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ORIGINAL CONTRIBUTION

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β-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers

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Summary *Background* Carotenoids contribute to the beneficial effects of fruits and vegetables consumption; however, the bioavailability of these compounds from fresh or processed foods is not well established. *Aim of the study* We evaluated the bioavailability of β-carotene (15 mg) from a single meal composed of cooked, pureed carrots and compared it to raw, chopped carrots. *Methods* Test meals were given to overnight-fasted-ileostomy volunteers (n = 8) along with skimmed-milk yogurt containing 40 g of added sunflower oil. Blood and complete ileal effluent samples were collected over a 24 h period. Samples were solvent-extracted and the β-carotene content measured by HPLC. *Results* Kinetics of excretion of cis and trans β-carotene were similar. More β-carotene was absorbed from puree as compared to raw carrots. Carotenoid mass-balance calculations indicated that 65.1 ± 7.4 % of the β-carotene was absorbed from cooked pureed carrot meals, vs. 41.4 ± 7.4 % from raw, chopped carrot meals. Gastrointestinal transit parameters did not differ significantly among the volunteers. As expected, the calculated lag phase was five times longer for raw vs. cooked carrots. Mean t-end, t-1/2 and rate of mass transit resulted in similar values for both raw and cooked carrot meals. A moderate response in carotenoid plasma profile was observed for cooked carrot test meals. *Conclusions* Significantly more β-carotene was absorbed from meals containing cooked, pureed carrots than from meals containing the raw vegetable. Moderate carotenoid plasma response was detected within 6 h following the administration of cooked processed carotenoid-containing single meal.

Key words carotenoids – β-carotene – ileostomy – transit time

Introduction

Epidemiological studies have shown that a high vegetable intake is associated with reduced risk of free radical-mediated degenerative diseases, such as epithelial cancers [1], cardiovascular disease [2] and age-related eye diseases [3–5]. Among the major carotenoids (α-carotene, β-carotene, lycopene, lutein, zeaxanthin, α-cryptoxanthin and β-cryptoxanthin) absorbed by humans and incorporated into their blood and tissues, β-carotene has been studied most extensively [6].

A significant positive correlation exists between the extent of vegetable intake and plasma concentration of carotenoids [7–9]. In addition, increased vegetable consumption results in increased plasma levels of carotenoids [10–12].

In studies in which β-carotene was given as supple-

EJN 430

ment, or in which β-carotene was a constituent part of foods, all have shown plasma alterations that are highly variable in magnitude and duration, and include 'non-responders' [13] to elevated levels that are still detectable 10 to 20 days post-dose [13, 14].

The absorption of carotenoids from test meals is reported to range between 2 and 90 % depending on the meal and the model used to assess absorption [15]. The benefit obtained from a high intake of carotenoids from fruits and vegetables is dependent on the bioavailability of these molecules, which is influenced by food type, mode of preparation and individual response [16].

Plasma concentrations have been erroneously used in the vast majority of the bioavailability nutritional studies to indicate the proportion of carotenoids that are available to the body. In addition, determination of absorption of carotenoids has been carried out in human volunteers using a large number of methodologies, such as, oral-fecal mass balance [17], chronic dosing [6, 18–20], acute dosing [16, 21], triglyceride-rich lipoprotein carotenoid from acute dosing [22, 23], radioactive tracers [24, 25], stable-isotope tracers [26–28], total gastrointestinal washout (mass balance) method [29], and more recently, the ileostomy mass-balance model [30].

This last methodology provides a system to assess the extent of carotenoid absorption and has been recently used to determine the extent of carotenoid intake from cooked spinach meals; however, it has not been tested on additional vegetables.

Mass-balance techniques in ileostomy patients [30] have been shown to provide useful absorption data while avoiding the confounding influence of the large intestinal microflora. Even though some residual microbial activity remains (colonization of the terminal ileum in ileostomy patients), this activity is not thought to cause any significant loss during transit.

The purpose of this study was to characterize the absorption and disposal of β-carotene in ileostomy subjects fed a single portion (equivalent to 15 mg carotenoids) of raw carrots compared to a single portion of cooked carrots.

Subjects and methods

Ethics

The study protocol was approved by the Ethics Committee for human studies of the Israeli Ministry of Health, and the experimental work was carried out in accordance with the Declaration of Helsinki (1989). Informed written consent was obtained from each volunteer.

