ELSEVIER

Journal of Nutritional Biochemistry 14 (2003) 656–662

THE JOURNAL OF Nutritional Biochemistry

Beta-carotene supplementation decreases leukocyte superoxide dismutase activity and serum glutathione peroxidase concentration in humans☆

Carla R. McGill[a,*], Nancy R. Green[a], Margaret C. Meadows[b], Sareen S. Gropper[c]

[a]*Tropicana Products, Inc., 1001 13th Avenue East, Bradenton, FL 34208, USA*
[b]*Atlanta Medical Center, Atlanta, GA, USA*
[c]*Dept. of Nutrition and Food Science, Auburn University, Auburn, AL, USA*

Abstract

The effects of a 30 mg/day beta-carotene supplement for 60 days on blood cell and serum antioxidant enzymes and selenium concentrations were examined in healthy adults. Serum beta-carotene concentrations increased significantly ($P < 0.05$) in response to supplementation. Forty percent of subjects exhibited hypercarotenemia of the skin after 30 days. There were no changes in the activity of red blood cell or leukocyte catalase activity, red blood cell copper,zinc-dependent superoxide dismutase activity or serum myeloperoxidase concentration in response to beta-carotene supplementation. Leukocyte superoxide dismutase activity decreased significantly ($P < 0.05$) at 30 and 60 days compared to baseline. Serum glutathione peroxidase concentration decreased significantly ($P < 0.05$) between baseline and days 45 and 60 of supplementation. Serum selenium and blood hemoglobin concentrations did not change during the study. Supplemental beta-carotene may alter the antioxidant capacity of plasma and/or blood cells in vivo.

Keywords: Beta-carotene supplements; Antioxidant enzymes; Superoxide dismutase; Glutathione peroxidase

1. Introduction

Carotenoids, a group of naturally occurring plant pigments that range in color from yellow to red, are well recognized for their antioxidant properties. Epidemiological evidence has shown that consumption of carotenoids from fruits and vegetables is protective against chronic diseases [1–5]. Specifically, individuals consuming the highest quartile of dietary beta-carotene (βC) have been shown to have lower risks of cardiovascular disease, strokes, and some cancers while increased risk of these diseases has been found in individuals having the lowest quartile of βC consumption or lowest plasma βC concentrations [4–8]. Beta-carotene's association with disease prevention and its role as an antioxidant has, in part, prompted increased consumer interest in over-the-counter βC supplements.

The use and effects of βC as a dietary supplement, however, are not without controversy. Beta-carotene, in doses up to about 180 mg, has been used daily without evidence of toxicity in individuals with erythropoietic protophyria [9]. However, human intervention studies with βC supplements have failed to show consistent protective effects. The Physicians Health Study randomly assigned 22,071 male physicians, aged 40 to 84 years, to receive either 50 mg βC or a placebo every other day for 12 years [10]. No significant effects of βC supplementation were found on cancer or heart disease mortality or incidence of cancer, strokes, or myocardial infarctions [10]. The Women's Health Study, a randomized, double blind trial, investigated the effects of 50 mg βC or a placebo every other day on cancer and cardiovascular disease incidence in 39,876 women aged 45 years and older [11]. No statistically significant differences in the incidence of cancer, cardiovascular disease, or total mortality were reported during the two year study and its follow-up [11].

In contrast to the lack of benefits in studies in healthy populations, the results of intervention trials with βC in high-risk population groups report increased mortality from cancer following βC supplementation. The Beta-Carotene

☆ This research was supported in part by the Alabama Agricultural Experiment Station 13-006, Hoffman-LaRoche® and East Alabama Medical Center.

* Corresponding author. Tel.: +1-941-742-3485; fax: +1-941-749-3938

E-mail address: carla.mcgill@tropicana.com (C. R. McGill).

0955-2863/03/$ – see front matter
doi:10.1016/j.nutbio.2003.08.003

and Retinol Efficacy Trial supplemented 18,314 men and women who were either smokers or had asbestos exposure with 30 mg βC and 25,000 IU retinol or a placebo each day [12]. A 17% higher mortality rate and a 28% higher incidence of lung cancer were found in the βC and vitamin A treatment group compared with the placebo group. The Alpha-Tocopherol, Beta-Carotene Trial provided 29,133 male smokers aged 58 to 76 years with 20 mg βC or a placebo for at least five years [13]. An 18% increase in the incidence of lung cancer, a 23% increase in prostate cancer, and a 25% increase in stomach cancer were demonstrated for those who received βC vs. those who received a placebo. Total mortality was 8% higher in individuals receiving βC.

