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CHANGES IN PLASMA CAROTENOID, ALPHA-TOCOPHEROL, AND LIPID PEROXIDE LEVELS IN RESPONSE TO SUPPLEMENTATION WITH CONCENTRATED FRUIT AND VEGETABLE EXTRACTS: A PILOT STUDY

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ABSTRACT

Studies over the last two decades equating diet with chronic diseases have linked the highest consumption of mixed fruits and vegetables to a reduced risk of coronary heart disease (CHD), stroke, cataracts, and cancer at multiple sites. High levels of natural antioxidants, including the carotenoids, tocopherols, and ascorbic acid, appear to be responsible for these reductions in risk. However, long-term intervention studies to alter chronic disease outcomes have generally used a single nutrient such as beta-carotene at high doses, and results have been disappointing. Because antioxidants have multiple and synergistic interactions and also exhibit compartmentalization and tissue specificity, it appears desirable to use supplementation that increases blood levels while simulating combinations of these chemoprotective substances in amounts more closely approximating amounts of mixed diets. This study measured carotenoid and tocopherol levels in human plasma after supplementation with dehydrated fruit and vegetable extracts (JuicePlus+™). Serum lipid peroxides were also measured to assess the effectiveness of supplementation in modifying oxidative processes. Fifteen healthy adults (10 women, 5 men; age range, 18 to 53 years) consumed supplements twice daily with meals for 28 days, with fasting plasma and serum samples taken at baseline and 7, 14, and 28 days. After 28 days, plasma antioxidant levels increased significantly: beta-carotene, 510%; alpha-carotene, 119%; lutein/zeaxanthin, 44%; lycopene, 2046%; and alpha-tocopherol, 58%. Serum lipid peroxides decreased fourfold after 7 days and remained significantly lower than baseline at 28 days (baseline, 16.85 ± 16.91 μmol/mL; 28 days, 4.22 ± 3.78 μmol/mL). Decreases in lipid peroxide levels were coincident with increases in carotenoids and alpha-tocopherol, and reflect functionally improved oxidative defense mechanisms. Because these bioactive compounds can act synergistically, the effect cannot be attributed to any one component, but it may reflect a combined mechanism of antioxidant defense. Marked increases in plasma

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levels of predominant dietary carotenoids and alpha-tocopherol in all subjects indicate that supplementation with fruit and vegetable concentrates may prove effective in future intervention studies.

INTRODUCTION

Free radical-induced damage to cell membranes, DNA, and other vital cell constituents may contribute to cardiovascular disease, cancer, and other degenerative disorders.[1,2] Vitamin E is the most effective scavenger of peroxyl free radicals in biological membranes.[1] Carotenoids, including beta-carotene and lycopene, and the oxycarotenoids—zeaxanthin and lutein—are potent quenchers of single molecular oxygen in the lipid phases.[3–6] The antioxidant properties of these nutrients may provide protection against the above disorders.

Epidemiologic evidence has equated high dietary intake of vitamins E and C and carotenoids with a reduced risk of coronary heart disease (CHD),[7] cancer,[8] stroke,[9] and cataracts.[10] Studies[7,11–14] in which blood levels of these same compounds were measured showed that as blood levels of these antioxidants increase, the risk of disease decreases. Furthermore, suboptimal levels of each factor increase the risk singly, whereas combined deficits of antioxidants increase risk by a multiple factor.[15]

Observed interactions between antioxidants indicate that ascorbic acid can reduce oxidized tocopherol,[16] glutathione can regenerate ascorbate from dehydroascorbate,[17] and beta-carotene can act synergistically with alpha-tocopherol.[18] Carotenoids also interact with each other, and the ability to use one may be affected by another.[19–21]

In recent intervention trials for the prevention of cancer or CHD, large oral doses of beta-carotene (15 to 50 mg) have not been shown to have any effect on the outcome of either disease[22–24]; other studies have shown beta-carotene to have adverse effects on cancer and CHD in smokers.[24,25] A previous report on the Harvard Physicians Health Study indicated a cardiovascular benefit of beta-carotene supplementation after 5 years,[26] but by the end of the 12-year trial, outcomes for patients with various cancers and cardiovascular disease who received beta-carotene supplementation were no different from outcomes for patients who received placebo.[24] These conflicting and disappointing studies failed to sufficiently consider the potential interactions of these bioprotective nutrients, and the effects of high doses of a single nutrient on absorption, transport, recycling, and tissue specificity of other dietary constituents.

