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Original Contribution

EFFECTS OF A CAROTENE-DEFICIENT DIET ON MEASURES OF OXIDATIVE SUSCEPTIBILITY AND SUPEROXIDE DISMUTASE ACTIVITY IN ADULT WOMEN

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Abstract—The effect of consuming a low carotene diet (~60 μg carotene/day) on oxidative susceptibility and superoxide dismutase (SOD) activity in women living in a metabolic research unit was evaluated. The diet had sufficient vitamins A, E, and C. The women ate the diet supplemented with 1500 μg/day β-carotene for 4 days (baseline), then the unsupplemented diet for 68 days (depletion), followed by the diet supplemented with > 15,000 μg/day carotene for 28 days (repletion). Production of hexanal, pentanal, and pentane by copper-oxidized plasma low density lipoproteins from carotene-depleted women was greater than their production of these compounds when repleted with carotene. Erythrocyte SOD activity was depressed in carotene-depleted women; it recovered with repletion. Thiobarbituric acid reactive substances in plasma of carotene-depleted women were elevated and diminished with repletion. Dietary carotene seems to be needed, not only as a precursor of vitamin A, but also to inhibit oxidative damage and decrease oxidation susceptibility.

Keywords—Carotene, Human, Oxidative damage, Superoxide dismutase, Free radicals

INTRODUCTION

About one third of the total dietary vitamin A activity is derived from carotenes, a group of brightly colored pigments that occur naturally in fruits and vegetables.[1] Carotenes are reported to protect against oxidative stress and chronic diseases, such as coronary heart disease, cataract, and cancer.[2] β-carotene has the highest vitamin A activity among 600 naturally occurring carotenoids. It has been reported to protect cells against oxidative damage, a function that is related to its effectiveness as an antioxidant and as a free radical scavenger.[3] Other functions of this antioxidant include an anticancer effect,[4,5] enhancing the immune response,[6,7] and quenching singlet oxygen.

At low concentrations, β-carotene exhibits good free radical-trapping antioxidant behavior at the partial pressures of oxygen found in most tissues under physiological conditions.[8,10] β-carotene and canthaxanthin are effective inhibitors of lipid peroxidation as measured by delay in malondialdehyde formation.[8] The effects of radicals generated by the xanthine oxidase/acetaldehyde peroxidation of linolenic acid can be decreased by β-carotenes.[9] Dixit et al.[10] reported that singlet oxygen may be involved in lipid peroxidation of microsomes from skin. Kunert and Tappel[11] observed significant reduction in expired ethane and pentane in vitamin C-deficient animals that were treated with vitamin C, isoascorbic acid, glutathione, α-tocopherol, or β-carotene.

Modification of plasma low density lipoproteins (LDL) by oxidation of their polyunsaturated fatty acids is thought to increase their atherogenicity. It was pro-

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posed recently that antioxidants including β-carotene and vitamin E play an important role against atherosclerosis by protecting LDL against oxidative damage.[12,13] In vitro autoxidation of LDL only occurs when they are depleted of their endogenous β-carotene and vitamin E.[14] Butylated hydroxytoluene and vitamin E inhibit modification of microsomes or LDL, potentially minimizing their atherogenecity and cytotoxicity.[15,16] Most studies of the antioxidant effects of carotenes have been conducted in vitro or in animals.[17,18] However, Mobarhan et al.[19] reported that a carotene-free liquid diet increased thiobarbituric acid reactive substances (an index of lipid peroxide levels) in humans.

This study was designed to test whether total carotene depletion increases susceptibility to oxidative damage even when the subject consumes a diet that contains RDA levels of "preformed" vitamin A, vitamin E, and vitamin C. We present data from a controlled human study investigating the effects of short-term total carotene depletion on antioxidant status of women.

METHODS AND MATERIALS

Subjects

Twelve healthy normal weight premenopausal women, 18 to 42 years of age were selected, and nine of them completed the study. Participants lived in the metabolic unit of the U.S. Dept. of Agriculture, Western Human Nutrition Research Center (WHNRC), Presidio of San Francisco, CA, for 100 days. The study was restricted to women because the communal living arrangements of the Center make mixed sex studies impractical. Procedures for this study were approved by the Human Subjects Review Committee of the University of California and the U.S. Dept. of Agriculture Review Committee at Tufts University, and they conformed to Helsinki Conference guidelines for research on human subjects.

