REVIEW

Nutritional and Potential Disease Prevention Properties of Carotenoids

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ABSTRACT: Epidemiological studies have shown that people who consume diets with a high content of vegetables have a reduced risk of degenerative diseases such as specific cancers, cardiovascular disease, age-related macular degenerative disease (AMD), and cataracts. There is no convincing evidence that the protective role of vegetables against cancer and cardiovascular disease is due to carotenoids. However, there is a strong possibility that lutein and zeaxanthin present in food materials may prevent AMD and cataract formation. Increased use of cooked tomato products also has been shown to reduce prostate cancer risk as a result of increased bioavailability of *cis*-lycopene. One of the most important biochemical mechanisms underlying the cancer-preventive activity of carotenoids is the stimulation of intercellular gap junction communications. β-Carotene, canthaxanthin, and lutein are efficient inducers of intercellular gap junction communication, whereas α-carotene and lycopene are less active.

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According to the American Institute for Cancer Research (1), there are more than 1.4 million new cancer diagnoses each year in the United States and approximately 600,000 deaths. Worldwide, about 10 million cancer diagnoses occur each year, and the number is increasing rapidly. It has been projected that if people were to eat plant-based diets rich in a variety of vegetables (broccoli, carrots, arugula, pumpkins, sweet potatoes, squash, tomatoes, watercress) and fruits (apricots, cantaloupes, mangos, papayas, peaches and persimmons, legumes) and minimally processed starchy staple foods each day, the overall cancer rates could decline by as much as 20%. Therefore, dietary choices, together with exercise and a healthy weight, could prevent three to four million cancer cases worldwide each year. It is estimated that as many as half of all breast cancers, one in every three cases of lung cancer, and three in every four cases of colon and rectal cancers could be prevented with healthier diets.

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In a recent article on carotenoids and gene regulation, Bertram (2) stated that lifestyle rather than genetics is the major contributing factor to cancer risk in Western societies. Vitamin A and its metabolites have been shown to be active cancer-preventive agents in trials with animals (3) and humans (4). Consumption of lycopene, a straight-chain hydrocarbon, has also been shown to lead to a decreased risk of both prostate cancer and cervical cancer (5).

Carotenoids, the basic source of yellow, orange, and red plant pigments, are widely distributed in nature. They are present in all living organisms, from bacteria and algae to higher plants, in both nonphotosynthetic and photosynthetic tissues. These natural pigments are not produced by animals but are supplied from the plant kingdom. Carotenoids are tetraterpenes with eight unconjugated double bonds and are made up of 40 carbon atoms. They are synthesized by a reductive dimerization of geranylgeranyl pyrophosphate. There are two major classes: (i) carotenes, which are hydrocarbons, and (ii) xanthophylls, which contain hydroxyl, methoxy, carboxyl, keto, or epoxy groups.

This is a review of recent progress in the use of carotenoid foods among the population and their impact on chronic diseases. Carotenoids are present in most commonly consumed vegetables and fruits. The most-studied carotenoids are α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and cryptoxanthin. Clevidence *et al.* (6), in their study on human consumption of carotenoid-rich vegetables, concluded that carotenoid-rich foods may promote immunocompetence as judged from the enhanced ability of T-lymphocytes to proliferate in test subjects when they ate the test vegetables. Additionally, lutein, zeaxanthin, and lycopene may provide protection from oxidative assaults. High intake of vegetables also has been associated with reduced risk of degenerative diseases, such as epithelial cancers, cardiovascular disease and age-related macular degeneration (AMD) (7).

XANTHOPHYLLS AND THEIR EFFECTS ON EYE DISEASES

AMD. AMD is the leading cause of irreversible blindness in adults and is the result of degenerative changes that occur in the central region of the retina and the macula. These changes eventually lead to the loss of vision. Lutein and zeaxanthin may play a major role in the prevention of AMD. They are not metabolized to vitamin A, and they accumulate in the macular region of the human retina, where they are thought

to prevent damage by absorbing high-energy blue light through their antioxidant properties (8–10).