Recent attention has focused on other carotenoids, which are predominant in common fruits and vegetables and appear at significant levels in human plasma. Lutein, lycopene, and their oxidative metabolites possess strong antioxidant activity and are promising chemopreventive agents.[27] Alpha-carotene is more potent in suppressing liver, lung, and skin carci-

nogenesis in mice than is beta-carotene.[28] High lycopene intake was shown to be associated with low prostate cancer risk,[29] and all types of digestive cancers were found to be lower in a population with a high dietary intake of lycopene.[30] High blood levels of lycopene have been inversely associated with pancreatic cancer,[31] and it is of interest that among the carotenoids, lycopene is the most effective singlet oxygen quencher.[5] A decreased risk of developing age-related macular degeneration[32] was strongly correlated with a high dietary intake of lutein and zeaxanthin. A reduced risk of lung cancer was related to dose-dependent inverse associations with beta-carotene, alpha-carotene, and lutein.[13] In a retrospective study of more than 15,000 women for 15 years, risk of developing cervical cancer was lowest in women with the highest plasma alpha-carotene, beta-carotene, and cryptoxanthin levels.[33] Strong tissue specificity for carotenoids has been observed, with lutein and zeaxanthin being the only carotenoids present in the macular region of the human retina[34,35] and the lens of the eye.[36] Lycopene is predominant in human testis,[29] and although it is found in equal amounts with beta-carotene in the skin, only lycopene appears to provide phytoprotection against ultraviolet light.[37]

It is apparent that any suggested biologically active component found in food should appear in the blood in significant amounts after consumption to elicit bioprotective effects in various tissues. To the extent that lycopene, lutein, zeaxanthin, alpha-carotene, and beta-carotene have important roles in chemoprotection, and given the complexity of their interactions, it is important that intervention studies use supplementation that elevates blood levels of each component proportionately, rather than using protocols that elicit large increases in beta-carotene alone.

The purpose of this study was to determine the ability of supplementation with fruit and vegetable concentrates to elevate plasma levels of carotenoids and tocopherols, and to evaluate the influence of these substances on the antioxidant defense status as measured by lipid peroxidation.

SUBJECTS AND METHODS

We enrolled 15 healthy subjects with no history of gastrointestinal surgery, diabetes mellitus, or other serious pathologic condition. Women were not pregnant or lactating. Subjects were not on a calorie-restricted diet, practicing vegetarians, or consuming vitamin or antioxidant supplements. Subjects were given a nutritional supplement that was taken twice daily for 28 days. Subjects agreed to abstain from any additional supplementation during the study. No other dietary restrictions were required. All subjects provided written informed consent prior to participation in the study.

The test supplements consisted of commercially available dried fruit and vegetable powders. Fruit juice extracts from apples, oranges, pineapples, papaya, cranberries, and peaches, and vegetable juice extracts from carrots, parsley, beets, broccoli, kale, cabbage, spinach, and tomatoes were cryoevaporated* to concentrate and preserve nutrients and particularly carotenoids. Most of the fruits and vegetables listed are rich sources of lutein, zeaxanthin, and beta-carotene, whereas carrots and tomatoes are primary sources of alpha-carotene and lycopene, respectively. The fruit and vegetable powders included *Dunaliella salina* (Henkle Corporation, Le Grange, Illinois), acerola cherry (Schweizerhall, Piscataway, New Jersey), and soy-derived d-alpha-tocopherol (Henkle Corporation) to provide standardized levels of natural beta-carotene, ascorbic acid, and alpha-tocopherol, respectively. The blends were encapsulated in hard gelatin capsules to provide 850 mg of fruit powder per fruit capsule and 750 mg of vegetable powder per vegetable capsule. Fasting plasma and serum samples were drawn on day −7 and day 0 for mean baseline determinations (variability between day −7 and day 0 were not significantly different) and on days 7, 14, and 28. Supplementation began on day 0 and consisted of two fruit blend capsules daily with a morning meal and two vegetable blend capsules daily with an evening meal.