Volunteers

Eight volunteers (38–75 years) were recruited for this study, which was conducted in Soroka Medical Center. The weight range of the volunteers was 52 to 77 kg and a mean BMI of 20.5 kg/m^2 (range 18.5–25.2). The subjects had undergone ileostomy surgery at least 2.5 years previous to the experiment. They were free of intestinal disease and otherwise healthy, as determined by medical histories, blood chemistry tests, lipidogram and complete blood count and urine analyses, which were all within normal limits.

Study protocol

The volunteers avoided consumption of food items known to contain large amounts of carotenoids (a list was provided) and recorded their habitual diet for 3 days prior to the test day. The volunteers fasted from 19:00 h the day before the test but were free to drink water. At 08:00 h on the test day (fasted, t = 0) blood samples were taken, participants then changed the stomal effluent collection bag and ate a breakfast containing fat-free yogurt to which 40 g of sunflower oil was added, homogenized in a low speed blender, and the carotenoid-containing test meal in the form of ground raw carrots or cooked pureed carrot. Each test meal contained an equivalent of 15 mg trans β-carotene. Volunteers were provided with carotenoid-free midday (12:00–12:30 h) consisting of fat-free chicken breast meat or tofu-based meal accompanied by potato puree and light refreshments. The evening meals consisted of sandwich with low fat cottage cheese, three slices of bread and tea or coffee. Venous blood samples were collected via antecubital cannula into 10-ml heparin tubes every 2 h up to 12 h and the last sample was collected after 24 h. Plasma was separated by centrifugation (5 min, 5000 x g) and frozen at –80 °C. Stomal effluents were collected into pre-weighed bags every 2 h up to 12 h and the last sample was collected after 24 h.

Analytical methods

The carotenoid extraction method from effluent and serum samples was evaluated by introducing the internal standard β-apo-8'-carotenal into the effluent or serum. This internal standard was prepared by dissolving β-apo-8'-carotenal to a concentration of 0.7 µg/ml in hexane. One ml of the internal standard was added to effluent or serum before extraction. The mean percent of recovery was 91 % (range 87–94).

Plasma samples (500 µl) were treated with sodium dodecyl sulfate (0.5 ml, 10 mmol/l) and ethanol (1 ml) to precipitate plasma proteins. The carotenoids were ex-

tracted three times by the addition of hexane (2 ml), and the pooled hexane fraction was dried with a stream of nitrogen gas. The dry residue was dissolved in dichloromethane (100 µl) before adding 400 µl of acetonitrile/methanol (79:21). The carotenoids were measured by HPLC [30]. Effluent samples (2 g) were placed in 50-ml screw-top glass centrifuge tubes and 20 ml of acetone added. The effluent was thoroughly dispersed using a small ultra-turrax, the mixture was centrifuged (800 x g, 10 min) to pellet the solids and the supernatant was transferred to a 100-ml volumetric flask. The pellet was resuspended in 20 ml acetone and the process repeated 3 more times. The acetone extract was brought to 100 ml, thoroughly mixed and a filtered sub-sample (20 ml) stored at –20 °C. A sub-sample (1 ml) of the filtered acetone extract was dried under N_2, resuspended in HPLC mobile phase, diluted if needed, and assayed by HPLC as above.

HPLC analysis

Samples were injected by using a JASCO Autosampler (model AS-950–10; JASCO, Tokyo, Japan) onto a C_{18}, RP (Vydac 201 TP 45) column fitted with a C_{18} guard column and biocompatible frits. Samples were eluted isocratically in the HPLC mobile phase at a flow rate of 1.2 ml/min with a model LC-1150 pump (GBC, Scientific Equipment, Victoria, Australia), a multiwave programmable detector (model MD 910, JASCO) and a Borwin PDA version 1.50 system controller (JASCO). Carotenoids were identified and quantified at 450 nm using known standards.

Data analyses

Cis and trans β-carotene was calculated as the subtraction of cis and betacarotene released into the effluent from the ingested concentration found in raw and puree carrot meals for each time point. The kinetics was drawn accordingly.

The normalized cumulative collection (percent) of the carrot meals was plotted against time to provide the transit profile of the carrot, and then the data were fitted using a logistics model to calculate t-1/2 of gastrointestinal (GI) residue time. The portion of the curve that appeared to be steeply increasing with time was fitted with a regression model. The slope was equivalent to the effluent production rate and the intercept (x at y = 0) was used to calculate the lag time. The normalized amount (percentage) of the original carrot meal collected at each time point was calculated from the total recovered β-carotene and the amount of β-carotene in each collection. This calculation took into consideration constant percentage absorption of β-carotene at all time points.