Research design

The first 4 days of the study were a baseline period. During this time all participants ate a low carotene diet supplemented with 1500 μg β-carotene/day (Dry Carotene Beadlets, lot 011605, Hoffmann-La Roche Inc., Nutley, NJ). This was followed by a carotene depletion period of 68 days. During this depletion period, the participants ate the low carotene diet supplemented with carotene free placebo beadlets (Placebo Beadlets, lot 0312581, Hoffmann-La Roche Inc., Nutley, NJ), which were very similar to the Dry Carotene Beadlets in appearance. The carotene depletion period was followed by a 28-day β-carotene repletion period,

during which the participants were fed the depletion diet supplemented with 15,000 μg β-carotene/day as Dry Carotene Beadlets. An additional supplement of 6 capsules per day of mixed carotenoids (Carotenoid Complex, lot 38564, Neo-life Company of America, Fremont, CA) was given during the last 12 days of the repletion period. This carotene complex consisted of approximately 1 g/kg β-carotene, 0.5 g/kg α-carotene, and 0.1 g/kg lycopene. The subjects participated in a controlled nonsedentary type exercise with activity level chosen to avoid changes in body conditioning throughout the study.

Diet

All meals were consumed under observation, with subjects leaving the facility only with an escort to insure absolute compliance. Subjects ate natural foods low in fruits, vegetables, and their juices. Foods were prepared mostly by baking, boiling, or microwaving. Canned and frozen foods were bought in manufacturers' lots to ensure that the macro- and micronutrient composition of the diet did not change significantly throughout the study. The diet averaged 8.6 ± 0.3 MJ energy and was 55% carbohydrate, 14% protein, and 31% fat, with a polyunsaturated/saturated fat ratio of 1. Four daily menus were served in a 4-day rotational pattern. Subjects were given daily vitamin and mineral supplements to maintain intake at approximately 100% of the U.S. RDA for all nutrients except carotenes and iron. Iron was supplemented at a relatively high concentration because participants in our metabolic unit studies often develop low hematocrits and hemoglobin concentrations, presumably because of the amount of blood collected during these studies. The vitamin A content of the experimental diet was calculated to be 302 ± 10.0 Retinol Equivalent (RE)/day. The carotene content of the diet was analyzed by reversed-phase chromatography (HPLC) as described later. Diet composition is given in Table 1. A more detailed description of the diet and its preparation will be given elsewhere.

Body composition and metabolic analysis

Body weights were measured in the same clothing each day just after awakening and voiding. Fat-free body mass was estimated by total body electrical conductivity (TOBEC). Oxygen consumption (VO₂ resting) was measured using an automated collection system 2900 Metabolic CART (SensorMedics, Anaheim, CA). The system was calibrated with a standard gas mixture before each measurement. Measurements were collected for 10 min shortly after rising, following a

Table 1. Micronutrients Intake From Supplement and Diet for
Women on Low Carotene Diet

Nutrient	Supplement	Diet	Total	RDA Value
Vitamin A (RE)	795.0	302.7 ± 10.0*	1097.7	800
Vitamin C (mg)	25.0	51.3 ± 1.7	76.3	60
Vitamin E (IU)	—[a]	13.5 ± 0.4	13.5	12
Iron (mg)		18.5	15.3 ± 0.5	33.815
Zinc (mg)		4.0	8.2 ± 0.3	12.212
Magnesium (mg)	100.0	249.0 ± 8.2	349.0	280

[a] No supplement given.
* Mean ± SD.

12-h fast. VO_2 max was predicted using the Astrand–Rhyming Bicycle test. The second of two tests was used for the baseline measurements.

