# Carotenoids as Cellular Antioxidants (43431)

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*Abstract.* **Consumption of carotenoids is associated with an enhanced immune response and protection against neoplasia and atherosclerosis. Because these effects have been achieved using carotenoids with no pro-vitamin A activity, they are assumed to be due to the antioxidant properties of carotenoids. Carotenoids protect against photosensitized oxidation by quenching singlet oxygen. In addition, β-carotene reacts chemically with peroxyl radicals to produce epoxide and apocarotenal products. To investigate the potential significance of these reactions to biological systems, we have used soybean lipoxygenase to generate peroxyl radical enzymatically. β-Carotene inhibits the oxidation of linoleic acid by soybean lipoxygenase as well as the formation of the hydroperoxide product. In addition, the absorption of β-carotene is diminished (bleached) by soybean lipoxygenase. The potential significance of these antioxidant reactions of carotenoids to biological function is discussed.** [P.S.E.B.M. 1992, Vol 200]

Carotenoids are widely distributed in nature. Over 600 have now been identified and characterized. Due to their highly conjugated double-bond system, carotenoids are extremely efficient quenchers of singlet oxygen. Singlet oxygen reactions of carotenoids are well known in bacteria and plants (1, 2). In fact, the first biological reaction described for carotenoids that did not involve their conversion to retinoids was protection against erythropoietic protoporphyria and other diseases of light sensitivity (3) by quenching of singlet oxygen. Although potentially important, a significant role for singlet oxygen in other molecular events *in vivo* has not yet been demonstrated. However, Kanofsky and Sima (4) have recently demonstrated that singlet oxygen is produced in high yield by the reaction of ozone with biological molecules, thereby suggesting that singlet oxygen may be an important intermediate in the biochemical damage caused by ozone.

Recently, reactions of carotenoids with peroxyl radicals in model systems were described (5–7). Two major epoxide products of β-carotene have been characterized and identified as the 5,6-epoxy-β,β-carotene and the 15,15′-epoxy-β,β-carotene (5, 6). These products appear to be formed by radical addition to the carotenoid molecule, in contrast with the electron or hydrogen transfer reactions of other cellular antioxidants, such as vitamin E. In addition, a variety of apocarotenals have been identified by radical-initiated autoxidation of β-carotene (6). Chemical reactions of carotenoids and active oxygen species have been discussed in detail earlier in this Journal (1). We discuss here ways in which these reactions may explain some of the actions of carotenoids as biomodulators of disease processes, as well as an enzymatic system which may serve as a molecular model for studying the *in vivo* actions of carotenoids.

## Relation of Carotenoids to Chronic Diseases

**Coronary Heart Disease.** In a preliminary report, dietary carotenoids were inversely related to heart disease and stroke in the Physicians Health Trial (8), and plasma β-carotene concentrations were inversely related to the risk of angina (9). It has been proposed that β-carotene, in combination with other antioxidants, protects against lipoprotein oxidation (10) and thus plays a potentially important role in retarding the progression of atherosclerosis. Oxidized low density lipoproteins (LDL) have been associated with atherosclerosis, and antioxidants that reduce rates of degradation of LDL also prevent progression of atherosclerosis in rabbits (11). In addition to oxidation of unsaturated fatty acids, lipid peroxidation results in fragmentation of the major apolipoprotein, apoB100. The chemotactic activity of oxidized LDL facilitates recruitment of blood monocytes, which become macrophages. Oxidized LDL inhibits migration of macrophages from the arterial wall, facilitating continued uptake of modified lipoprotein. Progression of this process generates foam

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cells. Because macrophages can also oxidize LDL, the process can be self-perpetuating. Oxidized LDL also promotes platelet aggregation (12). Lipoproteins of oxidized LDL and those extracted from homogenates of human aorta demonstrate similar electrophoretic mobility, as well as other properties. Autoantibodies against oxidized LDL have also been detected in human sera. It is of interest that smoking, which is correlated with lowered concentrations of plasma carotene (13), may result in LDL modification (14).