While carotenoids are known to protect against oxidation by quenching singlet oxygen, other effects on cellular antioxidant defenses remain unclear. It has been hypothesized that βC supplements may alter the antioxidant capacity of tissues in vivo. Carotenoids consumed as a supplement, however, did not alter activity of blood catalase, superoxide dismutase and glutathione peroxidase in humans [14]. Consumption of spinach lowered erythrocyte catalase activity in the same study [14]. In contrast, activity of glutathione peroxidase was significantly lower and those of catalase and manganese-dependent superoxide dismutase significantly higher in livers of canthaxanthin (4,4′-diketo-βC, a structural analogue of βC) treated mice [15]. No significant effect of canthaxanthin treatment on hepatic copper,zinc-dependent superoxide dismutase activity was observed in the mice [15].

Based on the results of epidemiological studies and intervention trials, studies assessing the effects of βC supplements on cellular functions important in disease prevention are necessary to better understand βC's safety and effectiveness. The purpose of this study was to determine the effects of βC supplementation on blood cell and serum antioxidant enzymes and selenium concentrations in healthy adults. Selenium, an essential trace mineral, functions as a component of glutathione peroxidase, an important enzyme along with superoxidase dismutase, catalase and myeloperoxidase of the body's oxidant defense system.

2. Methods and materials

2.1. Subjects.

Twenty-two males and females, 21 to 45 years old, were a convenience sample recruited from Auburn University, Auburn, Alabama and East Alabama Medical Center, Opelika, Alabama. Each subject signed an informed consent before participating in the study. Subjects were excluded if they were pregnant, used tobacco, or had a preexisting medical condition or acute infection as determined from a medical history questionnaire.

2.2. Study Design.

Subject volunteers at the start of the study reported for collection of dietary information and an initial (Day 0) blood sample. Subjects were then given a 60 day supply of βC supplements. Subjects were instructed to consume one oral dose of the supplement providing 30 mg of βC (Hoffmann-La Roche® Inc., Nutley, NJ) per day for 60 days. Subjects were directed to consume the supplement with a meal containing dietary fat at approximately the same time each day. The amount of supplemental βC was selected to be similar to amounts used in other intervention trials [10–13] and to approximate an amount available from over-the-counter antioxidant supplements. Subjects reported back to the laboratory on a scheduled basis for the 60 day study as described in the section blood sampling and analysis. Subjects served as their own control throughout the study. No separate placebo group was included as the use of such a group for objective outcomes has been questioned [16]. Moreover, for assessments of variables with large interindividual variations [17,18], the use of a repeated measures design has been shown to be valid.

2.3. Dietary information.

At the initial visit, subjects were given a food frequency questionnaire [19]. Subjects completed the questionnaire based on their usual eating habits. The daily number of servings of fruits and vegetables consumed by each subject was calculated from the food frequency questionnaires. Subjects were instructed to maintain their usual dietary consumption habits during the study period.

2.4. Blood sampling and analysis.

Fasting blood samples were collected by a certified phlebotomist from the antecubital vein using a Vacutainer® (Becton-Dickenson) collection system. Blood sampling was conducted at baseline (Day 0) before βC supplementation and on days 10, 20, 30, 45, and 60 of βC supplementation. Following collection, the blood samples were refrigerated at 2 to 8°C until a complete blood count (CBC) and cell isolation were performed. Serum was frozen at −70°C for carotenoid analysis. Serum samples were protected from white light.

Complete blood counts (CBCs) were performed by a clinical laboratory on a SYSMEX® NE8000 hematology analyzer (Toa Medical Electronics USA Inc., Los Alamedos, CA) with an automated leukocyte differential. Total serum cholesterol and serum triglycerides were analyzed for fasting blood samples on a DuPont Dimension® AR clinical chemistry system (DuPont Medical Products, Wilmington, DE). HDL cholesterol was determined on fasting samples by the Dimension® AR following quantitative precipitation of LDL and VLDL by a buffered phosphotungstate reagent [20].

Red blood cells (RBCs) and leukocytes were isolated by centrifugation and saline washing following completion of the CBCs. All leukocyte and RBC preparations were frozen at −70°C for analysis.