Increased risk of AMD also has been associated with increased risk of atherosclerosis (11). Atherosclerosis of the retina actually may induce AMD by limiting the flow of blood to and from the retina. Epidemiological studies have led to the hypothesis that carotenoids reduce the risk of AMD. To evaluate the relationship between dietary intake of carotenoids and vitamins A, C, and E and the risk of neovascular AMD, Seddon *et al*. (12) undertook a multicenter Eye Disease Case-Control study (EDCCS) at five ophthalmology centers in the United States. Case subjects (356) with advanced AMD were compared with 520 controls. By using multiple logistic-regression analysis, the relative risk for AMD was estimated by using dietary indicators of antioxidant status and controlling for smoking and other risk factors. It was concluded that a high dietary intake of carotenoids was associated with a lower risk of AMD. Adjusting for other risk factors for AMD, they found that those in the highest quintile of carotenoid intake had a 43% lower risk for AMD compared with those in the lowest quintile. Two specific carotenoids, lutein and zeaxanthin, were more strongly associated with a reduced risk for AMD. The intake of preformed vitamin A (retinol), vitamin E, or total vitamin C consumption was not associated with a statistically significant reduced risk for AMD, although there was a possibility that a lower risk for AMD might be due to a higher intake of vitamin C, particularly from foods. It was concluded that increasing the consumption of foods rich in certain carotenoids, in particular dark green, leafy vegetables, reduces the risk of developing advanced or exudative AMD. A separate analysis of 421 cases of persons with AMD and 621 controls from EDCCS showed that the risk of AMD was lower in subjects in the highest quintile for serum carotenoids than those in the lowest quintile (13).

Other epidemiological studies have shown no statistically significant reductions in risk of AMD with high serum or dietary intakes of lutein and zeaxanthin or β-carotene. A nested case-control study of 167 subjects with AMD and 167 controls in the Beaver Dam Eye Study found no relationship between α-carotene, β-carotene, lutein and zeaxanthin and the risk of AMD (14). This study did, however, show that the risk of AMD was 2.2-fold higher in persons in the lowest quintile for lycopene concentration than for all other carotenoids combined. Recently, Cooper *et al.* (15) stated that uncertainties concerning the relationships between light and the risk for AMD and between lifestyle, personal characteristics, and the risk of AMD need to be resolved before the role of carotenoids can be established.

Cataract. Cataract is clouding of the lens of the eye and/or of its surrounding transparent membrane, which obstructs the passage of light. This can lead to blindness. Cataracts become more common with increasing age and are an important cause of disability among older adults; more than 1 million extractions are performed annually in the United States (16).

It has been suggested that dietary antioxidants play a crucial role in the prevention of the oxidation of lens proteins and thus the formation of age-related cataracts (17). Though the relation between specific antioxidants and the risk of cataract is not clear, it has been observed that persons consuming low amounts of fruits and vegetables are at an increased risk of cataracts (17). Carotenoids such as β-carotene, lycopene and β-cryptoxanthin are absent in human lens, whereas lutein and zeaxanthin are present in human lens in small amounts. Dietary intake of lutein and zeaxanthin in the form of supplements or foods high in those two pigments has been shown to increase the amount of macular pigment. Hammond *et al*. (18), in a study involving 23 women ages 55–78 yr observed a significant inverse relation between macular pigment density and lens density, suggesting that lutein and zeaxanthin concentrations may be a suitable marker for lutein and zeaxanthin in the lens, which in turn may retard age-related increases in lens density. It has been hypothesized (18) that oxidative damage to lens protein and lipids present in lens epithelium may be a contributing factor for the development of cataractogenises. The intake of carotenoid antioxidants may prevent its development by blocking oxidative changes to the lens protein or by preventing lipid peroxidation within the epithelium of the lens (19). Carotenoids are very effective antioxidants at low partial pressures of oxygen, such as in the lens (20). Since lutein and zeaxanthin are the main carotenoids accumulating in the retina as well as other ocular tissue, they also may be effective in protecting the eye (21). To examine the association between carotenoid and vitamin A intake and cataract extraction in women and men between 45 and 75 yr, a cohort study was done (22,23). The group with the highest intake of lutein and zeaxanthin had 19–22% decreased risk of cataract extraction compared with those in the lowest quintile. It was also observed that other carotenoids, such as α-carotene and β-carotene, lycopene and crytoxanthin, vitamin A and retinol, were not associated with cataracts in a multivariate analysis. Mares-Perlman (24) noted that there is insufficient scientific evidence to support the protective role of lutein in the prevention of this or other age-related eye diseases. Although lutein and zeaxanthin are present in the human lens, their antioxidant properties and their photoprotective effect have not been unequivocally demonstrated.