Calculation and statistics

Values are given as means ± SD or SEM, as indicated. One or two-tailed t-tests were used to compare absorption, excretion and transit-time values between test meals in each volunteer.

Results

Carotenoid composition of carrot meals

The average composition of the raw carrot meal consisted of 54.8 µg/g trans β-carotene, 3.02 µg/g cis β-carotene, 25.2 µg/g trans α-carotene, 0.78 µg/g cis α-carotene, 1.42 µg/g lutein (total) and 0.08 µg/g zeaxanthin. We aimed at receiving 15 mg of trans β-carotene in each test meal; therefore the volunteers received 275 g of raw carrots in each test meal. The average composition of the cooked carrot puree meal consisted of 62.5 µg/g trans β-carotene, 2.52 µg/g cis β-carotene, 23.6 µg/g trans α-carotene, 1.18 µg/g cis α-carotene, 1.37 µg/g lutein (total) and 0.05 µg/g zeaxanthin. We aimed at receiving 15 mg of trans β-carotene in each test meal; therefore the volunteers received 240 g of pureed carrots in each test meal.

Excretion kinetics of cis and trans β-carotene

The concentrations of all-trans β-carotene (Fig. 1a) and cis-β-carotene (Fig. 1b) isomers "apparently" absorbed (calculated as secreted quantity subtracted from the measured amount present in the test meal) from raw and cooked carrots were determined. The kinetics for excretion of both isomers and the two meals was similar; however the final amounts absorbed differed. For trans-β-carotene the excretion of the carotenoid following consumption of the raw carrot meal was significantly higher starting 2 h following the meal and continued in a steeper rate onwards. Plateau was reached at 12 h. Fifty percent more trans-β-carotene was absorbed from cooked puree carrots than from raw carrots. Approximately 100 % cis-β-carotene was secreted into the effluent 24 h following the test meal consumption containing raw carrots (Fig. 1b). In contrast, volunteers consuming the cooked puree carrot meal secreted only 73 % of cis-β-carotene. For both differently processed test meals the kinetics of excretion was similar; most of the β-carotene was secreted within 12 hours following consumption. During the last 12 hours the excretion rate reached a plateau.

An average group calculation of β-carotene excretion to the effluent is shown in Fig. 2. The average concentration of β-carotene in the effluent showed that the amount of β-carotene secreted by volunteers fed cooked

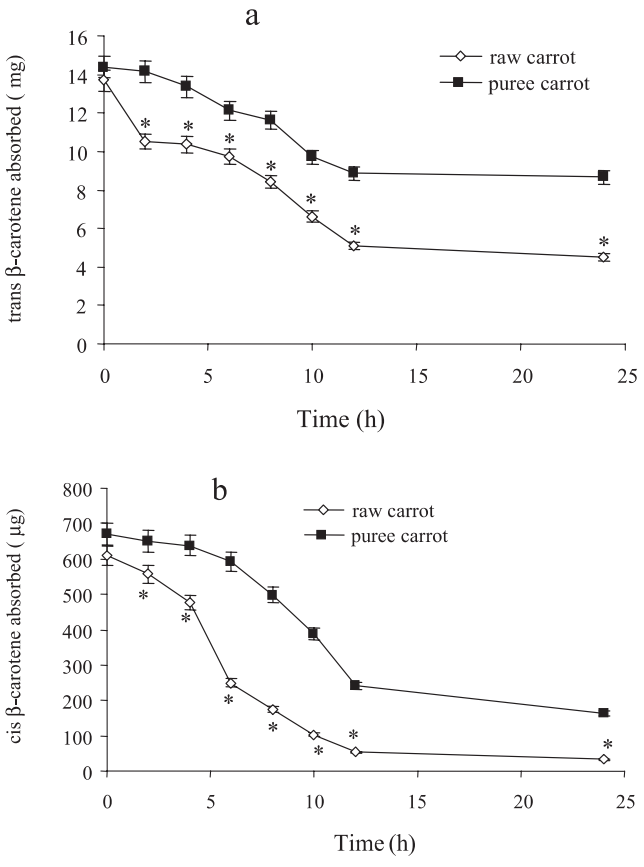

Fig. 1 **a** Kinetics of trans β-carotene excretion into the ileal effluent. The concentration (calculated as secreted quantity subtracted from the measured amount present in the test meal) of all-trans β-carotene isomer absorbed from raw and cooked carrots was drawn as a function of time. * $P < 0.001$ puree vs raw carrot. **b** Kinetics of cis β-carotene excretion into the ileal effluent. The concentration ± SD (calculated as secreted quantity subtracted from the measured amount present in the test meal) of cis β-carotene isomer apparently absorbed from raw and cooked carrots was drawn as a function of time. * $P < 0.001$ puree vs raw carrot