Sample collection and preparation

Blood was collected from fasting subjects between 0730 and 0830 h by venipuncture into heparinized tubes. Blood for the superoxide dismutase (SOD) and the thiobarbituric acid reactive substances (TBARS) assays were collected over a 2-day period (on days 1 and 2, 3 and 4, 29 and 30, 36 and 37, 43 and 44, 50 and 51, 64 and 65, 70 and 71, 92 and 93, and 99 and 100) with blood draw being done for the first four subjects on the first day and the second five subjects on the second day. Blood was centrifuged at 2,500 rpm for 15 min at 4°C, and the plasma was carefully removed. Plasma was used for the TBARS assay, and red blood cells were used for measuring SOD activity. TBARS and SOD were assayed at WHNRC.

Superoxide dismutase activity

Erythrocytes were washed three times by adding 0.9% saline to the original volume of erythrocyte pellet. Serum and buffy coats were removed following each centrifugation. Hemoglobin concentration was measured using a Baker system 9000 Hematology series cell counter (Allentown, PA). Cells were hemolyzed by adding 1.5 volumes of iced cold distilled water to the cells. Hemolysates were frozen and thawed three times in a dry ice–acetone mixture. Hemoglobin was extracted from the hemolysate by a modification of a previously published method.[20] Superoxide dismutase activity was measured by adapting the method of Wheeler et al.[21] to the Cobas FARA clinical automated analyzer and is expressed as U/mg hemoglobin.

Thiobarbituric acid reactive substances assay

Concentrations of TBARS in plasma were measured using the method of Warso and Lands.[22] Samples were heated to 95°C in a Reacti-Therm™ heating dry block (Pierce Chemical Co., Rockford, IL) for 30 min. Absorbance at 532 nm of the samples was measured on a Perkin–Elmer 557 double wavelength double beam spectrophotometer and compared to that of standard amounts of 1,1,3,3-tetraethoxypropane. Results are expressed as μmol/ml plasma.

Oxidative susceptibility of low density lipoproteins (LDL)

Blood for the LDL oxidative susceptibility tests was collected twice, at the end of depletion (days 70 and 71) and at the end of repletion (days 99 and 100), and analyzed at the University of California, Davis. Baseline determinations were not done for these tests because our collaboration had not been finalized in time. Blood was drawn from fasting subjects between 0730 and 0830 h into 10-ml tubes containing 100 μL of 860 mmol/L K_3-ethylenediamine tetraacetic acid (EDTA). Plasma was separated immediately by centrifugation at 2500 rpm for 15 min at 4°C. LDL particles were isolated from plasma by sequential density ultracentrifugation in the presence of EDTA and then dialyzed overnight in phosphate-buffered saline (PBS) at 20°C to remove the EDTA.[23] LDL protein was measured using a modified Lowry assay.[24] Oxidative susceptibility was assessed by headspace gas chromatography (HSGC) analysis of volatile fatty acid decomposition products of LDL exposed to $CuSO_4$.[25] Duplicate samples of 0.25 mg LDL protein in 250 μL PBS and standard solutions of hexanal were measured into special headspace 6-ml bottles, sealed with silicone rubber Teflon caps using a crimper, and incubated in a shaking water bath for 2 h at 37°C in the presence of 80 μM $CuSO_4$. After incubation, the bottles were inserted into the headspace sampler, heated to 40°C, and pressurized with the carrier gas for 30 s, and an aliquot of the gas phase was injected directly into the gas chromatograph through a stationary injection needle. Volatile products from oxidation of LDL were identified by comparing retention times with those of authentic reference compounds. Oxidative susceptibility and oxidation of n-6 and n-3 polyunsaturated fatty acids (n-6 to pentane and hexanal and n-3 to propanal) were measured.[25] Results are expressed as nmol product/mg LDL protein.