DIETARY AVAILABILITY OF CAROTENOIDS

Handleman *et al.* (25) examined the effects of dietary supplementation with egg yolk on plasma lutein and zeaxanthin concentrations in 11 moderately hypercholesterolemic men and women (6 men and 5 women, in the age group between 46 and 78 yr). The two baseline diets contained 20% of energy as either corn oil or beef tallow, while the two test diets contained an additional 1.3 egg yolks/d. Each diet was consumed for 4.5 wk with at least 2 wk of washout period. With egg yolk supplementation of the beef tallow diet, plasma lutein concentrations were increased by 28% relative to the baseline diet, and zeaxanthin was increased by 142% relative to tallow baseline diet. However, egg yolk supplementation of the corn oil diet increased plasma lutein by 50% relative to the baseline diet and

zeaxanthin was increased by 114% relative to the baseline corn oil diet. The only negative aspect about egg yolk supplementation was the increase in low-density lipoprotein (LDL) cholesterol level (8–11%). According to Handelman *et al.* (25), lycopene and β-carotene contents in plasma were variable with no consistent trend. In conclusion, egg yolk provides a highly bioavailable source of lutein and zeaxanthin. The benefits of introducing these carotenoids, which have been shown to protect against AMD, must be considered in light of the small increase in plasma total and LDL cholesterol levels.