2.5. Enzyme and mineral analysis.

RBC and leukocyte superoxide dismutase were analyzed spectrophotometrically using the Bioxytech® SOD-525 assay (OXIS International, Cedex, France). All superoxide dismutase analyses were performed in duplicate according to the method of Nebot et al. [21]. This procedure analyzes copper,zinc-dependent superoxide dismutase which is resistant to ethanol/chloroform treatment. Superoxide dismutase activity was calculated as a function of protein (leukocyte) and hemoglobin (RBC) content of the cell preparation.

Catalase activity in RBC and leukocyte preparations was analyzed according to a modification of the method of Aebi [22]. All analyses were performed in duplicate. The rate constant of a first order reaction was used to calculate units of catalase activity (k) and related to hemoglobin content of the sample for RBCs. Units of catalase activity were calculated and related to the protein content of the leukocyte preparation. For both superoxide dismutase and catalase analyses a control was prepared from normal erythrocytes. Control values for superoxide dismutase and catalase activity were calculated each day and agreed within 10% of previous values. Serum glutathione peroxidase (GPX3) and myeloperoxidase concentrations were measured using enzyme-linked immunosorbant assays (Bioxytech, Oxis International Inc., Portland, OR). Standards were run with both assays and all analyses were performed in duplicate. Serum selenium concentrations were measured using graphite furnace atomic absorption spectrophotometry (Perkin Elmer Model 5100, Norwalk, CT). Standard Reference Material (SRM) 1598 (National Institute of Standards and Technology, Gaithersburg, MD) bovine serum, which is certified for selenium, served as a standard. The mean serum selenium concentration of SRM 1598 analyzed in the lab was 259.3 mmol/L which was within the reported assay range, 255.5 to 293.9 mmol/L. Serum selenium assays were run in duplicate.

Protein content of leukocyte preparations was determined in duplicate by the Coomassie® Plus Protein Assay Reagent (Pierce Chemical Company, Rockford IL). This method is a modification of the Bradford method based on the protein binding properties of the dye Coomassie® Brilliant Blue [23].

2.5.1. Carotenoid analysis

Serum samples were thawed away from white light and saponified according to a modification of the method of Thompson et al. [24]. All stages of saponification, extraction and analysis were completed under yellow light to minimize oxidation of carotenoids. Carotenoid analysis was performed in duplicate for each sample. All reagents used were Optima® HPLC grade obtained from Fisher Chemical (Fisher Scientific, Fair Lawn, NJ). Following saponification, carotenoids were extracted three times with 5.0 mL of hexane containing 0.01% BHT. The combined hexane phase was evaporated to dryness and redissolved in a mobile phase containing acetonitrile and methanol (10/14 v/v) as modified from Bushway [25]. HPLC analysis was completed using a C_{18} 218TP54 column (5 μm, 4.6 mm X 25 cm)(Vydac, Hesperia, CA) and a Shimadzu Model LC-10AS pump equipped with a Shimadzu Model SPD-10AV uv-visible detector set at 450 nm with 0.004 AUFS (Shimadzu Scientific Instruments, Inc, Columbia MD). The mobile phase used was acetonitrile/methanol/tetrahydrofuran (10/14/1 v/v), and the flow rate was adjusted to 1.0 mL/min. Sample injection volume was 20 μL. Peak integration was completed using a Shimadzu CR501 Chromatopac integrator and software.

2.6. Statistical analysis.

The data were analyzed using SAS® software (SAS Institute Inc., Cary, NC) and InStat software (Graph Pad Software, Inc., San Diego, CA). Differences between baseline and supplemented samples were analyzed by repeated analysis of variance (ANOVA) general linear models and a least significant difference (LSD) multiple comparisons *post-hoc* test. If *P*-values of less than 0.05 were found for any parameter then Tukey-Kramer multiple comparisons tests were performed to further identify statistically significant differences. Statistical significance was set at the $P < 0.05$ level. Results are expressed as mean ± standard error of mean (SEM).

3. Results

Twenty subjects, age 31 ± 8 years, completed the study protocol. Two subjects did not complete the protocol for medical reasons (asthma and prescription of a retinoid analogue). The subject's mean height and weight were 1.72 ± 0.09 meters and 71.8 ± 14.4 kilograms, respectively. Subjects were healthy throughout the study. Fruit intake averaged 1.28 ± 0.75 servings/day while vegetable intake averaged 1.98 ± 0.75 servings/day. Side effects of βC supplementation were reported by 30% of subjects and included gastrointestinal symptoms such as gas and soft stools. Forty percent of subjects had hypercarotonemia of the palms and forehead with an orange tint observed after approximately 30 days of supplementation.

