Bioavailability of β-Carotene Is Lower in Raw than in Processed Carrots and Spinach in Women[1,2,3]

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ABSTRACT Populations at risk of vitamin A deficiency usually rely on dietary provitamin A carotenoids to meet vitamin A needs, yet bioavailability of these compounds is influenced by several factors as follows: location in the plant source, the presence of other influencing dietary components, and type and extent of processing. The purpose of this study was to examine the plasma β-carotene response to raw vs. processed carrots and spinach. Subjects were eight healthy females aged 23–36 y who consumed ∼9.3 mg β-carotene daily from either raw or thermally processed and pureed vegetables in two 4-wk treatment periods in a crossover study. Plasma concentrations of total, all-*trans*-, and *cis*-β-carotene and α-carotene were measured at base line and the end of each treatment period by using HPLC assays. Total and all-*trans* (but not *cis*) plasma β-carotene concentrations were significantly greater than base-line concentrations in the processed feeding period ($P < 0.04$) and tended to be greater in the raw feeding period ($P = 0.08$). Daily consumption of processed carrots and spinach over a 4-wk period produced an increase in plasma β-carotene concentration that averaged three times that associated with consumption of the same amount of β-carotene from these vegetables in the raw form ($P = 0.09$). Increased *cis* isomers provided in the processed vegetables did not result in significantly greater plasma *cis*-β-carotene isomer concentrations. These results suggest that isomerization of β-carotene by heat treatment does not negate the enhanced β-carotene uptake associated with consuming commercially processed vegetables compared with raw vegetables. J. Nutr. 128: 913–916, 1998.

KEY WORDS: • *β-carotene* • *bioavailability* • *vegetables* • *isomers* • *humans*

Vitamin A deficiency remains a major nutritional problem in most economically disadvantaged areas of the world (Olson 1994a, Sommer and West 1996), in which populations usually rely primarily on dietary sources of provitamin A carotenoids to meet vitamin A needs. Public health strategies that promote increased intakes of carotenoid-rich vegetables and fruits have been considered to be the most appropriate solution to the problem (Solomons and Bulux 1993), although recent studies of the efficacy of programs to improve vitamin A status by increased consumption of plant food sources of carotenoids have not been encouraging (de Pee et al. 1995).

An important consideration in the design and evaluation of such interventions is that the bioavailability of carotenoids is influenced by several factors such as characteristics of the food source, interactions with other dietary factors and various subject characteristics (Bowen et al. 1993, Erdman et al. 1993, Olson 1994b, Parker 1996). Particle size, the location of the carotenoid in the plant source (i.e., the pigment-protein complexes of cell chloroplasts vs. the crystalline form in chromoplasts) and the presence of factors that interfere with proper micelle formation are among the characteristics that can influence carotenoid uptake and absorption (Erdman et al. 1993, Rock and Swendseid 1992, Zhou et al. 1996). Although results from some studies suggest that heat treatment may improve the bioavailability of carotenoids from vegetables (Poor et al. 1993), such treatment promotes the isomerization of carotenoids from the *trans* to the *cis* forms in these foods (Chandler and Schwartz 1987). *Cis* isomers of β-carotene have been reported to be less bioavailable than all-*trans*-β-carotene, on the basis of results from laboratory studies and plasma response after administration of mixtures of isomeric forms (Erdman et al. 1993, Gaziano et al. 1995).

The purpose of this study was to examine provitamin A carotenoid bioavailability by comparing the plasma β-carotene response to daily consumption of raw vs. processed carrots and spinach in women for a 4-wk period with the use of a crossover study design. We also investigated whether an increase in the amount of *cis* isomers provided in the processed vegetables would result in a greater amount of *cis*-β-carotene isomers in the plasma. We hypothesized that consumption of processed compared with raw vegetables would result in a greater plasma β-carotene response despite a substantial increase in the proportion of *cis* isomers that were present in the processed vegetables.