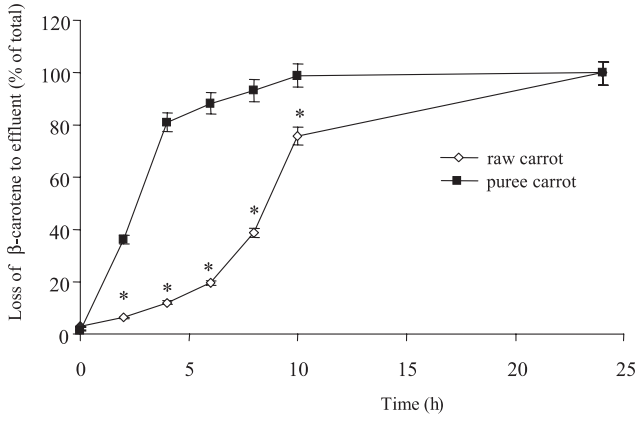

Fig. 2 The amount of trans β-carotene in effluent samples collected at 2-hour intervals was calculated. The calculations are mean ± SD for 8 volunteers. * $P < 0.05$

carrots was higher than for the volunteers fed raw carrots for the first 4-h time periods. However, at later time periods the trend was exchanged and showed an opposite pattern, i. e. the excretion of β-carotene in the effluent was more pronounced in volunteers fed cooked pureed carrots and less evident in effluents from volunteers fed raw carrots.

Gatrointestinal transit parameters

The results for the normalized excretion of β-carotene from the carrot meals to the ileal effluent are shown in Fig. 3 for the raw and processed meals. For cooked pureed carrots, 90 % of β-carotene was secreted within 6 h while in the effluent of the volunteers fed raw carrots it took 12 h to secrete a similar amount of the carotenoid. Additional meal-behavior parameters for variously processed carrot test meals along the GI tract are summarized in Table 1. The calculated mean lag phase of the initial appearance of effluent from the cooked carrot meal (0.66 ± 0.3 h) was shorter than that calculated from the raw carrot meal (2.55 ± 0.7 h). The mean half-life (t-1/2) of the cooked carrot meal in the GI tract (4.2 ± 0.3 h) was shorter than that for the raw carrot meal (6.55 ± 0.6 h). The mean rate of mass transit through the GI tract as estimated from the slope of the cumulative collection of ileal effluent was similar for the cooked carrot meal (12.4 ± 2.3 h) as for the raw carrot meal (12.7 ± 1.0 h). The

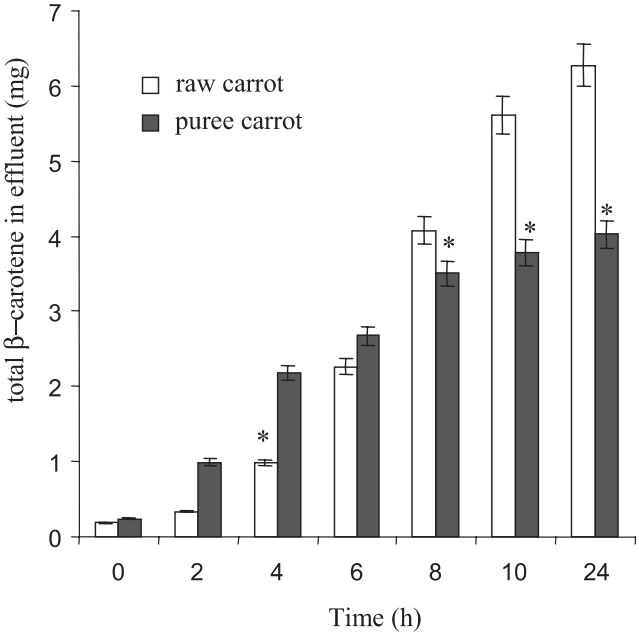

Fig. 3 Percent excretion of β-carotene in effluents collected for 24 h at intervals of 2 hours. The plot represents the calculations obtained from 8 volunteers fed raw carrots and the second plot that obtained from 8 volunteers following feeding cooked carrots. * $P < 0.01$

