The Bioavailability of β-Carotene in Stir- or Deep-Fried Vegetables in Men Determined by Measuring the Serum Response to a Single Ingestion[1]

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ABSTRACT To evaluate the bioavailability of β-carotene from plant foods, the serum β-carotene response to a single ingestion of various β-carotene sources was determined in 10 healthy men. Tested β-carotene sources included stir-fried shredded carrot, stir-fried water convolvulus leaves, deep-fried sweet potato ball, purified β-carotene in a capsule (beadlets) and beadlets with β-carotene free oriental radish (beadlets + radish). The maximal change in serum β-carotene concentration occurred at 24 or 32 h post ingestion. This response to beadlets was significantly higher than that to the other four tested β-carotene sources ($P < 0.05$). The maximal serum response to beadlets + radish was also significantly higher than that to the three food β-carotene sources ($P < 0.05$). The maximal serum response to sweet potato was significantly higher than that to water convolvulus leaves ($P < 0.05$). The bioavailability relative to β-carotene beadlets was calculated by dividing the maximal change in serum concentration to each test meal of each subject by his own serum maximal change in response to beadlets. Accordingly, the bioavailability was 65% for beadlets + radish, 33% for carrots, 26% for water convolvulus leaves and 37% for sweet potatoes. Concurrent ingestion of oriental radish reduced the bioavailability of beadlets to two-thirds of its original value, which partially accounted for the difference between the bioavailability of beadlets and natural foods. The relative bioavailability of β-carotene from stir-fried and deep-fried vegetables was about one-third to one-fourth that of the purified β-carotene beadlets. These bioavailabilities are higher than previously reported values. J. Nutr. 130: 534–540, 2000.

KEY WORDS: • *bioavailability* • *β-carotene* • *dark green leafy vegetable* • *sweet potato* • *humans*

Carotenoids possessing provitamin A activity are generally regarded as an important dietary source of vitamin A for humans (FAO/WHO 1988, NRC 1989). Among these carotenoids, β-carotene has the highest provitamin A activity (FAO/WHO 1988, NRC 1989). In addition, a potential beneficial health effect of β-carotene unrelated to its role as provitamin A has attracted more attention in the past few decades. A lower risk of certain cancers and cardiovascular disease associated with higher intake of fruit and vegetables (Van Poppel 1996) was attributed to the antioxidant action of carotenoids (Byers and Perry 1992, Krinsky 1993). Epidemiological evidence generally supports the idea that a high carotenoid diet is associated with a reduced risk of heart disease, although a random clinical trial did not support a protective role of β-carotene supplementation (Cooper et al. 1999, Kritchevsky 1999).

Bioavailability is defined as the fraction of an ingested nutrient that is available to the body for utilization in normal physiological functions or for storage (Jackson 1997). Factors affecting the bioavailability of carotenoids, proposed as "SLAMENGHI" for a mnemonic purpose, include species of carotenoids, molecular linkage, amount of carotenoids consumed in a meal, matrix in which the carotenoid is incorporated, effectors of absorption and bioconversion, nutrient status of the host, genetic factors, host-related factors and interaction (Castenmiller and West 1998, de Pee and West 1996).

The bioavailability of β-carotene from plant food sources generally is lower than that from the pure compound (Brown et al. 1989, Castenmiller et al. 1999, de Pee et al. 1995, Micozzi et al. 1992, Törronen et al. 1996). Moreover, de Pee et al. (1998) demonstrated that β-carotene is less available from dark-green leafy vegetables than from fruit. Some studies have indicated that β-carotene from raw vegetables is less available than that from cooked or processed vegetables (Rock et al. 1998, Torronen et al. 1996). The bioavailability of β-carotene from spinach leaves was elevated by treating leaves with pectinase, cellulase and hemicellulase to degrade the matrix (Castenmiller et al. 1999). While the bioavailability of β-carotene from dark green leafy vegetables has been questioned (de Pee et al. 1995), the value of β-carotene from dark

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TABLE 1

Anthropometric and serum biochemical data of men

Items	Beginning[2]	End[2]
Age, *y*	24.6 ± 1.43[1]	
Height, *cm*	171.0 ± 5.3	
Weight, *kg*	68.8 ± 8.0	69.9 ± 9.8
BMI[3], *kg/m²*	23.5 ± 1.6	23.5 ± 1.8
Serum		
Cholesterol, *mmol/L*	4.63 ± 1.22	4.34 ± 0.92
Triglyceride, *mmol/L*	0.92 ± 0.21	0.83 ± 0.38
Retinol, *μmol/L*	2.47 ± 0.39	2.91 ± 0.33**
α-Tocopherol, *μmol/L*	13.35 ± 4.28	11.22 ± 4.08*
γ-Tocopherol, *μmol/L*	4.38 ± 1.36	3.55 ± 1.44

[1] Values shown are mean ± SD, $n = 10$. Asterisks indicate significant difference between the values of beginning and end (*, $P < 0.01$; **, $P < 0.0005$) analyzed by paired *t* test.