**Cataract.** Dietary β-carotene has recently been reported to be protective for cortical, nuclear, and mixed cataract (15–17). As the ocular lens is physiologically damaged by oxyradicals, β-carotene is thought to exert its protective effect by counteracting the effects of these active oxygen species and thereby preserving membrane integrity (16). Thus, β-carotene may be useful for prophylaxis or therapy against cataracts.

**Cancer.** The best-studied association of carotenoids and disease has been that of carotenoids and cancer. Dietary β-carotene is associated with decreased risk for several types of cancer, including cervical cancer (18). However, the most consistent association is that of decreased dietary β-carotene with lung cancer, particularly squamous cell cancer. At least 16 studies have now shown a significant association between a decreased dietary intake of β-carotene and an increased risk of lung cancer (13, 14, 18–21). In addition, seven studies have shown an association between lowered serum levels of β-carotene and risk for lung cancer. These studies have been notably consistent. However, unresolved issues of gender differences, histological specificity, lifestyle (including cigarette smoking and alcohol consumption), as well as nutrient-nutrient interaction continue to confound the interpretation of these studies.

Carotenoids may also prevent the development of premalignant lesions. Significantly increased risk of cervical dysplasia/carcinoma was associated with low dietary intakes and low blood levels of β-carotene in five studies (22, 23), although deVet and co-workers (24) saw no association of β-carotene intake on regression of cervical dysplasia. One of the strongest associations of β-carotene and disease has been that with the potential prevention of squamous cell oral cancer. In addition to being associated with a reduction in frequency of micronucleated cells in buccal mucosa of betel nut chewers, β-carotene has brought about complete or partial regression of oral leukoplakia in several trials (25–27). Since carotenoids are not toxic, carotenoid treatment may be efficacious in the prevention and treatment of some types of oral cancer.

Somewhat surprisingly, in the one published study to date, retinoic acid protected against skin cancer, whereas β-carotene did not (28). These results may reflect different sites of action for retinoids and carotenoids in cancer chemoprevention, different dosage requirements, or different response times. Alternatively, it has been suggested that high dosages of β-carotene may have interfered with the actions of other antioxidants, particularly of vitamin E (29).

More than 30 studies have shown that carotenoids protect against neoplasia in animals. β-Carotene protects against UV-induced and chemically induced carcinogenesis and against tumor implants in a variety of regimens in mice, rats, and hamsters (30, 31). Interestingly, it was reported recently (32) that oral β-carotene was ineffective in protecting against UVB-induced dermal changes. These data suggest that route of administration, dosage, and duration of study can significantly affect the outcome of carotenoid treatment.

In single cell experiments, β-carotene inhibited dimethylbenzanthracene-induced malignant transformation in mouse mammary cells (33) and UV-induced and 3-methylcholanthrene-induced transformation in fibroblasts (34). In addition, carotenes inhibited proliferation of a human neuroblastoma cell line (GOTO) and suppressed the level of N-*myc* messenger RNA (35). Although carotenoid solubility and purity may have been a problem in these experiments, interestingly, α-carotene was more effective than β-carotene. In addition, β-carotene and canthaxanthin (a non-provitamin A carotenoid) inhibited proliferation of two human squamous cell lines (36). In these experiments, the onset of the response to β-carotene was accompanied by the appearance of a 70-kDa protein thought to be associated with regulation of cellular oxidation states. However, very little information is now available on the molecular mechanism(s) by which carotenoids exert their effects *in vivo*.

## Possible Mechanisms of Action of Carotenoids

**Metabolism.** It is likely that carotenoids, in addition to acting as antioxidants, also function by virtue of being metabolized to retinoids or apocarotenoids, which may either enhance or inhibit the effects of retinoic acid. Retinoic acid blocks promotion and proliferation and induces differentiation and cellular adhesion. At the biochemical level, retinoic acid interacts with cell membranes, binds to intracellular cytoplasmic proteins and to nuclear receptors, modulates enzyme action, and represses oncogene expression (37). Thus, it would be of great interest to test the oxidation products of β-carotene, particularly the apocarotenals, for their effects on these reactions. Indeed, proposed mechanisms by which oxidants promote carcinogenesis (38) include activation of protein kinases and induction of growth genes (c-*fos*, c-*myc*).