The measurement of lipophilic antioxidants in plasma allowed the separation and quantitation of beta-carotene, alpha-carotene, lycopene, lutein/zeaxanthin, alpha-tocopherol, gamma-tocopherol, and retinol. The extraction of plasma samples was done in subdued light. A 1-mL aliquot of plasma was mixed with 500 μL of ethyl alcohol containing 0.01% butylated hydroxytoluene (BHT), and the mixture was extracted twice using 1 mL of hexane each time. Before extraction, tocopherol acetate was added as an internal standard. The organic phase was pooled, dried under nitrogen, and reconstituted in 200 μL of mobile phase. A 25-μL portion of this solution was injected into a high-performance liquid chromatography (HPLC) device. The HPLC method was a modification from a previously published method.[38] The analysis apparatus was composed of a Perkin-Elmer series 4 LC (Perkin Elmer, Norwalk, Connecticut), a Shimadzu SIL-9A auto-injector (Shimadzu, Columbia, Maryland), a Bio-Rad model 1706 programmable UV/VIS monitor (BioRad, Richmond, California), and Axiom chromatographic software (Axiom Analytical, Irvine, California). A reverse-phase column (Alltech Econosil C18, Alltech, Deerfield, Illinois) was eluted at 1 mL/min by a mobile phase consisting of acetonitrile-dichloromethane-methanol (65:26:9). The detector was programmed to monitor at wavelengths of 325 nm from 0 to 5.2 minutes, at 450 nm from 5.2 to 6.6 minutes, at 290 nm from 6.6 to 9.4 minutes, at 470 nm from 9.4 to 11.09 minutes, and at 450 nm from 11.09 minutes to the end

*Trademark: JuicePlus + ™ (NSA International, Memphis, Tennessee).

of the run (15 minutes). A set of three standard solutions containing retinol, zeaxanthin, lycopene, alpha- and gamma-tocopherol, and alpha- and beta-carotene was analyzed with each analysis to verify calibration and reproducibility.

For analysis of capsule contents, a modification of the method of Khachik et al[39] was used. A 10-g sample of the dehydrated powder was extracted with a mixture of 100 mL water, ethanol, and pyridine (50:46:4 v/v) containing 0.05% BHT in the presence of 1-g magnesium carbonate, and the mixture was homogenized and filtered successively through 0.45-μm and 0.22-μm filters. The extraction was repeated, and the combined filtrate was partitioned into 50 mL of petroleum ether containing 0.05% BHT. The petroleum ether phase was evaporated to dryness and reconstituted in the mobile phase and evaluated by the HPLC method described above for plasma.

Measurement of lipid peroxides in serum samples was based on a reaction with 10-N-methylcarbamoyl-3, 7-dimethylamino-10 H-phenothiazine (MCDP), a derivative of methylene blue, in the presence of heme compounds such as hemoglobin.[40] The analysis was done using the Kassay LPO-CC assay kit (Kamiya Biomedical Company, Thousand Oaks, California). Duplicate 10-μL serum samples were aliquoted into a 96-well plate. A 50-μL aliquot of reagent 1 (14 μg/mL ascorbic oxidase and 1.2 μg/mL lipoprotein lipase in 100 mmol/L Good's buffer solution) was added, and the mixture was incubated at 30° C for at least 5 minutes, after which 110 μL reagent 2 (0.04 nmol MCDP and 67.5 μg/mL hemoglobin in 100 mmol/L Good's buffer solution) was added, followed by incubation for at least 10 minutes at 30° C. The colorimetric measurement of the reaction product was performed using a plate reader (Cambridge Instruments, Boston, Massachusetts) set at 650 nm using a single-wavelength filter. This method is linear over a range of 2 to 300 nmol/mL with sensitivity <1 nmol/mL, and has the advantage that no sample extraction is necessary; thus susceptible unsaturated lipids are not exposed to potential oxidative conditions. Because only lipid peroxides are measured and not their metabolites, the assay is superior to the commonly used thiobarbituric acid reaction method, which is known to be nonspecific for lipid peroxidation; prostaglandin endoperoxides, iron content, and antioxidants in the sample easily alter the thiobarbituric acid reaction.[41]

Statistical Analysis

Mean values and standard deviations were calculated for all variables. Student's two-tailed *t* tests were used to compare changes from baseline for each component. The Sigma Stat software program (Jandel Scientific, San Rafael, California) was used for data analysis. The Pearson correlation coefficient was used to determine the degree and direction of association

between plasma concentrations of individual components and serum lipid peroxide values. Values were considered significant at $P < 0.05$.