[2] Beginning is the time when subjects were recruited and before the washout period started. End is the time when subjects finished all the five test periods (26 wk).

[3] BMI, body mass index.

green leafy vegetables in improving vitamin A status was shown in the study of Takyi (1999).

Green leafy vegetables, orange-yellow colored roots, tubers and fruits, etc. contribute about 70% of the vitamin A intake of a Taiwanese population (Pan et al. 1991). However, the bioavailability of β-carotene in these provitamin A-contributing foods in the Taiwanese diet is still unclear. Instead of eating raw vegetables as in a Western-style salad, eating stir-fried vegetables is the most prevalent way of consuming vegetables in Taiwan. In the present study, healthy young men were the subjects used for examining the serum β-carotene response to a single ingestion of 12 mg β-carotene from stir-fried vegetables or deep-fried sweet potato ball in comparison to that from β-carotene capsule. Substantial interindividual variation in the magnitude of the serum response after supplementation has been reported by many investigators (Brown et al. 1989, Carughi and Hooper 1994, Dimitrov et al. 1988, Nierenberg et al. 1991, Torronen et al. 1996). We therefore employed a cross-over design so that each subject served as his own control in calculating the bioavailability data. To clarify the effects due to the presence of plant materials other than carotenoids, the β-carotene capsule was served without or with stir-fried shredded oriental radish.

MATERIALS AND METHODS

Subjects. Thirteen young men, aged 23–28 y, enrolled in this study. They were nonsmokers and healthy, as evaluated by a screening history, medical evaluation and laboratory tests at the beginning and rechecked at the end of the study. The study was approved by Department of Health, Executive Yuan, Taiwan. The subjects were senior, graduate or medical students of our University who were recruited by a post in the campus bulletin board system. After receiving thorough instruction, they completely understood the study and all gave their written informed consent. Three subjects withdrew in the middle because they could not adhere to the self-selected low β-carotene diet. Therefore, 10 subjects completed the whole period of study which lasted for 26 wk. During the study, the physical condition of the subjects was monitored and managed by a physician (C. Y. Chen). Subjects' data are listed in **Table 1**.

Study design. The study tested the serum response to the five test meals in which the β-carotene was from: *i*) β-carotene capsule (beadlets); *ii*) β-carotene capsule with stir-fried shredded oriental radish (beadlets + radish); *iii*) stir-fried shredded carrots, *iv*) stir-fried water convolvulus leaves and *v*) deep-fried sweet potato ball. Each of the sources provided 12 mg of β-carotene as assessed by HPLC (**Table 2**). Water convolvulus (*Ipomoea aquatica,* the Chinese name pronounced as "Kong-Hsin Tsai" means "hollow" vegetable) is a very popular and low-priced vegetable that is available all year in Taiwan. It was chosen as the source of β-carotene from dark green leafy vegetable. Sweet potato was chosen as the source of β-carotene from a tuber. Carrot was also included so that a comparison between our results with the existing literature would be possible. A beadlets + radish test meal was employed to examine the effect of the presence of plant materials on the bioavailability of β-carotene from the capsule. The oriental radish is a carotenoid-free root vegetable. The portion size was calculated so that this test meal provided nonfat dry matter in an amount equivalent to the carrot test meal.

A cross-over design was employed such that each subject ingested the five test meals in five test periods in a random order. The study started with a washout period of 6 wk, followed by five test periods. Each test period consisted of 2 wk of the experiment followed by a 2-wk washout period. On the morning of the 1st d (d 0) of each experimental period, blood from overnight fasting was drawn (0 h), and then the test meals were eaten as the breakfast. At the time points of 8, 24, 32, 48 h, 3, 7 and 14 d postingestion, blood samples from fasting men were drawn again (except that blood samples from nonfasting men were taken at 8 and 32 h postingestion). Blood samples were analyzed for serum β-carotene.

Components of each test meal are listed in **Table 3**. Based on the results of HPLC analysis of β-carotene content, the amount of test food samples that provided 12 mg of β-carotene was calculated. Other food items were adjusted so that each meal provided about 4200 kJ with about 55 g fat except that for the sweet potato meal (**Table 4**). To provide fat-free dry matter from a β-carotene-free root vegetable in an amount similar to that of 273 g of stir-fried shredded carrot, 636 g of stir-fried shredded radish was included in the "beadlets + radish" meal.

