) α -carotene, and (F) coenzyme Q10.

Table 4. Plasma antioxidant activity (ORAC and TRAP) following carrots and carrot juice intake

Variables	Carrots (n = 16)	Carrot juice (n = 16)
ORAC (μM TE)		
0 h	0.72 ± 0.04 ^a	0.77 ± 0.06 ^a
0.5 h	0.85 ± 0.08 ^{ab}	0.92 ± 0.04 ^{ab}
1 h	0.95 ± 0.06 ^{abc}	0.89 ± 0.09 ^{ab}
1.5 h	0.98 ± 0.09 ^{bc}	0.94 ± 0.06 ^{ab}
2 h	0.90 ± 0.06 ^{ab}	0.93 ± 0.06 ^{ab}
3 h	0.85 ± 0.08 ^{ab}	1.06 ± 0.12 ^{bc}
4 h	0.91 ± 0.06 ^{ab}	0.93 ± 0.07 ^{ab}
6 h	1.01 ± 0.08 ^{bc}	0.98 ± 0.08 ^{ab}
8 h	1.03 ± 0.09 ^{bc}	1.27 ± 0.14 ^c
12 h	1.15 ± 0.09 ^c	1.14 ± 0.04 ^{bc}
24 h	0.84 ± 0.09 ^{ab}	0.90 ± 0.05 ^{ab}
TRAP (mM)		
0 h	0.99 ± 0.06 ^{ns}	0.95 ± 0.05 ^{ns}
0.5 h	1.06 ± 0.06	0.99 ± 0.06
1 h	0.98 ± 0.06	1.01 ± 0.04
1.5 h	1.04 ± 0.05	1.02 ± 0.05
2 h	1.09 ± 0.05	1.03 ± 0.06
3 h	1.03 ± 0.04	1.03 ± 0.04
4 h	0.96 ± 0.07	0.99 ± 0.05
6 h	0.94 ± 0.07	1.03 ± 0.04
8 h	0.98 ± 0.06	0.99 ± 0.04
12 h	1.07 ± 0.06	1.00 ± 0.07
24 h	0.90 ± 0.07	1.01 ± 0.05

Values are presented as mean ± SE.

ORAC, oxygen radical absorbance capacity; TRAP, total radical-trapping antioxidant potential; μM TE, micromolar Trolox equivalents; ns, not significant.

^{a-c}Values with different letters are significantly different at $P < 0.05$ after analysis of variance with Duncan's multiple range test within each group.

DISCUSSION

In this study, despite standardizing the β -carotene content in the carrot juice and raw carrot groups, the bioavailability of β -carotene was significantly higher in the carrot juice group. The enhanced bioavailability is due likely to the liquid form of the juice, which disrupts the fibrous plant matrix in raw carrots, enhancing the release and absorption of β -carotene. Previous research has shown that food processing techniques, such as juicing, can increase carotenoid bioavailability significantly by breaking down the food matrix and improving carotenoid release and absorption [15]. Similarly, studies on citrus fruit reported higher bioavailability of carotenoids in juice compared to the pulp form, suggesting that the fibrous matrix in whole fruits may hinder carotenoid release and absorption [16].

The bioavailability and absorption of carotenoids are significantly influenced by the physical state of the food matrix and environmental conditions during processing and storage. Beyond simply breaking down the fibrous matrix, mechanical disruption of the food matrix, as seen in smoothies or finely processed foods, enhances carotenoid release by breaking down the cell walls and making the nutrients more accessible for digestion. In particular, a study comparing different carrots preparations found that smoothies, which disrupt the food matrix, resulted in higher plasma carotenoid concentrations than whole or minimally processed carrots. Moreover, the presence of dietary fats in smoothies also increases the solubility and absorption of these fat-soluble compounds [7]. Moreover, the pH of the food matrices, such as juices, can influence the chemical stability and bioavailability of carotenoids. The acidic conditions in carrot juice have been shown to increase total carotenoid content by improving

the solubility of crystallized carotenoids in plant cells [17]. Overall, these findings support the concept that the liquid forms of carrot juice enhance carotenoid release and bioavailability compared to consuming whole raw carrots.

Studies involving enzyme liquefaction treatments, such as pectinase and cellulase, which have been shown to increase the carotenoid content in carrot juice by breaking down the fiber matrix, provide supporting evidence [6,18]. Furthermore, ultrasound treatments have been found to improve the total carotenoid content and quality in carrot juice, likely through the breakdown of the cell walls and the release of bound carotenoids [19]. Overall, the enhanced bioavailability of β -carotene from carrot juice compared to raw carrots is well-supported by research, highlighting the importance of processing methods in optimizing nutrient absorption and utilization [16,20,21].

A study of the long-term effects of carrot juice consumption reported that the daily intake of fresh carrot juice for 3 mon significantly enhanced total antioxidant capacity and decreased the malondialdehyde levels in adults, indicating a decrease in oxidative stress [22]. Furthermore, after a three-week intervention period, carrot juice intake modulated the immune responses, as evidenced by the increased levels of pro-inflammatory and anti-inflammatory cytokines in lipopolysaccharide-stimulated blood samples. These results suggest that carrot juice may play a beneficial role in modulating inflammatory processes and enhancing the immune function [23]. In contrast, this study assessed the antioxidant activity following a single intake, which may have been insufficient to detect any significant changes in the antioxidant status attributable to carrots or carrot juice consumption.

In conclusion, carrot juice significantly enhances β -carotene bioavailability compared to raw carrots, as evidenced by the higher peak plasma concentrations and a greater AUC for β -carotene absorption. The liquid form of carrot juice facilitates the breakdown of the fibrous plant matrix in raw carrots, improving the release and absorption of β -carotene. Although both forms of carrots contribute to increased β -carotene levels, carrot juice provides a more efficient means of delivering this important nutrient. These findings support the potential of carrot juice as a superior dietary source of β -carotene for promoting overall health, particularly in populations requiring increased β -carotene intake.

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