RESULTS

Selected characteristics of study participants are shown in Table I. Ten women (mean age, 34.9 years; range, 23 to 53 years) and 5 men (mean age, 26.6 years; range, 18 to 40 years) were enrolled in this study. Subjects were of average weight (body mass index, 23.3 ± 2.40 kg/m^2 for men, and 23.0 ± 2.21 kg/m^2 for women), and 11 participants (2 men and 9 women) exercised regularly. Diets were not altered before or during the study, and daily consumption of fruits and vegetables was estimated by each subject. No attempt was made to calculate nutrient intake from these sources. Daily supplementation consisted of two dehydrated fruit blend capsules in the morning with a meal and two dehydrated vegetable blend capsules in the evening with a meal. Daily nutrient intake from supplementation is shown in Table I.

Baseline levels of plasma carotenoids and tocopherols were established by taking the mean of two analyses obtained 7 days apart (Table II). Levels of beta-carotene (0.07 ± 0.095 μg/mL) and lycopene (0.137 ± 0.168 μg/mL) were low at baseline compared with published values,[42,43] but increased significantly after 28 days of supplementation (510%, $P = 0.0006$; and 2046%; $P = 0.003$, respectively). Plasma levels of lycopene at

Table I. Subject characteristics.

	Men (n = 5)	Women (n = 10)
Age (y)	26.6	34.9
Range	18–40	23–53
Body weight (kg)*	77.6 ± 6.46	62.4 ± 7.10
Height (cm)*	182.4 ± 5.79	164.6 ± 2.76
Body mass index (kg/m^2)*	23.3 ± 2.40	23.0 ± 2.21
Cigarette smoking	0	2
Regular exercise (2–5 d/wk)	2	9
Serum total cholesterol (mg/dL)*	170.2 ± 15.2	214.4 ± 53.3
Diet		
Fruits and vegetables (servings/d)		
<1	0	2
1–2	2	3
3–4	2	2
>4	1	3
Supplementation (capsules/d)†		
Fruit capsule	2 in AM	2 in AM
Vegetable capsule	2 in PM	2 in PM

* Mean ± SD.
† Supplementation capsules were taken with meals. Daily supplement levels were as follows: beta-carotene, 6 mg; alpha-carotene, 0.5 mg; alpha-tocopherol, 60 mg; gamma-tocopherol, 2 mg; lutein/zeaxanthin, 1.2 mg; lycopene, 0.9 mg; and ascorbic acid, 300 mg.

Table II. Plasma levels of carotenoids, tocopherols, retinol, and lipid peroxides before and after supplementation with dehydrated fruit and vegetable capsules.

	Baseline	Day 7	Day 14	Day 28	% Change*	P †
Beta-carotene (μg/mL)	0.07 ± 0.095	0.405 ± 0.337	0.533 ± 0.412	0.427 ± 0.343	510	0.0006
Alpha-carotene (μg/mL)	0.036 ± 0.042	0.061 ± 0.058	0.084 ± 0.081	0.079 ± 0.062	119	0.034
Lutein/xeaxanthin (μg/mL)	0.36 ± 0.16	0.41 ± 0.16	0.46 ± 0.16	0.52 ± 0.17	44	0.013
Lycopene (μg/mL)	0.137 ± 0.168	2.21 ± 1.74	2.73 ± 1.95	2.94 ± 3.27	2046	0.003
Alpha-tocopherol (μg/mL)	8.86 ± 2.34	13.54 ± 2.43	13.49 ± 2.95	13.99 ± 4.19	58	0.0003
Gamma-tocopherol (μg/mL)	2.02 ± 0.69	1.11 ± 0.61	1.27 ± 0.70	1.25 ± 0.71	−38	0.005
Retinol (μg/mL)	0.50 ± 0.17	0.56 ± 0.17	0.57 ± 0.12	0.57 ± 0.14	14	NS
Lipid peroxides (nmol/mL)	16.85 ± 16.91	3.13 ± 3.14	3.41 ± 2.77	4.22 ± 3.78	−75	0.009

* Change between baseline values and day 28.
† Significance of change between baseline and day 28.

entry were below detectable levels in 7 of 15 subjects (data not shown). Baseline levels of lutein/zeaxanthin (0.36 ± 0.16 μg/mL) and alpha-carotene (0.036 ± 0.042 μg/mL) were within expected ranges, and increased significantly after 28 days (44%, $P = 0.013$; and 119%, $P = 0.034$, respectively). Alpha-tocopherol plasma levels were 8.86 ± 2.34 μg/mL at entry and increased 58% ($P = 0.0003$), whereas gamma-tocopherol levels were 2.02 ± 0.69 μg/mL at baseline and decreased 38% ($P = 0.005$). Retinol levels did not change significantly in response to supplementation.