The subjects consumed self-selected low β-carotene diets through

TABLE 2

Analysis of the proximate composition and β-carotene concentration of the prepared test food samples

	Stir-fried shredded carrot[1]	Stir-fried shredded radish[1]	Stir-fried water convolvulus leaves[1]	Deep-fried sweet potato ball[1]
			g/100 g	
Moisture	71.13	86.71	82.24	29.22
Crude protein	1.91	0.98	3.06	2.14
Crude fat	15.72	7.66	12.46	20.49
Crude fiber	1.18	0.73	0.49	0.67
Crude ash	1.17	0.67	0.7	0.43
Nitrogen-free extract	8.89	3.25	1.05	47.05
			mg/100g	
β-Carotene[2]	4.40	0	4.10	4.20

[1] Carrot and oriental radish were peeled and shredded. Water convolvulus leaves were picked free of stems and petioles. Each batch of 600 g shredded carrot or water convolvulus leaves was stir-fried in 90 g of soybean oil for 4 min in a Chinese wok. Shredded oriental radish was squeezed to remove 20 g/100 g juice before stir-fried in a similar way except less cooking oil was used. Sweet potato was peeled, steamed and mashed. Four kg of mashed sweet potato was blended with 1 kg of table sugar, 0.4 kg of cassava starch, 0.35 kg of all purpose wheat flour as well as 0.625 kg of soybean oil. The mixture was shaped into sweet potato balls (12–13 g/ball) and prefried in soybean oil at 180°C for 4 min and refried at 180°C for 2 min before served.

[2] Analyzed by reverse-phase HPLC.

TABLE 3

Composition of test meals

Test sample/meal	β-Carotene source	Other components[1]
Beadlets	1 Capsule containing 12 mg β-carotene	3 slices ham 2 slices toast 1 cup low-fat milk 3 sheets egg roll skin 90 g ground peanuts
Beadlets + radish	1 capsule containing 12 mg β-carotene	636 g stir-fried shredded radish 3 slices ham 2 slices toast 1 cup low-fat milk 3 sheets egg roll skin
Carrot	273 g Stir-fried shredded carrot	3 slices ham 2 slices toast 1 cup low-fat milk 3 sheets egg roll skin
Water convolvulus leaves	293 g Stir-fried water convolvulus leaves	3 slices ham 2 slices toast 1 cup low-fat milk 3 sheets egg roll skin 18 g ground peanut 10g table sugar
Sweet potato	286 g of Fried sweet potato ball	3 slices ham 1 cup low fat milk

[1] 1 slice of ham was 20 g, 1 slice of toast was 30 g, 1 cup of milk was 240 mL and 1 sheet of egg roll skin was 15 g. The formulation of the meals was aimed at providing 12 mg of β-carotene from the respective source while maintaining the energy providing food items similar. The distribution of energy in each test meal is shown in Table 4.

out the 26-wk study. Before the study started, they were instructed of how to choose a balanced diet excluding foods rich in β-carotene such as: dark-green leafy vegetables, yellow-orange colored root, tubers or fruits. To monitor their dietary intake, subjects were asked to complete dietary records for 4 d. They were consulted weekly by a dietician throughout the study period to check for the adherence of their self-selected low β-carotene diets. To ensure an adequate vitamin A status, a capsule containing 600 μg of vitamin A (retinol acetate) was provided to each subject every day, preferably to be taken after supper or dinner.

Preparation and analysis of foods. Purified β-carotene (10% beadlet form, from Hoffman-La Roche Co., Nutley, NJ) was packed in capsules, each providing 12 mg of β-carotene. A batch of high β-carotene sweet potato (TN-64) was kindly provided by Dr. Yung-Chung Lai of the Chia-Yi Agricultural Experimental Station (Chia-Yi, Taiwan). The sweet potato was peeled, steamed and mashed. Mashed sweet potato (4 kg) was blended with 1 kg of table sugar, 0.4 kg of cassava starch, 0.35 kg of all-purpose wheat flour as well as 0.625 kg of soybean oil. The mixture was shaped into sweet potato balls (12–13 g/ball) and prefried in soybean oil at 180°C for 4 min. A whole batch of prefried sweet potato ball was stored at −20°C. At each test period, a portion of two or three servings was thawed and fried at 180°C for 2 min before serving. Batches of carrots, oriental radish (daikon) and water convolvulus were purchased from the Central Market of the Taipei Fruits and Vegetables Distribution Company. Carrots were peeled, shredded and stir-fried. Every batch of stir-fried shredded carrots was prepared by heating 90 g of soybean oil in a Chinese wok heated by a gas stove to 240°C. Shredded carrots (600 g) were then added and stir-fried for 4 min under maximum heat of the stove. Stir-fried shredded radish was prepared in a similar manner except that 20 g/100 g juice was squeezed out from the shredded radish before stir-frying, and 28 g of soybean oil was used for stir-frying each batch of 600 g of shredded radish. Leaves of water convolvulus were picked free of petiole, washed and stir-fried in a manner similar to that for the preparation of stir-fried shredded carrots. The stir-fried samples were also frozen, and a portion of two or three servings was thawed and reheated in a microwave oven before serving. A total of at least 14 servings of each test food was prepared before the first test period started. One serving was used for chemical analysis.