**Immune Response.** In addition, the effects of carotenoids or their metabolites on cancer could be related to the immune response. The ability of carotenoids to protect against bacterial infection and tumor

growth has been known since 1959 (39). In the last decade, there has been a resurgence of interest in this phenomenon. Since 1985 alone, there have been more than 20 reports of enhancement of the immune response by β-carotene, e.g., lymphocyte proliferation, tumoricidal capacity, and enhanced graft-vs-host reactions (40–42). α-Carotene, β-carotene, and canthaxanthin have been associated with increased mitogenic response (43, 44) and enhanced cytokine production and responsiveness (45–51).

Retinoids as well have been demonstrated to enhance immune function in some systems (37). In some cases, β-carotene action is antagonistic to that of vitamin A. For example, β-carotene blocks the inhibiting effects of vitamin A on *in vitro* interferon-induced human monocyte receptor expression (46, 47). Conversely, in other cases, retinoids and carotenoids appear to act synergistically. In this regard, resistance to an antigenic tumor in mice developed when canthaxanthin plus retinyl palmitate were administered in the diet together, but not when either agent was given alone (52).

**Molecular Actions.** The molecular mechanisms by which carotenoids exert their effects are likely to involve a number of reactions. Carotenoid products, e.g., retinoic acid, retinol, or apocarotenals could antagonize or enhance retinoic acid action by binding to a retinoic acid receptor. In addition, carotenoids could protect in a rather unspecific manner against oxidative damage to membranes, organelles, and proteins. Carotenoids might also function together with other antioxidants, particularly with vitamins A, C, and E (10, 16, 49). Such interactions will be discussed in the following paper (53). Thus, no single model system mimics carotenoid actions *in vivo*.

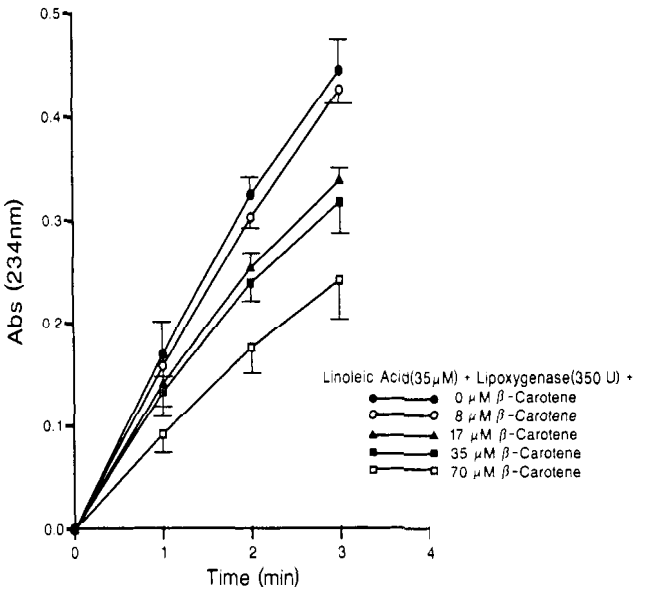


**Figure 1.** Inhibition of lipoxygenase catalyzed diene formation by β-carotene. β-Carotene solubilized in tethahydrofuran was added to micelles formed by sonicating linoleic acid in 0.2 *M* sodium borate (pH 9). The reaction was initiated by addition of soybean lipoxygenase (350 units). Diene formation was followed spectrophotometrically at 234 nm. Each point represents the average of ≥ six determinations, except for 8 μ*M* and 17 μ*M* β-carotene, in which cases duplicate determinations were made.