Table 1 Comparison of transit-time on ileal effluents from patients fed two different carrot test meals

Transit-time parameters	Raw carrots	Puree carrots
lag phase (h)	2.5 ± 0.7*	0.66 ± 0.3
t (1/2) (h)	6.5 ± 0.6**	4.2 ± 0.7
t (end) (h)	10.6 ± 0.7	9.8 ± 1.5
slope (%/h)	12.7 ± 1.0	12.4 ± 2.3

Data are expressed as Means ± SD of 8 volunteers.
lag phase (h) refers to the time taken for the test meal to appear at the terminal ileum; *t (1/2)* time (h) at which half the meal has been voided; *t (end)* time (h) at which the meal has been completely voided; *slope (%h)* rate expressing meal effluent accumulation (%/h)
* $p < 0.01$ raw compared to puree carrot meal, Student's *t* test
** $p < 0.07$ raw compared to puree carrot meal, Student's *t* test

mean t-end or time that the meal was completely voided of the two carrot meals in the GI tract was similar (10.6 ± 0.3 h for raw, 9.8 ± 1.5 h for cooked).

β-carotene absorption

All the meals contained 15 mg β-carotene. The absorption of trans-β-carotene was calculated as the difference between the level of trans-β-carotene in samples from raw carrot meals and the level obtained in the ileal effluent. Fig. 4 shows that the absorption of trans-β-carotene in volunteers who were fed raw carrots was 41.4 + 7.4 %, whereas volunteers fed cooked carrots, absorption of β-carotene reached 65.1 + 7.4 %.

Plasma b-carotene response

Significant plasma β-carotene response was observed when the volunteers consumed 15 mg β-carotene from processed carrot test meals, mainly 4–6 h following the consumption of the test meals. Thereafter, the plasma levels reached a plateau. Plasma β-carotene levels in volunteers fed raw carrot test meals differed from those measured in volunteers fed the cooked carrot meals; no significant increases were detected throughout the experiment. Some expected variability in the carotenoid plasma levels was observed among the different volunteers during the course of the experiment (Fig. 5).

Fig. 4 Absorption ± SD of trans β-carotene from raw compared to cooked carrot test meals. * P = 0.048 two-tail t-test comparing both test meals

Discussion

The mass-balance technique using the ileostomy model [30] was shown previously to produce reliable data on absorption and excretion of vegetable carotenoids, while avoiding the confounding influence of the activity in the large intestinal microflora.

The present study demonstrates that the rate of β-carotene absorbed from a single portion of cooked carrots is significantly higher (two-tailed t-test) than the rate values obtained by ileostomy volunteers eating raw carrot meals (P = 0.048). Absorption of an oral dose of 15 mg β-carotene provided in a raw carrot meal was 41.4 ± 7.4 %, whereas absorption of β-carotene from a cooked carrot meal reached 65.1 ± 7.4 %. These amounts are generally greater than previously published values [30] for β-carotene in oil, where the mass balance was taken as the difference between intake and fecal excretion over a non-specified period.

Cis β-carotene was completely excreted into the effluent 12 hours following ingestion of the raw carrot test meal. The volunteers apparently absorbed 21 % of cis β-carotene when they consumed the puree cooked carrot test meal. For trans β-carotene the excretion kinetics were similar for both raw and cooked carrot meals; however, more trans β-carotene was secreted from the raw carrot test meals. For both test meals the contribution of cis β-carotene to the general β-carotene profile was marginal; therefore, additional calculations relate only to trans β-carotene.