Retinol and β-carotene analyses

Vitamin A content of the diet menus were determined by adding 1.2 ml EtOH, 325 μL of 5.4 mol/L

KOH, and 100 μL of 1.2 mol/L pyrogallol to 150 mg composited menu and heating for 2 h at 80°C. Retinol was extracted with 3 ml diethyl ether/hexane:1/1, and 1 ml hexane containing the internal standards, retinal o-ethyloxime dried under argon, redissolved in 50 ml EtOH, and an appropriate aliquot analyzed by HPLC. Retinol content of the vitamin supplement was measured by dissolving tablet in 30 ml EtOH, 10 ml of 5.4 mol/L KOH, and 15 ml H_2O. The solution was saponified by adding 800 μL of 0.6 mol/L pyrogallol and heating the mixture for 2 h at 80°C. Retinol was extracted twice with 66 ml diethyl ether/hexane: 3/1, and the extracts were combined (106 ml total volume). A 1.0-ml aliquot of the retinol extract serially diluted 100-fold was dried and redissolved in 200 μL mobile phase, and a 50 μL portion was injected onto the HPLC. Complete saponification was assured by absence of retinyl ester peaks on the HPLC trace.

Carotene contents of the diet menus were measured by homogenizing 3 g diet with an equal volume of methanol containing approximately 10% KOH and 1% pyrogallol. This mixture was saponified for 1 h at 70°C, then cooled. Carotenes were extracted three times with 4 ml hexane (containing 100 mg BHT/L hexane) by vortexing for 1 min; then the extracts were combined and washed twice with 2 ml distilled water with 1 min vortexing. The distilled water was then washed once with 2 ml hexane, then all the hexane washes were combined and concentrated using a Speedvac SVC200H concentrator (Savant, Hicksville, NY). Extracts were reconstituted with 100 or 500 μL methylene chloride, then 5 or 15 μL (respectively) were injected for HPLC. Carotenes were analyzed by reversed phase HPLC using a polymeric Vydac TP201 column (Hesparia, CA). Carotenes were separated and quantitated using a methanol/acetonitrile/water eluent (88/9/3) at 2 ml/min.

β-carotene content of isolated LDL was measured by mixing 400 μL H_2O, 500 μL EtOh and 10 μL butylated hydroxytoluene with 100 μL of the dialyzed LDL to precipitate LDL protein. Two ml hexane + 1 ml hexane containing β-apo-12-carotenal-O-t-butyloxime as internal standard were added, and the mixture was shaken vigorously and centrifuged to separate the hexane and the aqueous layers. Two ml of the hexane layer were removed and dried under argon. The residue was dissolved in 30 μL EtOH and a portion injected on the HPLC with a 20 μL injection loop.

Apparatus for HPLC of LDL carotene consisted of Velosep RP18 (Applied Biosystems Brownlee) reverse phase HPLC columns (3μm particle size). Two columns (100 mm $\times$ 3.2 mm I.D.) were connected in series with an applied Biosystems Brownlee New-Gaurd system holder. An Applied Biosystems Brownlee NewGuard guard cartridge (15 mm $\times$ 3.2 mm I.D., 7 μm particle size) was installed upstream of the two columns in series. Conditions for the HPLC included an isocratic solvent of acetonitrile/isopropanol/methanol/ammonium acetate/water: 67/20/12/1/1 pumped at 0.9 ml/min. Detectors were set at 325 nm and 450 nm, and Chrom-1AT data acquisition hardware and Lab-Calc chromatography software (Galactic Industries Corp., Salem, NH) recorded the results.

Internal standards retinal-O-ethyloxime (for retinol) and β-apo-12-carotenal-O-t-butyloxime (for β-carotene) were synthesized from retinal and β-apo-12-carotenal, respectively, as described by van Kuijk et al.[26] and modified by Handelman et al.[27] For retinol standards, retinyl acetate (Sigma Chemical Co., St. Louis, MO) was saponified, and the retinol was purified on a preparatory HPLC column and quantitated spectrophotometrically at 325 nm using an extinction value of 52,554 $M^{-1}cm^{-1}$ for retinol. β-carotene (Type IV, Sigma Chemical Co.) was repurified by HPLC and quantitated using an extinction value of 140,642 $M^{-1}cm^{-1}$ at 450 nm. All standards were stored at -70°C in small quantities and were used once and discarded. Quality assurance procedures were established for each assay by routine checks of precision and linearity, batch analysis of quality control specimens, and analysis of external samples from the National Institute of Technology Micronutrient Quality Assurance Program (Gaithersburg, MD).