TABLE 4

The distribution of energy-containing nutrients in test meals

Test sample/meal[1]	Protein	Fat	Carbohydrate	Energy	Protein energy/ total energy	Fat energy/ total energy	Carbohydrate energy/total energy
	g			kJ	%		
Beadlets	48.0	53.4	86.3	4255	18.9	47.2	33.9
Beadlets + radish	32.1	59.1	90.6	4274	12.5	52.0	35.4
Carrot	31.0	53.3	94.2	4099	12.7	48.9	38.4
Water convolvulus leaves	39.2	55.5	86.2	4185	15.7	49.9	34.4
Sweet potato	20.8	52.5	180.6	5344	6.5	37.0	56.5

[1] Components of each test meal are shown in Table 3. The formulation of the meal was aimed at maintaining the energy providing food items similar.

The prepared food samples were analyzed for the proximate composition according to the methods of AOAC. For the analysis of β-carotene by HPLC, samples were homogenized in distilled water successively with a Waring Blender and a Potter-Elvehjem-type tissue homogenizer. Homogenate (5 mL) was added with β-apo-8′-carotenal as an internal standard and saponified solution consisting of 3 mL of absolute ethanol (containing 10 g/L of pyrogallol), 40 mg of L-ascorbic acid and 7 mL of saturated KOH. The mixture was saponified at 70°C for 2 h and extracted three times with 10 mL of *n*-hexane. The extract was washed twice with 10 mL of distilled water, and the solvent was evaporated off under vacuum. The residue was redissolved in 1 mL of a mixture of methanol/chloroform = 2:1 (v/v), and subjected to HPLC. The HPLC was performed using a Jasco 880-PU intelligent pump equipped with a Jasco 851-AS Auto-Sampler and a Jasco 970-UV Intelligent UV/Vis detector. A C18 reverse-phase column (Lichrospere 100-RP18, 4 X 125 mm, 5 μm; Merck, Darmstadt, Germany) was used. The flow rate was set at 1 mL/min, and the wavelength of the detector was set at 436 nm for the detection of β-carotene. The mobile phase was acetonitrile/chloroform/methanol = 46:8:46 (v/v/v). The method was originally developed by Dr. Samson Tsou of the Asian Vegetable Research and Development Center, Shan-Hua, Tainan, Taiwan (personal communication) and validated for the recovery and reproducibility in our laboratory. The retention time for the all-*trans*-β-carotene standard was 13.3 min.

Serum analysis. Blood samples were drawn into vacuum tubes (Vacutainer®; Becton Dickinson, Franklin Lakes, NJ). Serum was separated and stored in aliquots at −35°C. For the analysis of β-carotene, 0.5 mL serum was spiked with 1 μg of β-apo-8′-carotenal (in *n*-hexane) as an internal standard and mixed with 0.5 mL of absolute ethanol (containing 10 g/L pyrogallol). The *n*-hexane (5 mL) was added to extract the nonpolar fraction. The *n*-hexane layer was removed (4.5 mL), solvent evaporated under a vacuum and redissolved in 200 μL of a solvent mixture [methanol/chloroform (2:1, v/v)] for injecting into the HPLC. The instruments and conditions of the HPLC for the analysis of serum β-carotene were as described above for the analysis of β-carotene in food. Serum samples taken at the eight time-points for a subject within a test period were always analyzed in a same batch. Light was avoided by shielding tubes with aluminum foil. The CV for the batch-to-batch analysis over a year was 13%. To check the extent of change in serum retinol palmitate, aliquots of remaining serum samples were spiked with retinol acetate as an internal standard, extracted with a mixture of chloroform/methanol (1:1, v/v) and reextracted by chloroform. The chloroform extracts were washed with distilled H_2O and the solvent removed by a stream of N_2. The residue was dissolved in a mixture of methanol/chloroform (4:1, v/v) and analyzed by reverse-phase HPLC. The instruments, column and setting were similar to that described above for the analysis of β-carotene, except that pure methanol was used as the mobile phase and the wavelength of the UV/VIS detector was set at 325 nm. The retention time of retinol palmitate was 20.6 min.

Statistical analysis. Data are expressed as means ± SD except that in **Figure 1**. For the purpose of clarity of this figure, the error bar indicated half of the SEM. The maximal change in serum β-carotene of each subject after the ingestion of each test meal which usually occurred 24–32 h after the meal was used for the quantitative comparison of serum responses and for calculation of the bioavailability. The significance of differences in the serum response among the five test meals was analyzed by one-way ANOVA and Duncan's multiple range test. Due to heterogeneous variation, data were transformed to square roots before the statistical analysis was performed. To calculate bioavailability, the maximal change in serum β-carotene concentration after the ingestion of β-carotene capsule (beadlets) of each subject was considered as 100%, and the serum response to each of the remaining four test meals for each subject was divided by his own serum response to β-carotene capsule (beadlets). The significance of difference in the bioavailability among the test meals was also analyzed statistically by one-way ANOVA and Duncan's multiple range test after data were transformed to square roots. Other serum biochemical determinations were compared by paired *t* test for the significance of difference between the samples taken at the beginning of the study and at the end of the initial 6-wk washout period or after the 26-wk experiment. Differences of $P < 0.05$ were considered significant.