When lipid peroxides were measured, baseline levels of 16.85 ± 16.91 nmol/mL decreased 75% to 4.22 ± 3.78 nmol/mL ($P = 0.009$) in response to supplementation. Five subjects showed decreases below levels of detection by the assay method used (data not shown). The decrease in lipid peroxide levels is shown in relation to the increase in alpha-tocopherol and lycopene levels in Figure 1. Linear regression analysis of changes in plasma antioxidants showed a significant negative correlation between lipid peroxide levels and alpha-carotene levels ($r = -.523$; $P = 0.046$) and lutein/zeaxanthin levels ($r = -.642$; $P = 0.01$) (Figure 2). When changes between plasma antioxidants were analyzed, a significant positive correlation between alpha-carotene and lutein levels ($r = .633$; $P = 0.011$) and between alpha-carotene and beta-carotene levels ($r = .948$; $P = 0.001$) was found.

DISCUSSION AND CONCLUSIONS

Baseline plasma levels of subjects in this study showed alpha-carotene, lutein/zeaxanthin, and tocopherol levels to be in similar normal ranges reported by others[20,42–46]; baseline beta-carotene and lycopene levels appeared to be two- to fourfold lower than levels found in studies of healthy

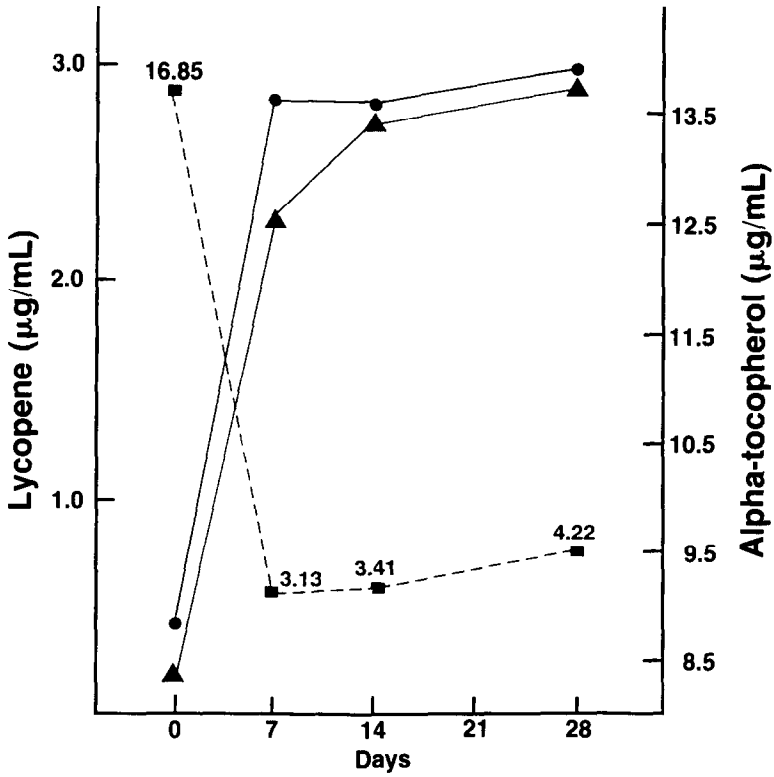

Figure 1. Plasma response of lycopene, alpha-tocopherol, and lipid peroxides to supplementation with dehydrated fruit and vegetable powders. ■ = lipid peroxides (nmol/mL); ▲ = lycopene; ● = alpha-tocopherol.