Serum retinol and carotene concentrations were assayed at the Centers for Disease Control and Prevention by a standard reversed-phase HPLC method.[28] Five carotenes are assayed by this method: β-carotene, α-carotene, lycopene, cryptoxanthin and lutein-zeaxanthin.

Statistical analysis

Data were analyzed using analysis of variance.[29] Means for different study periods were also compared by paired t-tests. Differences of $p < 0.05$ were considered statistically significant. Results are given as means $\pm$ *SD*.

RESULTS

The unsupplemented (and placebo supplemented) diet supplied 58 μg carotenoids/day, approximately 90% as β-carotene, with trace amounts of α-carotene and lutein isomers. All serum carotene concentrations decreased rapidly from baseline values on this diet, and all were significantly different ($p < 0.001$) by study Day 29. After Day 29, carotene concentrations

Table 2. Influence of Carotene Depletion Diet on Serum Carotene and Vitamin A Concentrations

Study Day	Study Period	β-Carotene	Vitamin A	Lutein-Zeaxanthin	Cryptoxanthin	Lycopene	α-Carotene
1	B	29 ± 13	45 ± 6	22 ± 7	16 ± 8	22 ± 8	6 ± 2
3	B	31 ± 11	45 ± 6	20 ± 6	14 ± 6	19 ± 6	9 ± 3
29	D	14 ± 5	43 ± 6	12 ± 3	7 ± 3	6 ± 2	4 ± 1
36	D	13 ± 5	45 ± 6	12 ± 2	7 ± 2	6 ± 2	4 ± 1
43	D	11 ± 5	42 ± 9	11 ± 3	6 ± 3	5 ± 2	3 ± 1
51	D	11 ± 4	44 ± 6	11 ± 3	5 ± 2	5 ± 1	3 ± 1
64	D	9 ± 4	42 ± 5	10 ± 2	4 ± 1	4 ± 2	3 ± 1
71	D	9 ± 3	45 ± 6	11 ± 3	5 ± 2	4 ± 1	3 ± 1
92	R	227 ± 64	45 ± 5	14 ± 3	8 ± 2	7 ± 3	23 ± 8
99	R	294 ± 86	42 ± 7	16 ± 3	11 ± 3	10 ± 4	45 ± 17

Means ± standard deviations. All concentrations are in $\mu g/dL$.
B = Baseline; D = Carotene Depletion; R = Carotene Repletion.

decreased very slowly. Although concentrations continued to decrease, all carotenes were still detectable throughout the depletion period. All serum carotene concentrations increased after carotene supplementation, and differences between values at the end of depletion and end of repletion were all significant ($p < 0.01$). Serum vitamin A concentrations did not change significantly with time on the study. Table 2 shows mean ($\pm SD$) serum carotene and vitamin A concentrations. Serum ferritin concentrations decreased gradually with time during our experiment, but this decrease was not significant.

Body composition (fat free mass), body weight, and oxygen consumption tests (VO_2 resting and VO_2 max) did not change during this study (Table 3).

Changes in oxidative damage and susceptibility due to depletion and repletion with carotene are summarized in Table 4. β-carotene concentrations in LDL of depleted women were 0.13 ± 0.08 nmol/mg LDL protein. β-carotene concentration in LDL of the same women when they were repleted with β-carotene was 5.89 ± 1.62 nmol/mg LDL protein. Zero time concentrations of hexanal, propanal, pentanal, and pentene in LDL were barely detectable. After the 2-h incubation with $CuSO_4$, concentrations of hexanal, pentanal, and pentene in LDL from carotene-depleted women were higher than those in LDL from the same women when

they were repleted. Propanal concentration in LDL from carotene-depleted and carotene-repleted women was not different. Hexanal, pentanal, pentane, and propanal in LDL were not measured during the baseline period.