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SUBJECTS AND METHODS

Subjects. Eight healthy nonsmoking female volunteer subjects, aged 23–36 y, were recruited for this study. Data from a previous study of the effect of fiber on plasma β-carotene response in similar subjects (Rock and Swendseid 1992) were used to estimate that a sample size of at least seven would allow the detection of a 20% difference in the total plasma β-carotene response after raw vs. processed vegetable feeding, with ≥80% power at the 0.05 significance level. All subjects were within 5% of desirable body weight (USDA and U.S. Department of Health and Human Services 1990), and their weights had been stable during the preceding year. Exclusion criteria included pregnancy or lactation, and any history or evidence of renal, hematological, gastrointestinal, cardiovascular, neurological or hepatic disease. All of the subjects reported that they had regular menstrual cycles. None of these subjects reported use of β-carotene supplements. Procedures for this study were approved by the Institutional Review Board of the University of Michigan School of Medicine.

Procedures. At recruitment, subjects were instructed to consume their normal diets but with limited amounts of β-carotene–rich foods (<5 mg β-carotene/d) throughout the study. Subjects were also instructed to record daily food intake during each of the two 4-wk feeding periods to enable monitoring of compliance with diet instructions. Subjects were assigned to either the raw and then processed vegetable feeding regimen or vice versa according to a crossover study design. Each feeding regimen was conducted by providing the carrots and spinach to subjects on a weekly basis with instructions to consume these vegetables daily as additional foods in the diet. Subjects were also instructed to include the vegetables in a meal in which dietary fat was present (at a level of >10 g fat or 10 mL oil or equivalent). Each feeding period began and ended at approximately the same time of the menstrual cycle (the early follicular phase). The washout period between the feeding periods was a minimum of 4 wk and was designed to achieve a similar base-line plasma concentration of total β-carotene at the beginning of each feeding period, as is the conventional approach (Bowen et al. 1993, Olson 1994b).

For the raw vegetable feeding period, the carrots and spinach were washed in cold water and sliced or torn into large pieces. For the processed feeding period, the spinach and carrots were purees that were thermally processed according to procedures accepted by the National Canners Association for in-container sterilization of low acid food products (National Canners Association 1976). The carrots were thermally processed a second time to further increase the content of *cis* isomers by transferring to conventional steel cans and heat processing at 121°C for 40 min. The carrots and spinach provided ~9.3 mg β-carotene/d in each feeding period. The amount of carrots and spinach required to achieve this dosage was determined on the basis of HPLC carotenoid analysis of the foods in the form they were to be eaten by using methods previously described (Chandler and Schwartz 1987, Lessin et al. 1997). To achieve the dosage, 113 g (~120 mL) each of processed and pureed carrots and spinach were consumed daily in the processed feeding period, and 54.9 g (approximately one medium) carrot and 39.0 g (approximately two cups) spinach were consumed daily in the raw vegetable feeding period. On the basis of HPLC analysis, the processed feeding regimen of spinach and carrots provided the β-carotene dosage as 76% all-*trans*-β-carotene and 24% *cis*-β-carotene. Of the *cis* fraction, 30% was 9-*cis*-, whereas the remainder was predominantly 13-*cis*- with negligible amounts of 15-*cis*-β-carotene. During the raw feeding regimen, the β-carotene was provided as 94% all-*trans*-β-carotene and 6% *cis*-β-carotene.

The amount of the vegetables fed was set to achieve the same dosage of β-carotene in the two feeding periods, but some α-carotene, another provitamin A carotenoid, was also present in the carrots. Although not the primary focus of this study, the amount of α-carotene provided was ~3.4 mg/d in the processed feeding period and 4.5 mg/d in the raw feeding period.

Blood samples were collected from fasting subjects on the first and last day of each feeding period. The samples were collected by venipuncture into heparin-treated tubes and were protected from light during processing and handling. Plasma was separated by centrifugation at 2300 × g at 4°C for 10 min. Plasma aliquots were stored at −70°C in cryogenic tubes until lipid extraction and analysis.