It is important to determine the bioavailability of carotenoids from vegetables and the effects of vegetable consumption on selected biomarkers of chronic diseases. In order to assess the bioavailability of β-carotene and lutein from vegetables and the effects of increased vegetable consumption, Van het Hof *et al.* (26) designed an experiment which lasted over 4 wk. Healthy adult subjects ($n = 22$) consumed a high-vegetable diet (490 g/d), 22 consumed a low-vegetable diet (130 g/d), and 10 consumed a low-vegetable diet supplemented with pure β-carotene (6 mg/d) and lutein (9 mg/d). The results indicated that the plasma concentrations of vitamin C and carotenoids such as α-carotene, β-carotene, lutein, zeaxanthin and β-cryptoxanthin were significantly higher after the high-vegetable diet than after the low-vegetable diet. The relative bioavailability of β-carotene and lutein from mixed vegetables compared with purified carotenoids was 14 and 67%, respectively. The pure carotenoid-supplemented diet caused an increase in plasma β-carotene and lutein and also induced a significant decrease in plasma lycopene concentration. The results of the this study suggested that carotenoid supplements competed with lycopene for absorption or transport in plasma. This was in line with studies made by others (27). However, some studies have shown that ingestion by men of a combined dose of β-carotene and lycopene does not affect the absorption of β-carotene but improves that of lycopene (28). This particular phenomenon is important for the risk of prostate cancer because an inverse association with lycopene intake has been reported (29). Others have reported the relatively low bioavailability of β-carotene from vegetables (30). According to Erdman (31), several factors may explain the high bioavailability of lutein compared with β-carotene. Firstly, some β-carotene converts to vitamin A, although it is reasonable to assume that there would be differences in the conversion efficiency among the groups as mentioned in Reference 26. Secondly, lutein is more polar than β-carotene and hence may be easily incorporated in the outer portions of lipid micelles within the gastrointestinal tract. Uptake by chylomicrons which would increase its bioavailability. Thirdly, lutein in the supplement (control) may have partially crystallized, reducing its bioavailability and therefore increasing the relative bioavailability of lutein from the high-vegetable diet. Lastly, β-carotene may be more difficult to digest and absorb from a food matrix than lutein. In a recent article, on dietary factors that affect the bioavailability of carotenoids, Van het Hof *et al*. (32) mentioned that both mechanical homogenization and heat treatment have the potential of increasing the bioavailability of carotenoids from vegetables (from 18% up to a sixfold increase). The amount of dietary fat required to ensure carotenoid absorption was 3–5 g per meal. Addition of a very low fat carotenoid-enriched spread to a meal (3 g fat/meal) was as effective in enhancing plasma α-carotene and β-carotene concentrations in plasma as a full-fat carotenoid-enriched spread (35 g fat/meal). However, when lutein was added as lutein esters, the plasma response was *ca.* 100% after consumption of the full-fat spread. The low amount of fat limits the solubilization of lutein esters and also the activity of enzymes like lipases and esterases. These enzymes are very important for the hydrolysis and absorption of esters. They also noted that the bioavailability of β-carotene is one order of magnitude higher when provided as a pure compound added to foods than when it is present naturally in foods. The average intake of total carotenoids in the United States is estimated as 6 mg/d (33). By increasing the total carotenoid intake 10 times through the use of a vegetable diet, the plasma carotenoid level was increased moderately, i.e., β-carotene 116%, lutein 67%, and lycopene 26% over baseline levels (34). The oxidative metabolites of lutein and zeaxanthin are carotene-3′-one, 3′-hydroxy-ε,ε-carotene-3-one, and ε,ε-carotene-3,3-dione. The formation of metabolites of lutein and zeaxanthin stems from a series of oxidation–reduction reactions. These metabolic pathways have been described in a series of studies on men taking purified lutein and zeaxanthin (35,36). Khachik *et al.* (36) also showed that by consuming high-vegetable diets there was no apparent lipid and DNA damage due to the presence of antioxidant carotenoids as determined by measurement of 8-hydroxy-2′-deoxyguanosine in urine.

Schlipalius *et al.* (37), in a patent on carotenoid formulation, carried out experiments with five groups of rats using a lycopene suspension in medium-chain triglycerides to determine the biologically acceptable dose range and the uptake and tissue deposition of dietary lycopene. The experiment was carried out for 10 wk. The composition of lycopene suspension was as follows: lycopene, 66%; β-carotene, 22%; phytofluene, 6%; phytoene, 5%; ζ-carotene, 0.7%; and 2,6-cyclolycopene-1,5-diol, 0.7%. Five groups of rats consumed, respectively, 1280, 512, 256, 128, and 51 mg carotenoids/kg diet. According to the authors, there was no deleterious effect on food intake or weight gains in either male or female rats. Of the estimated lycopene consumed, 55% was excreted in the feces. The authors concluded that lycopene was readily absorbed and stored in the rat liver, where it was metabolized in a manner similar to that in humans. In general, the uptake was dose-related; the lowest concentrations, particularly in liver lycopene concentration, were usually found in the group fed the lowest concentration. There was no evidence of lycopene toxicity at the dose range used in their study.

CAROTENOID INTERACTIONS AND BIOAVAILABILITY

In a recent review, Van den Berg (38) commented on the levels at which carotenoid interactions might occur, possibly leading to additional studies to understand the mechanism of

carotenoid functions and physiology. The interactions between carotenoids likely occur at the various stages of the absorption process, i.e., during micellar incorporation, during transport to different organs, or within tissues after absorption.