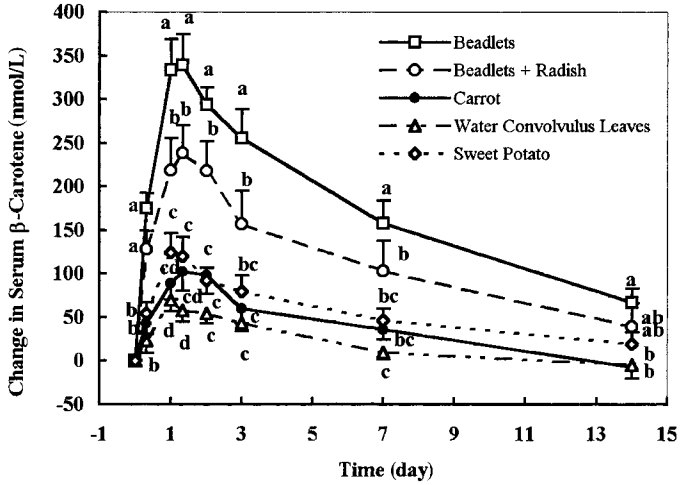

FIGURE 1 Changes in serum β-carotene concentration in men after the single ingestion of 12 mg of β-carotene from a capsule without (beadlets) or with stir-fried shredded radish (beadlets + radish) or from stir-fried shredded carrot (carrot), stir-fried water convolvulus leaves (water convolvulus leaves) or deep-fried sweet potato ball (sweet potato). A cross-over design was used such that each of the 10 subjects received the five test meals in five test periods in a random order. For each test period, the test meal was eaten as the breakfast only on d 0, and changes in serum β-carotene concentration were monitored for 2 wk. Each 2-wk experimental period was followed by a 2-wk washout period before the next test period started. Each data point is the mean and the error bar represents half of the SEM, $n = 9–10$. Data at each time-point labeled with different letters are significantly different ($P < 0.05$).

RESULTS

The body mass index and concentrations of lipids, retinol and tocopherol in serum of the 10 subjects were all in the normal range. There was no significant change ($P > 0.05$) in body weight, body mass index or serum lipids after the 26-wk experiment. The serum retinol concentration slightly but significantly increased $P < 0.05$, probably due to the daily vitamin A supplementation. In contrast, serum α-tocopherol, but not γ-tocopherol, was lower at the end compared to at the beginning ($P < 0.05$), probably due to the limited intake of deep-colored vegetables including dark-green leafy vegetables.

The serum β-carotene concentration of the 10 subjects at the beginning was 373.6 ± 165.3 nmol/L. The value presumably was indicative of normal status of the men. After the 6-wk washout period, the value significantly decreased to 187.6 ± 150.8 nmol/L ($P < 0.0005$). The average change between the two time points was 186.1 ± 53.4 nmol/L, representing a 50% reduction in serum β-carotene concentration.

After the ingestion of test meals, serum β-carotene concentrations increased promptly, reached maximal levels at 24 to 32 h, and then decreased gradually until 2 wk after the meal consumption (Fig. 1). Among the five test meals, β-carotene capsule only (beadlets) induced the highest serum response and water convolvulus leaves resulted in the lowest response. The increases in serum β-carotene concentration were significantly higher after consumption of β-carotene from capsules (beadlets and beadlets + radish) than that from food at 8 h after the test meal ($P < 0.05$). At 24 and 32 h after meal

TABLE 5

Maximal change in serum β-carotene concentration in men after a single ingestion of purified β-carotene in a capsule (beadlets) or of food sources and the bioavailability of β-carotene relative to the purified β-carotene[1]

Test sample	Maximal change in serum β-carotene[2]	Bioavailability[2]
	nmol/L	%
Beadlets	348.9 ± 106.4[a] (9)	100[a] (9)
Beadlets + radish	247.2 ± 96.1[b] (10)	65.35 ± 23.13[b] (8)
Carrot	111.8 ± 72.5[cd] (10)	32.97 ± 16.07[c] (9)
Water convolvulus leaves	77.1 ± 42.5[d] (10)	25.54 ± 12.00[c] (9)
Sweet potato	128.0 ± 65.6[c] (10)	37.27 ± 19.75[c] (9)

[1] Values shown are mean ± SD for the number of subjects indicated in the parentheses. Values with the same superscript letters are not significantly different ($P > 0.05$).

[2] Using a cross-over design, each subject ingested the five tested β-carotene sources in random order in five respective test periods. On each test period, the test meal was eaten as the breakfast only on d 0, and changes in serum β-carotene was monitored for 2 wk. Each 2-wk experimental period was followed by a 2-wk washout period before the next test period started. Maximal change in serum β-carotene occurred at 24 or 32 h post ingestion.