Plasma Carotenoid Analysis. Plasma carotenoid concentration was obtained by using the HPLC method of Stahl et al. (1993). The HPLC system consisted of a Waters model 501 pump and Waters model U6K injector (Waters, Milford, MA). The detector was a Linear model 203 UV-visible detector (Linear Instruments, Reno, NV). The column was a Suplex PKB-100 reversed phase column (4.6 i.d. × 250 mm) with a matching guard column (Supelco, Bellefonte, PA). The mobile phase consisted of 10:40:40:10 methanol/acetonitrile/isopropanol/water, with a flow rate of 1 mL/min and wavelength set at 450 nm. Lipid extracts were reconstituted in 200 μL 1:1 methyl-*tertiary*-butyl ether/methanol, of which 20 μL was injected. Duplicate injections were conducted for each sample. Day-to-day coefficients of variation were <10% and values for duplicate samples were within 5% for β-carotene concentration.

Statistical analysis. Initially, descriptive statistics were calculated on all variables, and distribution was examined for normality. Because the plasma concentration data were not normally distributed, the Wilcoxon Matched-Pairs Signed-Ranks nonparametric statistical test was used in the analysis of those data. Paired values for total, all-*trans*-, and *cis*-β-carotene and α-carotene plasma concentrations at base line and at the end of each feeding period were examined. Total β-carotene concentration is the sum of all-*trans*-β-carotene and the *cis*-β-carotene isomers. We also compared the percentage differences in the increase in total, all-*trans*-, and *cis*-β-carotene and α-carotene plasma concentrations in response to the two feeding regimens. Differences in percentage changes in carotenoid concentrations were compared using Student's *t* test, assuming unequal variances. All analyses were performed using the Statistical Analysis System (Version 6.11, 1995, SAS Institute, Cary, NC).

RESULTS

Review of the dietary records completed by subjects throughout the study confirmed their compliance with the feeding study guidelines. No β-carotene supplement use occurred during the study period. In **Table 1**, the medians and ranges are presented for total, all-*trans*-, and *cis*-β-carotene and α-carotene concentrations in the plasma at base line and at the end of the two feeding regimens. Only negligible or unmeasurable amounts of 9-*cis*-β-carotene were present in the plasma samples; thus concentrations of the *cis*-β-carotene isomers were combined into one value representing predominantly the 13-*cis*- and 15-*cis*-β-carotene isomers in the plasma. The processed vegetable period resulted in significant increases in plasma concentrations of both total and all-*trans*-β-carotene ($P < 0.04$). In contrast, the raw vegetable period resulted in marginally significant increases in the plasma concentrations of total and all-*trans*-β-carotene ($P = 0.08$). Plasma *cis*-β-carotene concentration did not change significantly in response to either feeding regimen. Plasma concentrations of α-carotene increased significantly in response to both raw ($P < 0.03$) and processed ($P < 0.04$) vegetable feeding regimens.

Analysis of base-line values for the two feeding periods revealed no differences in total or all-*trans*-β-carotene or α-carotene concentrations, but *cis*-β-carotene concentration was significantly higher at base line for the raw vs. processed feeding period ($P < 0.04$).

The percentage changes in plasma concentrations of total, all-*trans*-, and *cis*-β-carotene and α-carotene from base line to the end of each vegetable feeding period are also presented in Table 1. A marginally significant difference in the increase in plasma concentrations of total β-carotene ($P = 0.09$) and all-*trans*-β-carotene ($P = 0.08$) in these two regimens was observed. The percentage changes in plasma *cis*-β-carotene and α-carotene concentrations did not differ in the processed vs. raw vegetable feeding periods.