adults.[20,42–46] During supplementation, parallel increases in all of the carotenoids and alpha-tocopherol levels occurred, as well as a small but not significant increase in retinol levels. The exception was a decrease in gamma-tocopherol levels, which mirrored the increase in alpha-tocopherol levels, and has been observed in other studies.[47] Subjects in this study were not screened on the basis of dietary patterns, nor were they instructed to modify their diets. Therefore, the significant increases in all the carotenoids, which includes the hydrocarbon carotenoid group (beta-carotene, alpha-carotene, lycopene) and the oxycarotenoids (lutein, zeaxanthin), appear due to supplementation. It is interesting to note that baseline levels of beta-carotene comprise 11.7% of total carotenoids measured, which is somewhat below levels of 14% to 25% reported by others.[42,44–46] After supplementation, plasma beta-carotene levels increased approximately sixfold, but represented only 10.4% of plasma carotenoids, whereas alpha-carotene, lutein, zeaxanthin, and especially lycopene levels increased significantly. This finding is in contrast to numerous studies showing that

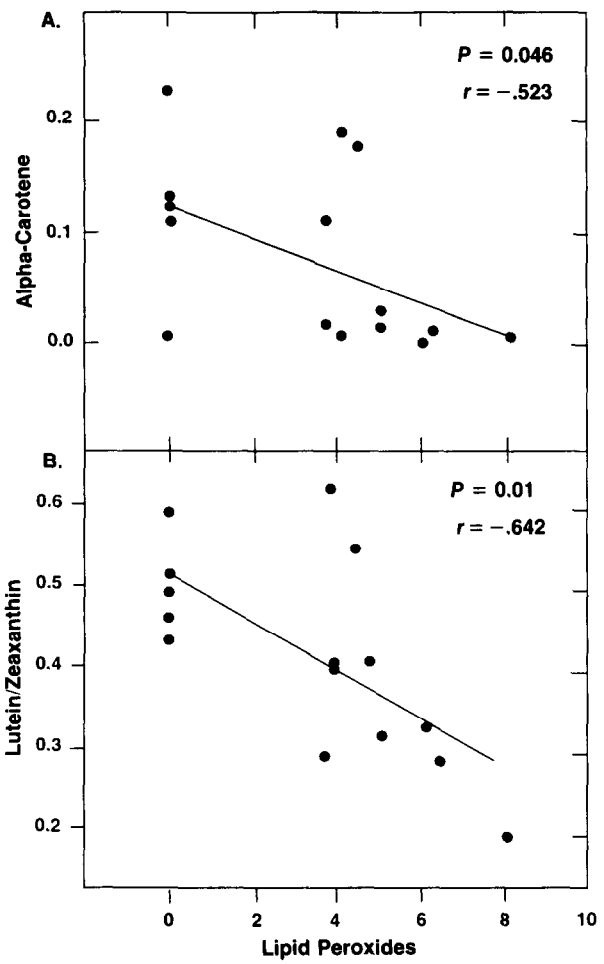

Figure 2. Pearson correlation coefficients (r) between changes in serum lipid peroxides and plasma (A) alpha-carotene and (B) lutein/zeaxanthin after supplementation.

beta-carotene supplementation increased the beta-carotene fraction as a percentage of total carotenoids,[20,48–51] while in some cases a decrease in lycopene and lutein levels also occurred.[19–21]

Overall, the increase in plasma carotenoids in response to supplementation with fruit and vegetable powders appears to be substantially greater than with similar quantities of carotenoids from food.[20] Given the modest level of beta-carotene (6 mg) and even lower levels of other carotenoids supplemented daily, it appears that they are much more readily absorbed than are the same micronutrients in foods.[20] The fact that the fruit and vegetable powders were derived from juice extracts may account for the level of bioavailability observed.

Because subjects were not requested to alter their diets before or during the study, changes were unlikely to be attributed to dietary sources. In fact, the study by Micozzi et al[20] attempted to raise plasma carotenoid levels by dietary means with only marginal success. Participants were asked to consume 272 g of carrots or 300 g of broccoli per day, which was attested to be the tolerable limit by many in their study. Given the slight plasma responses to these foods, the authors concluded that it may be difficult to significantly increase carotenoid blood levels by consumption of common foods alone.[20] In another study,[52] consumption of green leafy vegetables for 12 weeks failed to elevate serum beta-carotene or lutein/zeaxanthin levels in breast-feeding women with marginal serum retinol status (0.1–0.23 μg/mL).