Competition between the various carotenoids for micellar incorporation in the gastrointestinal tract has been suggested as a possible explanation especially at higher doses (39). Van den Berg and van Vilet (40) studied the interactions between β-carotene and lutein or lycopene in men. The comparison was made also between the carotenoid and retinyl palmitate response in triacylglycerol-rich lipoprotein (TRL) fraction of men given a single dose of 15 mg β-carotene with that of a dose of 15 mg β-carotene plus 15 mg lycopene or 15 mg lutein. They showed that the combined dose with lutein decreased the area under the serum response curve of β-carotene and retinyl palmitate in the TRL fraction, adjusted to the fat response, by 66 and 74%, respectively, whereas there was no significant effect with lycopene, i.e., there was no interference. However, interaction between the hydrocarbon carotenoids β-carotene and lycopene was suggested by the studies of high single oral doses that caused an increase of serum lycopene (28). Johnson *et al*. (28) showed that the absorption of lycopene in men was improved by using a single oral dose of 0.11 mmole (60 mg) separately or with an equimolar mixture of β-carotene and lycopene. Van den Berg and van Vilet (40) speculated, along with Kostic *et al.* (41), that lutein might interfere with the *in vivo* cleavage of β-carotene, but they found a decrease in the area under the curve for β-carotene and retinyl palmitate in all male volunteers. This suggested that lutein interferes with β-carotene absorption rather than cleavage. An increase of serum α-carotene level after β-carotene supplementation has also been shown. This may be due to reduced cleavage of α-carotene in the presence of excess dietary β-carotene (41).

PROPERTIES OF NONPROVITAMIN CAROTENOIDS AND THEIR EFFECT ON CHRONIC DISEASES

An inverse association between consumption of tomato products and prostate cancer risk was confirmed in the U.S. Health Professional Follow-up Study (29). High concentrations of lycopene are present in tomato products. An almost 35% risk reduction was observed based on a consumption frequency of more than 10 servings of tomato products per week. The lycopene protective effect was even stronger when the analysis was focused on more advanced or aggressive prostate cancer.

Nonprovitamin carotenoids such as lycopene and canthaxanthin have been proposed as cancer-preventing compounds because of their biological activity. Many factors influence absorption and bioavailability of dietary lycopene. Lycopene when supplied in low dosages is absorbed more efficiently than high doses. The release of lycopene from food matrices occurs during the processing of food. The *cis*-isomer of lycopene, present in cooked tomatoes, is more bioavailable than the *trans*-isomer that occurs in raw tomato (42). Ingestion of tomato juice cooked in oil increases serum lycopene levels threefold, whereas ingestion of uncooked juice has no effect (43).

Lycopene is the most prominent carotenoid in human plasma and has a half-life of 2–3 d (43). In human plasma, lycopene is an isomeric mixture containing 50% of the total lycopene as the *cis*-isomer. There are some indications of *in vivo trans-cis* isomerization (43). Because of the lipophilic nature of lycopene, it concentrates in the LDL and very low density lipoprotein (VLDL) fractions of serum but not in the high-density lipoprotein (HDL) fraction (44). With prolonged and increased consumption of tomato juice, serum lycopene levels increase, resulting in coloration of skin and liver, a condition identified as lycopenemia (43). The distribution of lycopene in various tissues is not uniform. Lycopene has been found in the adrenal glands, testes, liver, and prostate (45,46). According to Rao and Agarwal (43), adipose tissue may serve as a marker for the assessment of body lycopene status since adipose is a potential source of easily available biological material. Lycopene and β-carotene have been found in human seminal plasma. Their levels are lower in immunoinfertile men compared to normal individuals (43).