[3] To calculate bioavailability, maximal change of serum β-carotene concentration from the ingestion of β-carotene capsule (beadlets) of each subject was taken as 100%; the serum response to each of the remaining four test meal of each subject was respectively divided by his own serum response to β-carotene capsule (beadlets).

consumption, when the serum reached the peak value, the response to the beadlets was significantly higher than to the other four test meals ($P < 0.05$). At these same time points, the change in serum β-carotene for beadlets + radish was significantly lower than for beadlets ($P < 0.05$) but significantly higher than for carrot, water convolvulus leaves and sweet potato ($P < 0.05$). The increases in serum β-carotene 24 and 32 h after the ingestion of sweet potato were not significantly different from that of carrot ($P > 0.05$), but significantly higher than that after eating water convolvulus ($P < 0.05$). The maximal changes in serum β-carotene concentration after consumption of the five test meals are shown in **Table 5**.

To calculate the β-carotene bioavailability, the maximal change in serum β-carotene concentration after the ingestion of β-carotene capsule (beadlets) was considered 100%. The bioavailability of β-carotene from carrot, water convolvulus leaves and sweet potato did not differ significantly but were significantly lower than that of the β-carotene capsule (beadlets and beadlets + radish) (Table 5, $P < 0.05$). The bioavailability of β-carotene capsule was significantly lowered by the simultaneous intake of radish ($P < 0.05$).

After the ingestion of β-carotene from test meals, a small increase in serum retinol palmitate was noticed which peaked at 8 h after the meal. The maximal increases in serum retinol palmitate for the five dietary treatments did not differ and were 28.4 ± 25.8 (beadlets), 24.9 ± 28.2 (beadlets + radish), 23.3 ± 32.3 (carrot), 11.2 ± 6.8 (water convolvulus leaves) and 17.9 ± 13.9 (sweet potato), nmol/L, respectively. There was no significant correlation between maximal increases in serum retinol palmitate and serum β-carotene response.

DISCUSSION

The increase in serum β-carotene concentration in response to the ingestion of β-carotene has been an accepted method for the assessment of its bioavailability. The drawback most researchers commonly encountered is substantial inter-individual variation. There is no exception with the present study; for example, the CV was over 50% for the maximal changes after the ingestion of carrots, water convolvulus leaves and sweet potatoes (Table 5).

Most of the recent bioavailability studies measured serum response after repeated daily consumption of a food for a period of weeks or months. To achieve a complete cross-over design within a reasonable period of time, we adapted the method of a single ingestion used by Brown et al. (1989), so that a long washout period between two experimental periods could be avoided.

The serum responses to β-carotene capsules (beadlets) in this study and to 12 mg of purified β-carotene reported by Brown et al. (1989) are remarkably similar. Although both studies used similar amounts of carrots, our carrot sample (273 g) provided 12 mg of β-carotene, while in the Brown et al. 1989 study, 272 g of carrot provided 29 mg of β-carotene. We repeated and confirmed our HPLC analysis. Moreover, our carrot β-carotene content was close to that listed in the newly developed Taiwan food composition database (FIRDI 1997). The discrepancy may arise from different varieties or production conditions, etc. Based on the change in serum β-carotene concentration, the reported bioavailabilities of carrot β-carotene were 21% (Brown et al. 1989, 29 mg of β-carotene one ingestion, cooked), 18% (Micozzi et al. 1992, 29 mg of β-carotene daily for 6 wk, cooked), 26% (raw) or 45% (carrot juice) (Törronen et al. 1996, 12 mg of β-carotene daily for 6 wk). Our result for stir-fried shredded carrot (33%) seems to be higher (Table 5) than these reported data for solid carrots. Some major differences in our study design include the cooking method, the consumption of a high fat meal, the dose and the single ingestion method. The small difference between the bioavailability data for cooked carrots from the study of Brown et al. (1989) and from the study of Micozzi et al. (1992) (21% vs. 18%) implies that the bioavailability obtained did not vary greatly when the experimental design was switched from single ingestion to repeated dosing.

The bioavailability of β-carotene from dark green leafy vegetables was reported to be as low as 7% (de Pee et al. 1995) and 5–6% (Castenmiller et al. 1999). In the present study, the bioavailability of β-carotene from stir-fried water convolvulus leaves was higher (26%, Table 5) than the reported data. In the study of Castenmiller et al. (1999), 10 mg of β-carotene from blanched spinach was supplemented to healthy subjects daily for 3 wk. In addition to the differences in the dark-green leafy vegetable sample used (spinach vs. water convolvulus) and the single vs. repeated ingestion design, the stir-frying, as well as the high fat nature of our test meals, were other major differences between our study and the study of Castenmiller et al. (1999). In the study of de Pee et al. (1995), 3.5 mg of β-carotene from stir-fried dark green leafy vegetables were supplemented daily to lactating women for 12 wk. The dose used in the study of de Pee et al. (1995) is lower than the dose used in our study. Furthermore, the physiological/health conditions and the nutritional status of their subjects, lactating women with marginal vitamin A and iron status, were in greater physiological needs. Nevertheless, the prevalence of parasite infection in their subjects may be a strong adverse effecter upon the absorption and utilization of nutrients from