Several other factors may account for the significant increases in plasma carotenoids and alpha-tocopherol levels observed in this study. The poor antioxidant status of our study group on entry may partially explain the increases observed, particularly in lycopene levels. By supplementing the diet with a balance of antioxidant micronutrients normally found in fruits and vegetables, this response may reflect physiologic mechanisms operating to increase the antioxidant defense capabilities. Supplementation with a vegetable juice cocktail containing natural mixed carotenoids has also been shown to elevate plasma levels of each carotenoid measured.[53] However, consumption of carrot juice containing alpha-carotene at 15% to 25% the level of beta-carotene resulted in increases in plasma alpha-carotene levels twice the increases in beta-carotene levels when measured as a dose response[20,54]; presumably this effect was the result of greater absorption of alpha-carotene.

The current study used smaller amounts of natural beta-carotene consisting of equal amounts of all *trans* and 9-*cis* isomers. At present, the interaction of carotenoids appears to be complex and poorly understood. Binding at the intestinal mucosa, uptake, transport, and compartmentalization of individual carotenoids may be affected by the dose, as well as the isomeric form.[44,55,56] In addition, through antioxidant interactions, sparing, recycling, or making antioxidant enzymes more available may influence the plasma and tissue levels of individual carotenoids and tocopherols.

To our knowledge, the only reported study[53] similar to ours used a vegetable juice beverage from carrots, celery, and tomatoes, containing appreciable levels of alpha-carotene (6.6 mg), beta-carotene (14.7 mg), and lycopene (5.2 mg), and lesser amounts of lutein/zeaxanthin (0.66 mg). These levels were two to eight times greater than the levels supplemented in this study. After 2 months of daily juice consumption, all of the serum carotenoids were elevated, with lycopene showing the smallest elevation (40%) and alpha-carotene the highest (436%).

Accumulating evidence has recognized an interactive role of the antioxidants, as well as potential site specific roles in bioprotection. In their

role as bioprotective agents, carotenoids and alpha-tocopherol participate in the antioxidant defense to protect biologic membranes and lipoprotein particles against oxidative stress.[57–59] Combinations of alpha-tocopherol and beta-carotene have been shown to substantially reduce lipid peroxidation in humans,[60,61] and a greater reduction of lipid peroxidation was observed when beta-carotene and alpha-tocopherol were combined rather than administered singly. Ojima et al[62] demonstrated that carotenoids protect alpha-tocopherol from oxidative loss and reduce hydroperoxide formation in lipoproteins. The substantial reduction in lipid peroxides observed in this study was associated with increases in plasma carotenoids and tocopherols. All 15 subjects in this study showed a significant decrease in lipid peroxides, with 5 patients showing nonmeasurable levels by day 28. This effect was most strongly correlated with increases in alpha-carotene and lutein/zeaxanthin.

Other constituents of fruits and vegetables, such as plant polyphenols, were not measured in this study but may also act to inhibit the formation of reactive oxygen species,[63] and in so doing, may spare the antioxidant carotenoids and alpha-tocopherol and increase their levels in tissues.[64] Additionally, these nutrients may interact during uptake and transport, as well as function to spare or recycle each other.[65]

Lycopene and lutein are the most abundant carotenoids in the human diet, as well as in human plasma.[21] A study[5] has shown that lycopene is a more powerful singlet oxygen quencher than beta-carotene. During the oxidation of human low-density lipoproteins, lycopene disappearance preceded beta-carotene,[66] whereas irradiation of human skin preferentially destroyed skin lycopene with little effect on beta-carotene levels.[37] These studies implicate an important protective role for lycopene in vivo. Increased dietary levels of lycopene have been associated with a 45% reduction in prostate cancer,[29] and reduction in risk of most forms of digestive cancer including the mouth, esophagus, stomach, intestine, colon, and rectum.[30] Lutein is a major dietary oxycarotenoid that can be converted in vivo to the isomeric form zeaxanthin[21]; both carotenoids are the dominant pigments found in the macula, the small central part of the retina.[35] Beta-carotene and lycopene are virtually absent in the macula.[35] When dietary intake of carotenoids was analyzed in relation to risk of age-related macular degeneration, the sum of lutein and zeaxanthin had the strongest protective effect.[32] The carotenoid alpha-carotene may have unique bioprotective properties based on its ability to inhibit human cancer cells in vitro, with the inhibitory effect increasing with dose.[67] Alpha-carotene also proved to be a potent inhibitor of carcinogenesis in liver, lung, and skin in mice, compared with beta-carotene.[28]