## Co-oxidation of Fatty Acids and Carotenoids by Lipoxygenases

An attractive system to study some of these reactions is that of the co-oxidation of fatty acids and carotenoids by lipoxygenases (54–57). Lipoxygenases, enzymes of fatty acid metabolism both in plants and animals, have been extensively studied and have been used to model human lipoxygenase action. They were first shown to co-oxidize carotenoids by Sumner and Sumner (55) in 1940. More recently, it was proposed that lipoxygenases metabolize carotenoids to retinoids *in vivo* (58) and that arachidonic acid metabolism is inhibited by β-carotene.

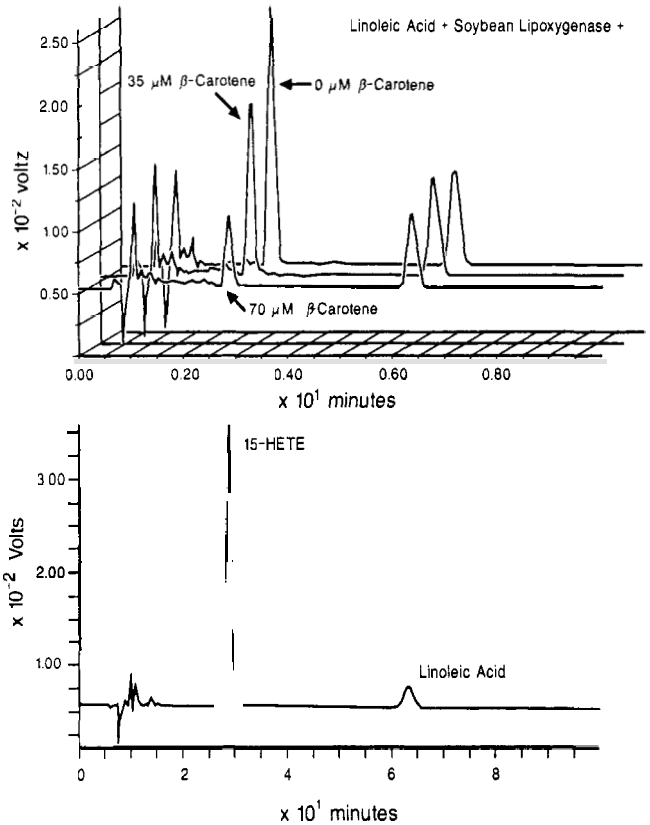


**Figure 2.** Inhibition by β-carotene of hydroperoxide formation by soybean lipoxygenase. β-Carotene was reacted with linoleic acid and lipoxygenase as described in Figure 1. Products were acidified with 3% formic acid to pH 3.5, eluted on $C_{18}$ filters, injected on high performance liquid chromatography, and eluted with acetonitrile and water (75:25 v/v), with sufficient acetic acid to achieve a pH of 3.5. Lipoxygenase products are shown in the top panel. Reference compounds (15-hydroxyeicosatetraenoic acid [15-HETE] and linoleic acid) are shown in the bottom panel. Peroxide products of linoleic acid were tentatively identified using 15-HETE, the corresponding ω-6 oxidation product of arachadonic acid.