Serum βC concentrations are listed in Table 1. There were no significant gender differences on day 0 (baseline) for serum concentrations of βC. Males had slightly lower levels than females of βC (0.190 ± 0.033 μmol/L vs. 0.301 ± 0.060 μmol/L) at baseline but the difference was not significant. There were no significant differences between males and females for any of the serum βC measurements

Table 1
Mean ± SEM (μmol/L) serum beta-carotene concentration in subjects before supplementation at baseline (Day 0) and on days 10, 30 and 60 of supplementation

	Males (n = 12)	Females (n = 8)	All Subjects (n = 20)
Day 0	0.190 (0.033)[a]	0.301 (0.060)[a]	0.234 (0.033)[a]
Day 10	1.358 (0.145)[b]	2.154 (0.208)[b]*	1.676 (0.147)[b]
Day 30	2.370 (0.251)[c]	3.273 (0.516)[c]	2.732 (0.267)[c]
Day 60	2.951 (0.303)[c]	3.630 (0.684)[c]	3.222 (0.326)[c]

[a] Values in columns with different letters differ significantly ($p < 0.05$).
[b] Values in columns with different letters differ significantly ($p < 0.05$).
[c] Values in columns with different letters differ significantly ($p < 0.05$).
* Significant difference between males and females ($p < 0.05$).

with the exception of day 10. Females had a significantly higher level of βC on day 10 than males (2.154 ± 0.208 μmol/L vs. 1.358 ± 0.145 μmol/L, respectively).

Serum βC was significantly increased from day 0 (0.234 ± 0.033 μmol/L) at day 10 (1.676 ± 0.147 μmol/L), day 30 (2.732 ± 0.267 μmol/L) and day 60 (3.222 ± 0.326 μmol/L). The increase in βC from day 10 to day 30 was also significant. The increase in βC from day 30 to day 60 was not significant.

To allow for comparison with the varying published units of enzyme activity, RBC superoxide dismutase is expressed in two units of activity, units/mL extract and units/g hemoglobin (Hb), (Table 2). RBC catalase activity is expressed as k/g Hb according to the method of Aebi [22]. Leukocyte superoxide dismutase and catalase activities are expressed as units/mg protein. The increase in RBC superoxide dismutase expressed as units/mL of extract at day 30 (113.1 ± 2.6 units/mL) compared to day 0 (105.3 ± 2.7 units/mL) tested by LSD did not reach statistical significance ($P = 0.09$). The value at day 60 (106.6 ± 2.4 units/mL) did not differ from day 0 or day 30. There were no significant differences in RBC superoxide dismutase expressed as units/g Hb at day 0 (2518 ± 103 units/g Hb) compared to day 30 (2460 ± 59 units/g Hb) and day 60 (2387 ± 69 units/g Hb). There was a significant decrease in leukocyte superoxide dismutase activity at days 30 (4.69 ± 0.41 units/mg protein) and 60 (5.98 ± 0.33 units/mg protein) compared to day 0 (7.54 ± 0.62 units/mg protein).

Values for blood cell catalase activity are also listed in Table 2. There was no significant change in RBC catalase activity expressed as k/g Hb [22]. RBC catalase activity at baseline (326.8 ± 13.2 k/g Hb) was not significantly different at day 30 (329.9 ± 16.0 k/g Hb) or day 60 (298.5 ± 18.7 k/g Hb). There was no significant change in leukocyte catalase during the supplementation period. Leukocyte catalase at day 0 (1.81 ± 0.15 units/mg protein) did not differ from levels at day 30 (1.68 ± 0.11 units/mg protein) and day 60 (1.95 ± 0.13 units/mg protein).

Serum glutathione peroxidase and selenium concentrations at baseline and days 20, 45, and 60 following βC supplementation are shown in Table 3. Serum glutathione peroxidase concentrations decreased significantly between baseline (63 ± 4 ng/mL) and day 45 (48 ± 4 ng/mL), and between baseline and day 60 (49 ± 5 ng/mL). Serum selenium and myeloperoxidase concentrations did not significantly differ between baseline and days 20, 45, or 60 of βC supplementation (Table 3). Blood hemoglobin concentrations, assessed to ensure adequate iron status which has been shown to diminish glutathione peroxidase [26], also did not differ significantly between baseline and days 20, 45, and 60 of βC supplementation (Table 3).