Much of the epidemiologic data linking high beta-carotene intakes with reduced risks of cancer and cardiovascular diseases have evaluated diets based on established nutrient values of various foods. Until recently,

values for the individual carotenoids in fruits and vegetables were not available.[39] When researchers reanalyzed a case-controlled study[13] of diet and lung cancer, the highest total intake of alpha-carotene, lutein, and beta-carotene was associated with the lowest risk of lung cancer. Similarly, in a 13-year study, subjects in the highest quartile of total serum carotenoids had a 36% lower risk for CHD compared with subjects in the lowest quartile.[7]

Analysis of published studies equating plasma levels of beta-carotene to risk of cancer and CHD showed that minimal risk was associated with plasma levels >0.22 μg/mL of beta-carotene or levels of alpha-carotene and beta-carotene >0.27 μg/mL.[14,15] Similar analysis of alpha-tocopherol indicated that >12.9 μg/mL in plasma was related to minimal risk of CHD and cancer.[14,15] In this study, the mean baseline levels of beta-carotene (0.07 ± 0.095 μg/mL) would be equated with high risk for cancer and CHD.[14,15] After 28 days of supplementation, the mean plasma levels of beta-carotene were elevated to 0.427 ± 0.343 μg/mL, and alpha- plus beta-carotene to 0.506 ± 0.405 μg/mL; these levels were almost twofold greater than the potentially protective levels cited above.[14,15] The same comparison of alpha-tocopherol in this study showed mean baseline levels of 8.86 ± 2.34 μg/mL, equating to a high risk for cancer and CHD, and 28-day plasma levels of 13.99 ± 4.19 μg/mL, which is well above the levels associated with minimal risk for both diseases.[14,15] One other antioxidant, ascorbic acid, was analyzed in the same retrospective study, and plasma levels of >8.8 to 10.6 μg/mL appeared to provide minimal risk for the same diseases.[14,15] Although we did not measure ascorbic acid levels in our study, daily intakes of >140 mg have been shown to be sufficient to achieve plasma levels above 10 μg/mL even in smokers.[15] Because intakes of ascorbic acid exceeded 300 mg/d in this study, presumably plasma levels in our subjects exceeded 10 μg/mL. We recognize that the small number of subjects, the short duration of the study, and the lack of a control group limit any conclusions that might be made concerning actual disease risk reduction.

In other intervention trials, large supplementary doses of beta-carotene were consumed over extended periods (1 year or more) and showed no effects[22–24] or were shown to have adverse effects.[24,25] Clinical trials with beta-carotene or other antioxidant vitamins failed to prevent colorectal adenomas.[22,23] The Harvard Physicians Health Study, conducted for 12 years, found that beta-carotene supplements provided neither benefit nor harm for cancer or cardiovascular disease.[24] A cancer prevention trial in Finland, using smokers at high risk for lung cancer, found an increased mortality due to ischemic heart disease and lung cancer among patients who received 20 mg of beta-carotene daily for 5 to 8 years.[25] Similar findings were reported in the Beta Carotene and Retinol Efficacy Trial, involving smokers and asbestos workers at high risk for

lung cancer.[24] The intervention phase was terminated almost 2 years prematurely when the investigators announced increased death rates from lung cancer and cardiovascular disease in the beta-carotene/vitamin A treatment group.[24]

Although these results have been disappointing, they do not negate dozens of studies, including some cited here, that have shown that beta-carotene was positively associated with reduced risk of cancer and CHD. Certainly it is not reasonable to expect a single nutrient, such as beta-carotene, to reverse the negative effects and risks associated with a lifetime of smoking. However, it has prompted a reevaluation of study design in randomized intervention trials with a look toward combinations of mixed carotenoids and antioxidants.[19,68,69]

Future studies should consider the well-established interactions between antioxidants, as well as tissue specificity and compartmentalization. This study indicates that supplementation with fruit and vegetable extracts is an effective means to elevate plasma levels of the major carotenoids and alpha-tocopherol while reducing deleterious oxidative processes. Such supplementation, with a mixture of bioprotective agents in amounts or ratios more closely resembling those found in a mixed fruit and vegetable diet, should be considered in the design of new intervention studies.

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