GI-tract transit time parameter calculations indicate

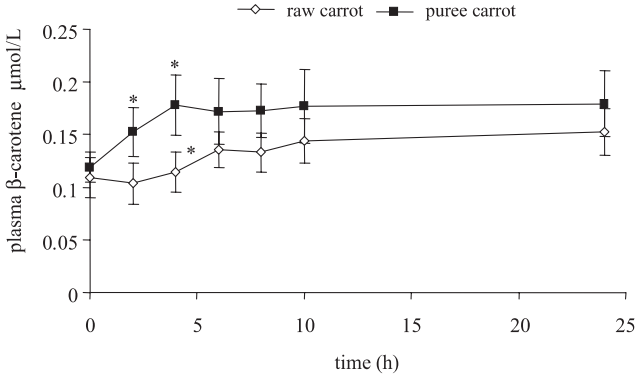

Fig. 5 Plasma β-carotene response recorded for 24 h period. The plots represent the results of plasma trans β-carotene response (ng/ml ± S. E. M.) of 8 different volunteers fed both raw and then processed carrots. * P = 0.01

some clear differences in values between raw and processed carrots: the lag phase, i. e. the initial appearance of β-carotene in the effluent was longer for the raw carrot meal than for cooked carrots (physical change of the vegetable from raw to cooked stage resulted in a quicker passage through the GI tract). This difference became less significant after t-1/2 (the mean half life) and disappeared towards the t-end (see Fig. 3). The mean rate of mass transit through the GI tract as estimated from the slope of the cumulative collection of β-carotene in the ileal effluent was the same for both meals. The cooked carrot meal passed through the GI tract at 12.4 g/h, the same rate as the raw carrot meal (12.7 g/h).

A study using a computerized alimentary tract model demonstrated that β-carotene absorption falls in the range of 13 to 18 % without a meal whereas it rises to 40 to 65 % with a meal of 4184 kJ, containing 40 % dietary fat [31], results that correlate well with our findings. The values found in the present mass-balance study, however, may still be an overestimation of absorption, since destruction of carotenoid by the intestinal microflora cannot be calculated. We have previously shown levels of 90 % β-carotene absorption (range 74.3–97 %) in mass-balance studies carried out in fasted ileostomy subjects given an oral dose of β-carotene extracted from *Dunaliella salina* and administered dispersed in vegetable oil [30]. The physical form of carotenoids may be an important factor in the absorption process. Crystalline β-carotene was shown to be less available than dissolved β-carotene [32–34]. In carrots, β-carotene is present as crystals and this may also explain the low availability of β-carotene from carrots as compared to extracted β-carotene previously dissolved in oil [19, 33]. Processing, such as mechanical homogenization or heat treatment, can enhance the bioavailability of carotenoids from vegetables [35, 36]. Pureeing vegetables results in smaller particle sizes and also mechanically disrupts the plant cells, so that the carotenoids are presumably more available in the intestinal lumen for absorption [33, 36–38]. Heat treatment is suggested to enhance the bioavailability of carotenoids by loosening their binding to proteins [39].

Mass-balance technique using this model allowed us to demonstrate that when a cooked carrot meal containing 15 mg β-carotene is consumed it is possible to detect plasma β-carotene response, whereas consumption of 15 mg β-carotene from a raw carrot meal does not allow detection of the plasma β-carotene response. Van het Hof et al. [6] showed a very significant plasma response to carotenoid meals 4 days after consumption of palm oil carotenoids, which could explain the lower levels we found in plasma collected after a single serving. It seems that 24 h after the consumption of a single processed meal is sufficient to induce a new steady state of plasma carotenoid levels. However, a similar single dose of raw carrot meal is not sufficient to induce a significant serum response. There is less variability in the plasma response to continuous supplementation of dietary carotenoids than after administration of a single dose [16, 19]. In addition, the amount of β-carotene in the meal (15 mg) was lower relative to the dose (20–60 mg) given in similar studies, which have resulted in significant changes in plasma carotenoid concentration [16, 18, 19]. Nevertheless, a short intervention period as used here has several advantages: It mirrors a more realistic stage of the carotenoid changes in a subject and compliance is probably better during a short period.

It has been suggested that carotenoids are absorbed by enterocytes in the chylomicrons as are fatty acids, and then slowly released into the plasma [13]. Small changes in plasma β-carotene following a single raw carrot meal may represent poor absorption due to matrix present in the raw carrots that interferes with free transfer of the carotenoid crystals to the enterocytes [37]. In the processed cooked carrots newly absorbed carotenoids are measured in plasma chylomicrons. Resecretion of the carotenoid from the liver packed in VLDL particles may be evident much later and at lower peak concentrations in LDL or HDL fractions.

The extent of carotenoid absorption varies among individuals. In healthy, well-nourished populations, large variability of the response of plasma or serum β-carotene concentrations to supplementation was reported [16, 40]. Some volunteers identified as so-called non-responders: people who show no, or only a very small increase in β-carotene concentration in chylomicrons or plasma, following β-carotene supplementation [13, 41]. The mechanism underlying this variation may not only be related to differences in carotenoid absorption but also to differences in lipoprotein metabolism among individuals [42].