There were no significant changes in subjects lipid profiles, serum cholesterol concentrations or hematology parameters as assessed by CBC during the course of the study (data not shown).

Table 2
Mean ± SEM red blood cell (RBC) and leukocyte superoxide dismutase (SOD) and catalase activity in subjects before supplementation at baseline (Day 0) and on days 30 and 60 of beta-carotene supplementation

Parameter		Baseline	Supplementation	
		Day 0	Day 30	Day 60
SOD				
RBC				
	units/mL extract	105.3 (2.7)[a]	113.1 (2.6)[a]	106.6 (2.4)[a]
	units/g Hb[1]	2518 (103)[a]	2460 (59)[a]	2387 (69)[a]
Leukocyte				
	units/mg protein	7.54 (0.62)[a]	4.69 (0.41)[b]	5.98 (0.33)[b]
Catalase				
RBC				
	k/g Hb[2]	326.8 (13.2)[a]	329.9 (16.0)[a]	298.5 (18.7)[a]
Leukocyte				
	units/mg protein	1.81 (0.15)[a]	1.68 (0.11)[a]	1.95 (0.13)[a]

[a] Values in rows with different letters differ significantly ($p < 0.05$).
[b] Values in rows with different letters differ significantly ($p < 0.05$).
[1] Hb = hemogoblin.
[2] k = unit of catalase activity by Aebi [22].

Table 3
Mean ± SEM serum glutathione peroxidase, selenium, and myeloperoxidase and blood hemoglobin concentrations in subjects before supplementation at baseline (Day 0) and on days 20, 45, and 60 of beta-carotene supplementation

Parameter	Baseline	Supplementation		
	Day 0	Day 20	Day 45	Day 60
Glutathione Peroxidase (ng/mL)	63 ± 4[a]	58 ± 3[a,b]	48 ± 4[b]	49 ± 5[b]
Selenium (mmol/L)	710.8 ± 44.8[a]	614.7 ± 32.0[a]	678.7 ± 44.8[a]	653.1 ± 38.4[a]
Hemoglobin (g/L)	152 ± 4[a]	151 ± 3[a]	156 ± 3[a]	150 ± 3[a]
Myeloperoxidase (ng/mL)	133 ± 23[a]	Not Available	129 ± 44[a]	202 ± 61[a]

[a] Values in rows with different letters differ significantly ($p < 0.05$).
[b] Values in rows with different letters differ significantly ($p < 0.05$).

4. Discussion

Individual subject tolerance to supplementation with a 30 mg/day of βC was variable with 30% of subjects reporting gastrointestinal side effects. Similar effects were reported in the Physicians Health Study with a 50 mg dose of βC on alternate days [10]. Forty percent of subjects had hypercarotenemia of the palms and forehead with the discoloration appearing after approximately 30 days of βC supplementation. Yellowing of the skin has been reported at doses of 15 mg/day [27] but is more common at higher doses of 30 mg/day or more [28,29].

Subjects in this study reported a mean intake of 3.26 servings of fruits and vegetables daily. This is in agreement with other surveys of the American public [4,30] and is below the intake recommended by the National Cancer Institute [30]. Mean intake of carotenoids for adults has been reported to be 2.3 mg per day with 10% of the population consuming less than 0.5 mg [31]. Dietary carotenoids were not assessed in this study since the supplemental dose was more than 10 times the reported mean intake for US adults and questions concerning the completeness of the carotenoid database have been raised [32].

Baseline serum concentrations of βC reported in Table 1 are close to the reported reference ranges for the U.S. population from the 1988 to 94 NHANES III survey [33]. There was no gender difference in baseline βC concentration in the present study. Women have been reported to have higher serum carotenoid concentrations than men [34–36]. In this study women had a significantly higher mean serum βC concentration than men only on day 10 of supplementation. Interindividual variations in baseline carotenoid values and responses to supplementation have been reported [17,18,37,38]. Baseline serum concentration of βC was the factor most strongly associated with serum levels after supplementation [39]. This suggests individual homeostatic mechanisms that influence carotenoid concentrations both under normal conditions and in response to supplementation.

Serum βC concentrations significantly increased in response to supplementation. Lack of an increase in serum βC concentration after day 30 suggests a leveling of the serum response to a 30 mg/day supplement. Costantino et al. [40] observed a similar increase and then leveling in serum βC in response to oral supplementation of 15 mg/day. Mean serum βC concentration increased after four months with no further increase when supplementation continued for 10 months.