Formation of lipid peroxidation products, measured as plasma TBARS and erythrocyte superoxide dismutase activity, during the three study periods are shown in Figure 1. Plasma TBARS concentrations were 1.1 ± 0.2 < 5.5 ± 0.9 > 1.2 ± 0.4 $\mu mol/ml$ during the baseline, carotene depletion and carotene repletion periods, respectively. Erythrocyte superoxide dismutase activity values were 2.3 ± 0.3 > 1.8 ± 0.4, < 2.5 ± 0.5 u/mg hemoglobin during the baseline, depletion, and repletion periods, respectively.

DISCUSSION

All serum carotene concentrations decreased while our subjects were fed the low carotene diet—first rapidly, then very slowly. This suggests that carotenes are present in at least two body pools: a rapidly metabolized pool, probably corresponding to blood; and at least one slower metabolizing pool, probably stored in tissues such as body fat. All carotene concentrations increased rapidly after supplementation. Surprisingly, all carotenes appeared to increase, even though lutein/

Table 3. Body Composition and Metabolic Status of Study Participants

Period of Study	VO_2[a] Resting (mL/min)	VO_2[a] Max (L/min)	Body Weight (kg)	Fat-Free Body Mass (kg)
Baseline	0.201 ± 0.007[b]	2.09 ± 0.12	60.4 ± 1.1	42.6 ± 1.3
Mid-Depletion	0.202 ± 0.004	2.11 ± 0.14	59.8 ± 1.1	42.4 ± 1.3
End-Depletion	0.203 ± 0.005	2.14 ± 0.14	59.7 ± 1.1	42.9 ± 1.5
Repletion	0.201 ± 0.005	2.08 ± 0.13	59.6 ± 0.9	42.6 ± 1.4

[a] Oxygen consumption.
[b] Values are means ± SEM.
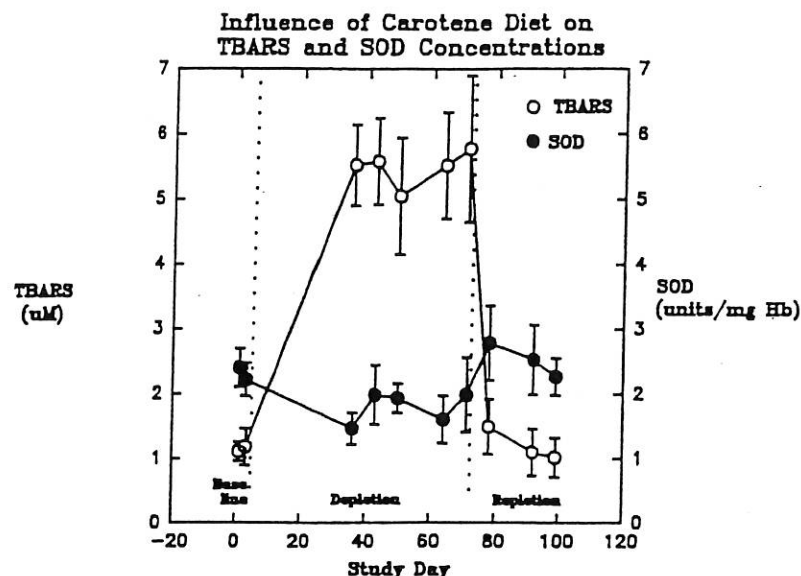
Fig. 1. Variation of plasma TBAR and red blood cell SOD during baseline, depletion of carotene, and repletion of β-carotene. Methods are described in text.

zeaxanthin and cryptoxanthin concentrations were negligible in these supplements. These increases may be artifacts, because concentrations of these carotenes were small; however, it is possible that these carotenes may share some storage sites or metabolic pathways so that the large increase in β-carotene in the diet after supplementation caused their release from tissue. The small decrease in serum ferritin concentrations we observed is common in our metabolic unit studies, even though we collect less blood than the subject could donate to a blood bank. The only well established functions for carotenes in humans are to serve as vitamin A precursors. However, epidemiological studies show strong inverse correlations between eating foods containing large amounts of carotenes and chronic diseases, for example, coronary heart disease,[30,31] and cancer.[32,33] This suggests that carotenes have important functions in human health.