The processing of foods involves changes in the structural integrity of the matrix and this produces both negative (loss of carotenoids due to oxidation) and positive (increased bioavailability) effects. The ileostomy model used herein is proved to be a good method to perform bioavailability studies of feeding meals for short time periods with low carotenoid levels.

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References

1. Wright ME, Mayne ST, Swanson CA, Sinha R, Alavanja MC (2003) Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri women's health study (United States). Cancer, Causes & Control 14:85–96
2. Ness AR, Powles JW (1997) Fruit and vegetables, and cardiovascular disease: a review. Int J Epidemiol 26:1–13
3. Jacques PF, Chylack LT (1991) Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr 53: 352s–355s
4. Hankinson SE, Stampfer MJ, Seddon JM, Colditz GA, Rosner B, Speizer FE, Willet WC (1992) Nutrient intake and cataract extraction in women: a prospective study. Br Med J 305: 335–339
5. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA, Willet W (1994) For the Eye Disease Case-Control Study Group. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 272:1413–1420
6. van het Hof KH, West CE, Weststrate JA, Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503–506
7. Campbell DR, Gross MD, Martini MC, Grandits GA, Slavin JL, Potter JD (1994) Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiol Biomarkers Prev 3:493–500
8. Drewnowski A, Rock CL, Henderson SA, Shore AB, Fishler C, Galan P, Preziosi P, Hercberg S (1997) Serum β-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am J Clin Nutr 65:1796–1802
9. Polsinelli ML, Rock CL, FADA, Ahlstrom Henderson S, Drewnowski A (1998) Plasma carotenoids as biomarkers of fruit and vegetable servings in women. J Am Diet Assoc 98:194–196
10. Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI (1996) Human plasma ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr 64:594–602
11. Rock CL, Flatt SW, Wright FA, Faerber S, Newman V, Kealey S, Pierce JP (1997) Responsiveness of carotenoids to high vegetable diet intervention designed to prevent breast cancer recurrence. Cancer Epidemiol Biomarkers Prev 6: 617–623
12. Zino S, Skeaf M, Williams S, Mann J (1997) Randomized controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipid and antioxidants. Br Med J 314: 1787–1791
13. Johnson EJ, Russell RM (1992) Distribution of orally administered beta-carotene among lipoproteins in healthy men. Am J Clin Nutr 56:128–135
14. Kostic D, White WS, Olson JA (1995) Intestinal absorption, serum clearance, and interactions between lutein and beta-carotene when administered to human adults in separate or combined oral doses. Am J Clin Nutr 62:604–610
15. van den Berg H, Faulks R, Fernando Granado H, Hirshchberg J, Olmedilla B, Sandman G, Southon S, Stahl W (2000) The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J Sci Food Agric 80:880–912
16. Brown ED, Micozzi MS, Craft NE, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, Smith JC (1989) Plasma carotenoids in normal men after a single ingestion of vegetables or purified beta-carotene. Am J Clin Nutr 49: 1258–1265
17. Klaui H, Bauernfeind JC (1981) Carotenoids as food colors. In: Klaui H, Bauernfeind JC (eds) Carotenoids as Colorants and Vitamin A Precursors. New York: Academic Press, pp 47–317
18. Dimitrov NV, Meyer C, Ullrey DE, Chenoweth W, Michelakis A, Malone W, Boone C, Fink G (1988) Bioavailability of beta-carotene in humans. Am J Clin Nutr 48:298–304
19. Micozzi MS, Brown ED, Edwards BK, Bieri JG, Taylor PR, Khachic F, Beecher GR, Smith JC (1992) Plasma carotenoid response to chronic intake of selected foods and beta-carotene supplements in men. Am J Clin Nutr 55:1120–1125
20. Castenmiller J, West C, Linssen J, van het Hof K, Voragen G (1999) The food matrix of spinach is a limiting factor in determining the bioavailability of beta-carotene and to a lesser extent of lutein in humans. J Nutr 129:349–355
21. Henderson C, Mobarhan S, Bowen P, Stacewicz-Sapuntzakis M, Langenberg P, Kiani R, Lucchesi D, Sugerman S (1989) Normal serum response to oral beta-carotene in humans. J Am Coll Nutr 8:625–635
22. van Vliet T, Schreurs W, van den Berg H (1995) Intestinal beta-carotene absorption and cleavage in men; response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. Am J Clin Nutr 62:110–116
23. O'Neill ME, Thurnham DI (1998) Intestinal absorption of beta-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction. Br J Nutr 79: 149–159
24. Goodman D, Blomstrand R, Werner B, Huang H, Shiratiori T (1966) The intestinal absorption and metabolism of vitamin A and beta-carotene in man. J Clin Invest 45:1615–1623
25. Blomstrand R, Werner B (1967) Studies on the intestinal absorption of radioactive beta-carotene and vitamin A in man. Scand J Clin Lab Invest 19: 339–345
26. Parker R, Swanson B, Marmor B, Goodman K, Spielman A, Brenna J, Viereck S, Canfield W (1993) Study of beta-carotene metabolism in humans using ^{13}C-beta-carotene and high precision isotope ratio mass spectrometry. Ann N Y Acad Sci 691:86–95
27. Dueker S, Jones A, Smith G, Clifford A (1994) Stable isotope methods for the study of beta-carotene-d8 metabolism in humans utilizing tandem mass spectrometry and high performance liquid chromatography. Anal Chem 66: 4177–4185
28. Novotny J, Dueker S, Zech L, Clifford A (1995) Compartmental analysis of the dynamics of beta-carotene metabolism in an adult volunteer. J Lipid Res 36: 1825–1838
29. Shiau A, Mobaraham S, Stacewicz-Sapuntzakis M, Benya R, Liao Y, Bowen P, Friedman H, Frommel TO (1994) Assessment of the intestinal retention of beta-carotene in humans. J Am Coll Nutr 13:369–375
30. Faulks R, Hart D, Wilson P, Scott J, Southon S (1997) Absorption of all trans and 9-cis beta-carotene in human ileostomy volunteers. Clin Sci (Lond) 93:585–591
31. Bowen PE, Mobarhan S, Smith JC (1993) Carotenoid absorption in humans. Methods Enzymol 214:3–17
32. Williams AW, Boileau TW, Erdman JW (1998) Factors influencing the uptake and absorption of carotenoids. Proc Soc Exp Biol Med 218:106–108
33. Zhou JR, Gugger ET, Erdman JW (1996) The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta and alpha-carotene in the ferret model. J Am Coll Nutr 15:84–91
34. Bierer TL, Merchen NR, Erdman JW (1995) Comparative absorption and transport of five common carotenoids in preruminant calves. J Nutr 125: 1569–1577
35. van het Hof KH, Gartner C, Wiersma A, Tijburg LB, Weastrate JA (1999) Comparison of the bioavailability of natural palm oil carotenoids and synthetic beta-carotene in humans. J Agric Food Chem 47:1582–1586