In response to supplementation, no changes in the activity of RBC or leukocyte catalase or cytosolic RBC copper, zinc-dependent superoxide dismutase activities were found. Myeloperoxidase concentrations also did not change in response to supplementation. RBC catalase activity at all time points was within the proposed reference range for adults over age 20 [41]. Similarly, RBC superoxide dismutase activity expressed as units/mL extract are close to values published by the authors of the method of 93 units/mL extract for human RBCs [21]. Baseline leukocyte superoxide dismutase activity in this study also compared closely with values reported by Ciuffetti et al. [42].

In contrast the other enzymes assayed, two enzymes were affected by βC supplementation. Leukocyte superoxide dismutase activity decreased significantly at 30 and at 60 days of βC supplementation compared to baseline. Serum glutathione peroxidase concentrations decreased significantly between baseline and day 45 and day 60 of supplementation. No significant changes in serum selenium concentrations were observed and serum selenium concentrations remained within normal ranges suggesting that the change in glutathione peroxidase was not due to changes in selenium availability [43,44]. Similarly, blood hemoglobin concentrations, assessed to ensure adequate iron status which has been shown to diminish glutathione peroxidase [26] also did not differ from baseline during the supplementation period.

The reason for the observed decrease in leukocyte superoxide dismutase activity and serum glutathione peroxidase concentration is not clear. Superoxide dismutase, catalase and glutathione peroxidase function as part of the primary antioxidant defense system in mammals. Superoxide dismutase catalyzes the formation of hydrogen peroxide from the superoxide radical. Glutathione and the iron-dependent catalase catalyze the removal of hydrogen peroxide to water and oxygen. Myeloperoxide, found in the primary granules of neutrophils, has a different function and is a major component of the bactericidal capacity of phagocytes.

It catalyzes the production of hypochlorous acid, a powerful oxidant, from chloride ion and hydrogen peroxide as part of the intracellular destruction of engulfed foreign material [45]. Supplemental βC may act as a direct scavenger of reactive oxygen species and decrease the body's need for certain antioxidant enzymes. Alternately, βC may be suppressing the synthesis or shortening the half-life of some antioxidant enzymes. The knowledge of the mechanisms by which βC may function as an antioxidant in vivo is limited.

Few studies have examined the effects of βC on antioxidant enzymes. Moreover, the results of the studies have not been consistent. Lawlor and O'Brien [46] reported a decrease in catalase activity in chick embryo fibroblasts incubated with low levels of βC with no change in superoxide dismutase. In contrast, Blakely and associates [47] found a significant decrease in hepatic superoxide dismutase but not catalase activity in rats supplemented with ten times the recommended level of βC. Canthaxanthin (4,4′-diketo-βC, a βC analogue) significantly increased hepatic catalase, significantly decreased hepatic glutathione peroxidase activity, but had no effect on hepatic copper,zinc-dependent superoxide dismutase in mice [15]. Consumption of spinach, which contained a mixture of carotenoids, significantly decreased RBC catalase activity in healthy humans; however, consumption of mixed carotenoids as a supplement did not alter blood catalase, superoxide dismutase or glutathione peroxidase [14]. Omaye and associates [48] found that a 28-day repletion period providing 15 mg βC after a low βC diet in nine women did not significantly affect glutathione peroxidase activity, but significantly increased catalase concentrations. This study is the first to investigate the effects of βC supplements on several antioxidant enzymes in healthy humans.

Because of their antioxidant properties dietary carotenoids such as βC from fruits and vegetables are believed to be beneficial in the prevention of disease. Reactive oxygen species, formed as a by product of aerobic metabolism and deliberately during cellular metabolism, can result in damage to cell membrane lipids, proteins and DNA. The products formed by oxidative damage are thought to play significant roles in the pathogenesis of disease, including cancer and cardiovascular disease.

Many questions concerning the safety and efficacy of βC supplements in disease prevention remain unanswered. Whether the observed decreases in oxidant defense enzymes results in radical-induced damage to cell components and induces disease is unknown. Reasons for the increased incidence of cancer and mortality observed with βC supplementation in several intervention trials are also unknown. This study observed a significant decrease in serum glutathione peroxidase concentrations and leukocyte superoxide dismutase activity with βC supplementation. A decrease in leukocyte SOD activity may result in a decreased capacity to generate ROS as part of the oxidative burst. Preservation of the inducibility of leukocyte superoxide dismutase activity has been correlated with longevity [49]. Recently the U.S. Preventive Services Task Force (USPSTF) recommended against βC supplements, either alone or in combination, for the prevention of cancer and cardiovascular disease. The USPSTF stated that "β-carotene supplements are unlikely to provide important benefits and might cause harm in some groups" [50]. Additional research is needed to characterize the effects of supplemental βC on body processes and their interactions with cell antioxidants.