TABLE 1

*Comparison of total, all-*trans-*, and* cis-*β-carotene and α-carotene plasma response from base line to the end of the 4-wk raw and processed vegetable feeding periods in 8 healthy women*

	Raw vegetable period			Processed vegetable period		
	Base line	End	Increase	Base line	End	Increase
	μmol/L[1]		*%*[2]	*μmol/L*[1]		*%*[2]
Total β-carotene	0.490 (0.349–1.581)	0.597 (0.388–2.208)[3]	30 ± 13	0.405 (0.148–1.835)	0.829 (0.254–4.324)[4]	94 ± 31[5]
All-*trans*-β carotene	0.348 (0.211–1.367)	0.448 (0.255–1.806)[3]	38 ± 16	0.305 (0.114–1.665)	0.722 (0.222–4.078)[4]	105 ± 33[6]
cis-β-Carotene	0.142 (0.133–0.215)	0.149 (0.134–0.402)	17 ± 12	0.051 (0.033–0.170)	0.065 (0.032–0.246)	26 ± 15
Total α-carotene	0.087 (0.034–0.369)	0.129 (0.085–0.512)[4]	79 ± 27	0.103 (0.038–0.394)	0.222 (0.029–1.246)[4]	87 ± 36

1 Values are medians (ranges).
2 Values are means ± SEM.
3 A trend for an increase from base line to end for this feeding regimen, $P = 0.08$.
4 Significant increase from base line to end for this feeding regimen, $P < 0.05$.
5 A trend for a greater percentage increase in processed vs. raw vegetable feeding period, $P = 0.09$.
6 A trend for a greater percentage increase in processed vs. raw vegetable feeding period, $P = 0.08$.

DISCUSSION

Daily consumption of processed carrots and spinach over a 4-wk period produced a plasma β-carotene response that averaged three times that associated with the consumption of the same amount of β-carotene from these vegetables in the raw form ($P = 0.09$), despite the greater proportion of *cis-β*-carotene isomers provided by the processed forms. Because plasma β-carotene response is an indicator of bioavailability, these results suggest that the isomerization of β-carotene produced by heat treatment does not negate the enhanced β-carotene uptake associated with consuming cooked and pureed vegetables vs. raw vegetables. Although the processed vegetables provided proportionately greater amounts of the *cis* isomers of β-carotene in this study, a significant increase in the plasma concentrations of these isomers was not observed in the processed vegetable period.

Several characteristics of the food source have been shown to influence the bioavailability of dietary carotenoids. Pureeing vegetables results in smaller particle size and also mechanically disrupts the plant cells, so that the carotenoids are presumably more available in the intestinal lumen for absorption (Erdman et al. 1993, Parker 1996, Zhou et al. 1996). Dietary pectin, which is present in vegetables (such as carrots) along with the carotenoids, has been previously shown to adversely influence β-carotene absorption (Erdman et al. 1986, Rock and Swendseid 1992). Pectin increases the viscosity of the gastrointestinal contents, which disrupts mixing of these contents and proper micelle formation and thus interferes with carotenoid uptake (Schneeman and Tietyen 1994). Studies of the effect of heat treatment on provitamin A carotenoid bioavailability have been inconsistent. Poor et al. (1993) found a small improvement in β-carotene bioavailability associated with mild heat treatment of carrot slurries in preruminant calves. In comparison, Zhou et al. (1996) did not observe a difference in the tissue uptake of α- and β-carotene from heated and nonheated carrot juice in ferrets.

Heat treatment promotes isomerization of the carotenoids in foods, from *trans* to *cis* isomeric forms, and the degree of isomerization is directly correlated with the intensity and duration of heat processing. Fresh sweet potatoes, carrots, and tomatoes contain negligible quantities of *cis-β*-carotene, but when canned, the proportion in these vegetables is ~25, 27 and 47%, respectively (Chandler and Schwartz 1987). Analysis of the processed vegetables fed to the subjects in this study confirmed that the thermal processing had substantially increased the proportion of *cis-β*-carotene isomers in the vegetables.

Results from some studies have suggested that *cis-β*-carotene isomers have less provitamin A activity than all-*trans-β*-carotene, and that some absorption or transport discrimination between isomers may explain a (possibly) lower bioavailability of the 9-*cis-β*-carotene isomer (Erdman et al. 1993, Gaziano et al. 1995, de Pee et al. 1995). In two recent studies (Ben-Amotz and Levy 1996, Gaziano et al. 1995), a substantially greater increase in total plasma β-carotene concentration was observed after dosages of synthetic all-*trans-β*-carotene compared with provision of the same dosages from algal preparations consisting of a 50:50 mixture of all-*trans*- and 9-*cis-β*-carotene. However, differences in the formulations that were provided in the two conditions (despite identical dosages) may also have influenced the plasma responses in these studies. When equal amounts of all-*trans*- and 9-*cis-β*-carotene from an algal preparation were administered, a greater accumulation of all-*trans*- versus 9-*cis-β*-carotene occurred postdosage in the triglyceride-rich lipoproteins (Stahl et al. 1995).