The TBARS method for estimating oxidative damage is widely used; however, the method can be nonspecific. Results from headspace gas chromatography (HSGC) analysis, which measures the specific decomposition products of LDL fatty acid, were in agreement with the TBARS determination in plasma. Both methods showed significant decreases in the formation of oxidation products in carotene-repleted women compared to when they were carotene-depleted. These changes reflect a decrease in oxidative susceptibility, presumably resulting from an increase in β-carotene concentration in LDL after repletion. Researchers[34,35] have shown that the ability of LDL to withstand oxidation is related to fatty acid and antioxidant composition

Table 4. Changes in Oxidative Damage and Susceptibility due to Depletion and Repletion of Dietary Carotenes

Response Variable	Baseline (4 days)	Depleted (68 days)	Repleted (28 days)	p Value
β-carotene*	—[c]	0.13 ± 0.08	5.89 ± 1.62	0.0001
Hexanal*	—	113.1 ± 27.3	91.5 ± 25.2	0.01
Pentanal*	—	10.7 ± 3.5	6.8 ± 2.8	0.005
Pentane*	—	7.3 ± 2.9	3.9 ± 2.0	0.005
Propanal*	—	2.2 ± 0.5	2.2 ± 0.6	NS
TBARS[†]	1.1 ± 0.2[b]	5.5 ± 0.9[a]	1.2 ± 0.4[b]	0.0001
Superoxide dismutase[‡]	2.3 ± 0.3[a]	1.8 ± 0.4[b]	2.5 ± 0.5[a]	0.0001

Values are means ± *SD* for $n = 9$. NS = differences not significant at $p > 0.05$. Units are *nmol/mg LDL protein, [†]μmol/mL plasma, and [‡]μ/mg hemoglobin.
[a,b] Values within a row with different superscripts are different.
[c] These measurements were not made during the baseline period.

of the LDL particle. In this study, fatty acid composition of the lipid in the diet was constant across all three dietary periods to avoid differences in oxidation being due to changes in the fatty profile of LDL. We suggest that increased oxidative susceptibility might occur in carotene deficiency. The increase in oxidation products caused by total carotene depletion can lead to modification of LDL that have been associated with development of atherosclerotic plaques.[34]

In this study, marked increases in the oxidative indices, as measured by HSGC and plasma TBARS, were observed during the carotene depletion period followed by decreases after repletion with carotene. Thus carotene appears to inhibit formation of secondary oxidation products.

Antioxidant enzymes are capable of inhibiting free radical reactions.[36] Superoxide dismutase is involved in the elimination of the superoxide radical. Low antioxidant enzymes were reported to cause accumulation of free radicals, which can result in damage to DNA, RNA, and proteins. Data from our study showed significantly decreased SOD activity during carotene depletion. During repletion, SOD activity was increased, suggesting that β-carotene may have acted as a direct scavenger of superoxide radicals or indirectly affected SOD activity in another, as yet unexplained, way.

Recent studies in which humans ate pharmacological concentrations of β-carotene[37], or β-carotene, tocopherol, and vitamin C[38] showed little or no improvements in oxidative status. Our study also shows no benefit from supplementing the diet with pharmacological amounts of β-carotene. TBARS and SOD values at the end of the baseline and carotene-supplemented diet periods were not different. Thus, our study suggests that carotene supplements may have little benefit for people already eating a healthy diet.

In conclusion, dietary β-carotene appears to function not just as a precursor of retinol and thus as a source of vitamin A, but also serves a protective role against LDL oxidation. Even though the women received adequate preformed vitamin A in their diets, indices of oxidative damage increased during carotene depletion and decreased after repletion. These results, showing increased lipid peroxidation products and decreased SOD activity during depletion, suggest that a regular intake of carotenes is necessary to prevent accumulation of oxidative products in healthy women.

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ABBREVIATIONS

EDTA—ethylenediamine tetraacetic acid

HPLC—high performance liquid chromatography

HSGC—headspace gas chromatography

LDL—low density lipoprotein

PBS—phosphate buffered saline

RDA—recommended dietary allowances

SOD—superoxide dismutase

TBARS—thiobarbituric acid reactive substances

TOBEC—total body electrical conductivity

WHNRC—Western Human Nutrition Research Center