natural foods. By pretreating helminthic worm infestation with mebendazole for 3 d, malnourished preschool children significantly improved their vitamin A status after daily consumption of dark green leafy vegetables with fat for 3 mo (Takyi 1999). Moreover, the preparation of test samples and the high fat nature of our test meals may be attributed to the discrepancy of the bioavailability data. The β-carotene content of the test samples used in the study of de Pee et al. (1995) (3.5 mg/100–150 g portion) revealed that stem and petiole might all have been included in their samples. This not only decreased the β-carotene concentration but also increased the dietary fiber level of the test vegetable samples. Furthermore, in addition to the high fat nature of our test meal, we seem to have used more cooking oil in our stir-frying. The fat concentration of our stir-fried water convolvulus leaves was 12.5 g/100 g, compared to the value of 7.8 g/100–150 g portion in the study of de Pee et al. (1995). Castenmiller and West (1998) pointed out that there is some increase in serum β-carotene concentration in response to a high fat diet. Takyi (1999) also demonstrated that because of the fat content of the test meal, malnourished children could obtain more serum retinol by consuming dark green, leafy vegetables.

Sweet potato was an important staple food in Taiwan four decades ago before the economic development started. The sweet potato sample used in this study (TN-64) is a locally developed variety that is especially high in β-carotene. Due to the potential importance of fat in the absorption of β-carotene, deep-frying was chosen as the cooking method. The serum response to the deep-fried sweet potato ball was significantly higher than that to the stir-fried water convolvulus leaves ($P < 0.05$, Fig. 1 and Table 5). Because of the carbohydrate-rich nature of sweet potato and the carbohydrate added through the preparation of the deep-fried sweet potato ball, the test meal of this β-carotene source provided more energy, mainly from carbohydrate, than the remaining test meals. However, it seems unlikely that the absorption and utilization of β-carotene would be influenced by a higher intake of carbohydrate.

Since the subjects consumed a low β-carotene diet for as long as 26 wk, 600 μg of retinol acetate (recommended daily intake in Taiwan) were supplemented daily to lower the risk of vitamin A deficiency. Although this was for ethical purposes, maintaining an adequate vitamin A status may also be important since it presumably can minimize the bioconversion of β-carotene to vitamin A and thereby maximize its recovery in serum as β-carotene (Castenmiller and West 1998). Results from animal studies indicated that high vitamin A intake decreased dioxygenase activity and bioconversion (van Vliet et al. 1996).

Simultaneous ingestion of radish, a root vegetable free of β-carotene significantly reduced the serum response and bioavailability of β-carotene from the capsule ($P < 0.05$, Table 5). Thus, plant materials or dietary fiber hinders the bioavailability of β-carotene that has been demonstrated (Castenmiller et al. 1999, Erdman et al. 1986, Rock and Swendseid 1992). This partially accounted for the difference in bioavailability of β-carotene between capsules and root vegetables such as carrots. However, the difference between beadlets + radish and carrot was significant ($P < 0.05$, Table 5), suggesting that there are other factors such as the matrix effect of food (Castenmiller et al. 1999, Törronen et al. 1996), the presence of other carotenoids and the physicochemical form of β-carotene affecting the bioavailability. In dark-green leafy vegetables, carotenoids are entrapped and complexed with protein in chloroplasts and within cell structures, and hence are the least available. The β-carotene is dissolved in oil droplets in chromoplasts in sweet potato and can be extracted readily during digestion. The α- and β-carotenes exist as crystals in carrots so it takes a longer time for solubilization in the gastrointestinal tract; this time may exceed the normal transit time (Castenmiller and West 1998).

In conclusion, the bioavailability of β-carotene from capsules plus radish, stir-fried shredded carrot, stir-fried water convolvulus leaves and deep-fried sweet potato ball were 65, 33, 26 and 37% respectively, relative to β-carotene from the capsules alone which was considered to be 100%. Concurrent ingestion of β-carotene-free plant materials such as oriental radish decreased the bioavailability of β-carotene from the capsules. The bioavailabilities of β-carotene from dark-green leafy and root vegetable were not as low as the values reported in the existing literature. It is unclear if this was because the experimental conditions we used in this study were optimal for the absorption and utilization of β-carotene from food.

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LITERATURE CITED

Brown, E. D., Micozzi, M. S., Craft, N. E., Bieri, J. G., Beecher, J., Edwards, B. K., Rose, A., Taylor, P. R. & Smith, J. C., Jr. (1989) Plasma carotenoids in normal men after a single ingestion of vegetables or purified β-carotene. Am. J. Clin. Nutr. 49: 1258–1265.

Byers, T. & Perry, G. (1992) Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Ann. Rev. Nutr. 12: 139–159.