In this study, the processed vegetable feeding that provided an increased proportion of the 9-*cis-β*-carotene isomer was not associated with an increase in the plasma concentration of the *cis-β*-carotene isomers. However, addressing this issue was confounded by the finding that the base-line concentrations of the *cis* isomer fractions were different for the two feeding periods despite similar total plasma β-carotene concentrations, which had guided the plan for the washout period. A difference at base line suggests the possibility that a longer washout period might have enhanced the ability to examine this variable; the ability to detect a period effect or treatment-period interaction, if present, is impaired by the small sample size. Notably, the base-line difference that occurred would have increased the likelihood of finding a greater *cis-β*-carotene response from the processed (compared with the raw) vegetable feeding, which did not occur. In stable isotope studies, isomerization of 9-*cis*- to all-*trans-β*-carotene has been shown to occur in human intestinal mucosa at physiologic doses (You et al. 1996), which suggests that the isomeric distribution of β-carotene in the diet may be less important than other factors in determining the proportions of the isomers in the body pools.

The difference between the plasma β-carotene response to the raw vegetables compared with the cooked vegetables was

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not as great as had been anticipated at the time the study was planned and subjects recruited. A greater number of subjects would have increased the power to detect differences between the two feeding regimens. Differences between these treatments were of marginal significance, and the results may not be generalizable.

Similar to the effect on β-carotene, heating tomato juice in the presence of oil slightly increases the proportion of *cis* isomers of lycopene present; this is associated with an apparent increase in total lycopene absorption (Stahl and Sies 1992). In an evaluation of lycopene bioavailability similar to the present study, the lycopene concentration in chylomicra after the consumption of tomato paste was found to be markedly higher than the concentration after the same lycopene dosage from fresh tomatoes (Gartner et al. 1997).

Compared with β-carotene supplements, providing carotenoid-rich foods that have had mild heat treatment has sometimes, but not consistently, been observed to promote an increased serum β-carotene or retinol concentration in populations and groups with poor or marginal vitamin A status (Bulux et al. 1994, de Pee et al. 1995, Lala and Vinodini 1970, Rao and Rao 1970, Solomons and Bulux 1993, Solomons 1996). Among healthy subjects in developed countries, the superior bioavailability of β-carotene supplements compared with mildly heat-treated carotenoid-rich foods has also been observed (Brown et al. 1989, Micozzi et al. 1992). Results from this study suggest that processing vegetables with heat treatment and pureeing may enhance provitamin A carotenoid uptake from these foods considerably, which may have applicability to public health efforts to combat vitamin A deficiency. However, populations that are the focus of these efforts typically have high rates of parasitic infections, consume very low fat diets, and generally have impaired absorptive capabilities as a result of malnutrition, which are all factors known to adversely influence carotenoid absorption (Bowen et al. 1993, Erdman et al. 1993, Olson 1994b, Parker 1996). Thus, results similar to those observed in healthy subjects may not be achievable in the populations at high risk for vitamin A deficiency. Nonetheless, approaches that address how carotenoids may be made more bioavailable from the food sources, through the processing and preparation methods used with these foods, deserve further investigation.

In conclusion, providing cooked and pureed vegetables rather than raw vegetables would appear to be a better approach to providing bioavailable β-carotene from these carotenoid-rich foods, which may have applicability for populations who rely on these foods to meet vitamin A requirements. Although this approach provides an increased proportion of the β-carotene as *cis* isomers, the plasma response is characterized primarily by an increase in the plasma concentration of total and all-*trans*-β-carotene.

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