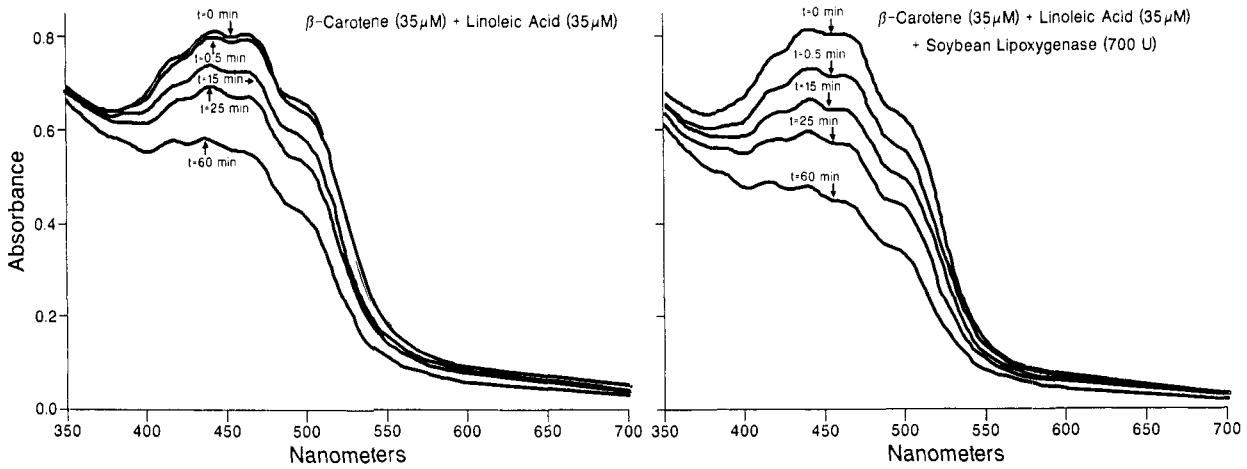


**Figure 3.** Loss of absorbance at 452 nm ("bleaching") of β-carotene in the presence of soybean lipoxygenase and linoleic acid. Reaction mixtures were prepared as described in Figure 1 and a decrease in absorbance followed spectrophotometrically using photodiode array detection.

Oxyeicosanoids are the first-formed products of the reaction of fatty acids with *in vivo* lipoxygenase. In addition to being highly reactive biomolecules, these compounds are precursors of leukotrienes and other cytokines that are involved with chemotaxis, natural killer cell activity, production of γ-interferon, and activation of T suppressor cells (59, 60).

**Carotenoid Products of Lipoxygenase Action.** We reported recently that β-carotene inhibits the reaction of soybean lipoxygenase and linoleic acid (61). Methodology for these reactions has been described elsewhere (62). As shown in Figure 1, β-carotene inhibits the rate of conjugated diene formation from linoleic acid in a concentration-dependent fashion. Concomitantly, the formation of the major peroxide product (Fig. 2), the putative 13-hydroperoxy-9,11-octadecadienoic acid, decreases (60). When β-carotene and linoleic acid are added to soybean lipoxygenase at concentrations of 35 $\mu M$, the carotenoid absorbance at 452 nm is significantly diminished by 5 min (Fig. 3). Two major fractions of oxidized products were detected: (i) a polar fraction possibly containing aldehydes or apocarotenals, and (ii) a more hydrophobic fraction with a retention time similar to epoxide products formed by reaction of carotene with peroxyl radical. However, at these concentrations, we have not been able to detect the formation of any enzyme-dependent carotenoid products, absorbing in the range of 300 to 600 nm, that are different from the auto-oxidation products identified by reaction with peroxyl radical. Similar, if not identical, oxidation of β-carotene was reported by Handelman *et al.* (5). Thus, the enzymatic products indicated by the enzyme-dependent diminution in $A_{452}$ have not been identified. They may be too polar to be retained by our high performance liquid chromatography column or, conversely, too hydrophobic to be eluted. Alternatively, products may be formed that do not have absorption maxima in the range of 300 to 600 nm.

**Relation of Cartenoid Bleaching to Hydroperoxide Formation.** The relationship of carotene bleaching to inhibition of hydroperoxide product formation is not yet clear. Enzymatically produced peroxyl radicals might oxidize carotene directly, resulting in carotene bleaching (54). However, we observed no relationship between the rate of decrease in carotenoid absorbance at 452 nm and the rate of peroxide product formation. Thus, the oxidation of β-carotene may or may not be directly linked to the peroxidation of linoleic acid. In this regard, it is of interest that the carotenoid bleaching reaction in photosensitized liposomes is not explained by reaction with peroxyl radical (63).

## Summary

β-Carotene is clearly linked to the prevention of a variety of disease processes. Possible mechanisms by which carotenoids may function include antioxidant action, singly or in combination with other antioxidants, metabolism to retinoids or apocarotenoids, and modulation of enzyme activity. By any one or a combination of these mechanisms, carotenoids may serve as biomodulators of important biological pathways. The reaction of β-carotene with soybean lipoxygenase

offers a useful model system for the study of biomodulation of metabolic pathways by carotenoids.

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