Carughi, A. & Hooper F. G. (1994) Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture. Am. J. Clin. Nutr. 59: 1369–1375.

Castenmiller, J. J. M. & West, C. E. (1998) Bioavailability and bioconversion of carotenoids. Ann. Rev. Nutr. 18: 19–38.

Castenmiller, J. J. M., West, C. E., Linssen, J. H., van het Hof, K. H. & Voragen, A. G. J. (1999) The food matrix of spinach is a limiting factor in determining the bioavailability of β-Carotene and to a lesser extent of lutein in humans. J. Nutr. 129: 349–355.

Cooper, D. A., Eldridge, A. L. & Peter, J. (1999) Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: a review of recent research. Nutr. Rev. 57: 201–214.

de Pee. S. & West, C. E. (1996) Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur. J. Clin. Nutr. 50: S38-S63.

de Pee, S., West, C. E., Muhilal, Karyadi, D. & Hautvast, J. G. A. (1995) Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 346: 75–81.

de Pee, S., West, C. E., Permaesih, D., Martuti, S., Muhilal & Hautvast, J. G. A. J. (1998) Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentration of retinol and β-Carotene in schoolchildren in Indonesia. Am. J. Clin. Nutr. 68: 1058–1067.

Dimitrov, N. V., Meyer, C., Ullrey, D. E., Chenoweth, W., Michelakis, A., Malone, W., Boone, C., & Fink, G. (1988) Bioavailability of β-carotene in humans. Am. J. Clin. Nutr. 48: 298–304.

Erdman, J. W. Jr, Fahey, G. C. Jr. & White, C. B. (1986) Effects of purified dietary fiber sources on β-carotene utilization by the chick. J. Nutr. 116: 2415–2423.

FAO (Food and Agriculture Organization) (1988) Requirements of Vitamin A, Iron, Folate, and vitamin B_{12}. Report of a joint FAO/WHO Expert Consultation. FAO Food and Nutrition Series No. 23: Food and Agriculture Organization, Rome.

FIRDI (Food Industry Research and Development Institute) (1997) Taiwan food and nutrients composition database. Food Industry Research and Development Institute, Shin-Chu, Taiwan.

Jackson, M. J. (1997) The assessment of bioavailability of micronutrients: introduction. Eur. J. Clin. Nutr. 51: S1-S2.

Krinsky, N. I. (1993) Actions of carotenoids in biological systems. Ann. Rev. Nutr. 13: 561–587.

Kritchevsky, S. B. (1999) β-Carotene, carotenoids and the prevention of coronary heart disease. J. Nutr. 129: 5–8.

Micozzi, M. S., Brown, E. D., Edwards, B. K., Bieri, J. G., Taylor, P. R., Khachik, F., Beecher, G. R. & Smith, J. C., Jr. (1992) Plasma carotenoid response to chronic intake of selected foods and β-Carotene supplements in men. Am. J. Clin. Nutr. 55: 1120–1125.
National Research Council (1989) 7. Fat Soluble vitamins. In: Recommended Dietary Allowances. 10th Ed. pp. 78–92, National Academy Press, Washington, D.C.
Nierenberg, D. W., Stukel. T. A., Baron, J. A., Dain, B. J., Greenberg, E. R. & Skin Cancer Prevention Study Group (1991) Determinants of increase in plasma concentration of β-carotene after chronic oral supplementation. Am. J. Clin. Nutr. 53: 1443–1449.
Pan, W. H., You, S. L., Hsu, C. P., Chou, J. & Huang, P. C. (1991) Major food contributors of various nutrients consumed by Chinese population in Taiwan, 1980–1981. (II) Vitamins and Minerals. J. Chinese Nutr. Soc. 16: 21–27.
Rock, C. L., Lovalvo, J. L., Emenhiser, C., Ruffin, M. T., Flatt, S. W. & Schwartz, S. J. (1998) Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. J. Nutr. 128: 913–916.
Rock, C. L. & Swendseid, M. E. (1992) Plasma β-carotene response in humans after meals supplemented with dietary pectin. Am. J. Clin. Nutr. 55: 96–99.
Takyi, E. E. K. (1999) Children's consumption of dark green, leafy vegetables with added fat enhances serum retinol. J. Nutr. 129: 1549–1554.
Törronen, R., Lehmusaho, M., Hakkinen, S., Hanninen, O. & Mykkanen, H. (1996) Serum β-Carotene response to supplementation with raw carrots, carrot juice or purified β-Carotene in healthy non-smoking women. Nutr. Res. 16: 565–575.
Van Poppel, G. (1996) Epidemiological evidence for β-carotene in prevention of cancer and cardiovascular disease. Eur. J. Clin. Nutr. 50: S57-S61.
van Vliet, T., van Vlissingen, M. F., van Schaik, F. & van Den Berg, H. (1996) β-carotene absorption and cleavage in rats is affected by the vitamin A concentration of the diet. J. Nutr. 126: 499–508.

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