36. Edwards AJ, Nguyen CH, You C-S, Swanson JE, Emenhiser C, Parker RS (2002) α- and β-carotene from a commercial carrot puree are more bioavailable to humans than boiled-mashed carrots, as determined using extrinsic stable isotope reference method. J Nutr 132: 159–167
37. Erdman JW, Bierer TL, Gugger ET (1993) Absorption and transport of carotenoids. Ann N Y Acad Sci 691: 76–85
38. Parker RS (1996) Absorption, metabolism and transport of carotenoids. FASEB J 10:542–551
39. Erdman JW, Poor CL, Dietz JM (1988) Factors affecting the bioavailability of vitamin A, carotenoids and vitamin E. Food Technol 10:214–221
40. Nierenberg DW, Dain BJ, Mott LA, Baron JA, Greenberg ER (1997) Effects of 4 y of oral supplementation with beta-carotene on serum concentrations of retinol, tocopherol, and five carotenoids. Am J Clin Nutr 66: 315–1319
41. Stahl W, Schwarz W, von Laar J, Sies H (1995) All-trans beta-carotene preferentially accumulates in human chylomicrons and very low density lipoproteins compared with the 9-cis geometrical isomer. J Nutr 125: 2128–2133
42. Borel P, Grolier P, Mekki N, Boirie Y, Rochette Y, Le Roy B, Alexandre-Gouabau MC, Lairon D, Azais-Braesco V (1998) Low and high responders to pharmacological doses of beta-carotene: proportion in the population, mechanisms involved and consequences on beta-carotene metabolism. J Lipid Res 39: 2250–2260