Acknowledgments

This research was supported in part by the Alabama Agricultural Experiment Station 13-006. The authors wish to thank Hoffman-LaRoche® for providing beta-carotene supplements and East Alabama Medical Center for donating phlebotomy supplies. We also wish to thank Patti West for technical assistance with atomic absorption spectrophotometry and Amy Simone for assistance with carotenoid analysis.

References

[1] Verlangier AJ, Kapeghian JC, el-Dean S, Bush M. Fruit and vegetable consumption and cardiovascular mortality. Med Hypotheses 1985;16:7–15.
[2] Acheson RM, Williams DRR. Does consumption of fruits and vegetables protect against stroke? Lancet 1983;2:1191–3.
[3] Ziegler RG. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 1989;119:116–22.
[4] Steinmetz KA, Potter JD. Vegetables, fruit and cancer prevention: a review. J Am Diet Assoc 1996;96:1027–39.
[5] Block G, Patterson BH, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; 18:1–29.
[6] Manson JE, Stampfer MJ, Willett WC. A prospective study of antioxidant vitamins and incidence of coronary heart disease in women. Circulation 1991;84(II):2168.
[7] Gey KF, Stahelin HB, Eichholzer M. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: basel prospective study. J Clin Invest 1993;71: 3–6.
[8] Bolton-Smith C, Woodward M, Tunstall-Pedoe H. Dietary intake by food frequency questionnaire and ratios for coronary heart disease risk. Eur J Clin Nutr 1992;46:85–93.
[9] Bendich A. The safety of β-carotene. Nutr Cancer 1988;11:207–14.
[10] Hennekens CH, Buring JE, Manson JE, Stampfer MJ, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9.
[11] Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. β-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 1999;91: 2102–6.
[12] Omenn GS, Goodman GE, Thornquist MD, Rosenstock L, Barnhart S, Gylys-Collwell I, Metch B, Lund B. The carotene and retinol efficacy trial (CARET) to prevent lung cancer in high-risk populations: pilot study with asbestos-exposed workers. Cancer Epidemiol Biomarkers Prev 1993;2:381–7.
[13] The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of

lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
[14] Castenmiller JM, Lauridsen ST, Dragsted LO, van het Hof KH, Linssen JP, West CE. β-carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. J Nutr 1999;129:2162–9.
[15] Palozza P, Calviello G, De Leo M, Serini S, Bartoli G. Canthaxanthin supplementation alters antioxidant enzymes and iron concentration in liver of Balb/c mice. J Nutr 2000;130:1303–8.
[16] Hrobjartsson A, Gotasche PC. Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. N Engl J Med 2001;344:1594–602.
[17] Brown ED, Micozzi MS, Craft NE, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, Smith JC. Plasma carotenoids in normal men after a single ingestion of vegetables or purified β-carotene. Am J Clin Nutr 1989;49:1258–65.
[18] Carughi A, Hooper FG. Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture. Am J Clin Nutr 1994;59:896–9.
[19] Block G. Health habits and history questionnaire: diet history and other factors. Personal Computer System Packet, National Cancer Institute. Washington, DC: 1989.
[20] Lopes-Virella MF, Stone P, Ellis S, Coldwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882–4.
[21] Nebot C, Moutet M, Huet P, Xu JZ, Yadan JC, Chaudiere J. Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. Anal Biochem 1993; 214:442–51.
[22] Aebi H. Catalase. In: Bergmeyer HU, editor. Methods in enzymology. New York: Academic Press, 1974. p. 673–8.
[23] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 1976;72:248–54.
[24] Thompson JN, Duval S, Verdier P. Investigation of carotenoids in human blood using high-performance liquid chromatography. J Micronut Anal 1985;1:81–91.
[25] Bushway RJ. Determination of α- and β-carotene in some raw friuts and vegetables by high-performance liquid chromatography. J Agric Food Chem 1986;34:409–12.
[26] Moriarty PM, Picciano MF, Beard JL. Classical selenium-dependent glutathione peroxidase expression is decreased secondary to iron deficiency in rats. J Nutr 1995;125:293–301.
[27] Barber DA, Harris SR. Oxygen free radicals and antioxidants: a review. Am Pharm 1994;NS34:26–35.
[28] Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993;85:1483–92.
[29] Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.
[30] Havas S, Heimendinger J, Reynolds K, Barabowski T, Nicklas TA, Bishop D, Buller D, Sorenson G, Beresford S, Cowan A. 5 a Day for Better Health: a new research initiative. J Am Diet Assoc 1994;94: 32–6.
[31] Patterson BH, Block G. Food choices and the cancer guidelines. Am J Public Health 1988;78:282.
[32] Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: an evaluation of analytic data. J Am Diet Assoc 1993;93:284–96.
[33] Briefel R, Sowell A, Huff C, Hodge S, Koncikowski J, Wright J, Gunter E. The distribution of serum carotenoids in the U. S. population: results from the Third National Health and Nutrition Examination Survey (NHANES III). FASEB J 1996;10:A813.
[34] Olmedilla B, Granado F, Blanco I, Rojas-Hidalgo E. Seasonal and sex-related variations in six serum carotenoids, retinol, and a-tocopherol. Am J Clin Nutr 1994;60:106–10.
[35] Brady WE, Mares-Perlman JA, Bowen PE, Stacewicz-Sapuntzakis M. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J Nutr 1996;126:129–37.
[36] Hallfrisch J, Muller DC, Singh VN. Vitamin A and E intakes and plasma concentrations of retinol, β-carotene, and α-tocopherol in men and women of the Baltimore Longitudinal Study of Aging. Am J Clin Nutr 1994;60:176–82.
[37] Van Vliet T, Schreurs WHP, van den Berg H. Intestinal β-carotene absorption and cleavage in men: response of β-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of β-carotene. Am J Clin Nutr 1995;62:110–6.
[38] Johnson EJ, Russell RM. Distribution of orally administered β-carotene among lipoproteins in healthy men. Am J Clin Nutr 1992;56: 128–35.
[39] Albanes D, Virtamo J, Rautalahi M, Haukka J, Palmgren J, Gref CG, Heinonen OP. Serum beta-carotene before and after beta-carotene supplementation. Eur J Clin Nutr 1992;46:15–24.
[40] Costantino JP, Kuller LH, Begg L, Redmond CK, Bates MW. Serum level changes after administration of a pharmacologic dose of β-carotene. Am J Clin Nutr 1988;48:1277–83.
[41] Guemouri L, Artur Y, Herbeth B, Jeandel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin Chem 1991;37:1932–7.
[42] Ciuffetti G, Mannarino E, Paltriccia R, Malagigi V, Sergi F, Paulisch P, Pasqalini L, Lupattelli G. Leucocyte activity in chronic venous insufficiency. Int Angiol 1994;13:312–6.
[43] Hill KE, Xia Y, Akesson B, Boeglin ME, Burk RF. Selenoprotein P concentrations in plasma is an index of selenium status in selenium deficient and selenium supplemented Chinese subjects. J Nutr 1996; 126:138–45.
[44] Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids Washington, DC: National Academy Press: 2000, p. 284–324.
[45] Moslen MT. Reactive oxygen species in normal physiology, cell injury and phagocytosis. In: Armstrong D, editor. Free radicals in diagnostic medicine. New York: Plenum Press, 1994. p. 17–27.
[46] Lawlor SM, O'Brien NM. Modulation of oxidative stress by β-carotene in chicken embryo fibroblasts. British J Nutr 1995;73:841–50.
[47] Blakely SR, Slaughter L, Adkins J, Knight EV. Effects of β-carotene and retinyl palmitate on corn oil-induced superoxide dismutase and catalase in rats. J Nutr 1988;118:152–8.
[48] Omaye ST, Burri BJ, Swendseid ME, Henning SM, Briggs LA, Bowen HT. Blood antioxidant changes in young women following beta-carotene depletion and repletion. J Am Coll Nutr 1996;15:469–74.
[49] Niwa Y, Ishimoto K, Kanoh T, Induction of superoxide dismutase in leukocytes by paraquat: correlation with age and possible predictor of longivity. Blood 1990;76:835–41.
[50] US Preventive Services Task Force. Routine vitamin supplementation to prevent cancer and cardiovascular disease: recommendations and rationale. Ann Intern Med 2003;139:51–5.