Recent studies have shown that lycopene is at least twice as active as β-carotene in protecting lymphocytes against $NO^{\bullet}_2$ radical-induced membrane damage and cell death (47). Since lycopene is highly conjugated and highly lipophilic, it is concentrated in membrane and other lipoprotein components permitting reaction with reactive oxygen species. Lycopene also protects human LDL against photooxidative damage (48). When humans are exposed to ultraviolet energy, skin lycopene is degraded preferentially over β-carotene. This suggests that lycopene is superior to β-carotene in mitigating oxidative damage in tissues (49).

Lycopene is a moderate hypocholesterolemic agent because it inhibits 3-hydroxy-3-methyl glutaryl CoA reductase, the rate-limiting enzyme in cholesterol synthesis (43).

Paetau *et al*. (50) recently studied carotenoids in human buccal mucosa cells (BMC). After a 4-wk supplementation with tomato juice (476 mL/d containing 74.9 mg lycopene), oleoresins (soft-gel capsules; 4 capsules/d containing 75.4 mg lycopene), lycopene beadlets (15 capsules/d containing 70.2 mg lycopene) or placebo, the results from 15 healthy subjects showed that lycopene in BMC increased significantly after ingestion of oleoresin and lycopene beadlets to 4.95 and 3.75 μg/g protein, respectively. The tomato juice treatment did not cause any significant change. The placebo treatment produced a significant decrease in BMC lycopene concentration. A moderate increase (~40%) occurred in plasma lycopene concentration after tomato juice, oleoresin, and beadlet treatments from a baseline plasma lycopene concentration. They noted that the moderate increase of lycopene plasma concentration, when compared with other carotenoids such as lutein, β-cryptoxanthin, α-carotene and β-carotene may be due to several factors: (i) decreased absorption of lycopene by the intestine, (ii) increased excretion, or (iii) increased tissue uptake. In rats *ca.* 55% of the lycopene from a lycopene suspension feed was excreted (37). Paetau *et al*. (50) showed that there was a significant correlation between plasma and BMC concentration of lutein, β-cryptoxanthin, α-carotene, and β-

carotene, suggesting that cellular carotenoid concentrations were good biomarkers for the plasma concentration of these carotenoids. Since BMC can be collected by noninvasive procedures, the collection of blood samples to assess the carotenoid status of individuals is eliminated. It has been shown that β-carotene and other carotenoids increased substantially in plasma and BMC concurrent with ingestion (51). The authors also noted a weak correlation between lycopene concentration in plasma and in BMC. For α-carotene there was a significant correlation with oleoresin, lycopene beadlets, and placebo treatments**,** but not with tomato juice treatment. These authors concluded that cellular content of lycopene and other tomato carotenoids can be increased through prolonged use of the products.

CAROTENOIDS AND CARDIOVASCULAR DISEASE (CVD)

The Alpha-Tocopherol β-Carotene Study (ATBC) and the β-Carotene and Retinol Efficacy Trial (CARET) (52,53) showed that in smokers a β-carotene supplement seems to cause an increase in death from coronary artery disease (CVD). Since smokers often consume alcohol and alcohol has been shown to cause an increase in β-carotene concentration, and since cardiovascular complications are apparently associated with elevated β-carotene levels, it is possible that β-carotene is cardiotoxic. Because β-carotene is an antioxidant, it is generally believed to have a beneficial effect on CVD. Greenberg *et al.* (54) showed that there was no evidence of lower mortality after β-carotene supplementation among patients with initial β-carotene concentrations below the median for the study group, and there was no strong support for a strong effect of supplemental β-carotene in reducing mortality from CVD or other causes. Hennekens *et al*. (55) in a similar study showed that there was no reduction in the incidence of mortality from CVD with β-carotene supplementation (50 mg β-carotene/d or every other day) for an average of 12 yr. Among many studies regarding CVD and carotenoids, almost all have contradictory results.

Kritchevsky (56) noted a number of possible confounding factors that may explain the inconsistency between the trials and the epidemiological evidence. In the circulating carotenoids, there are other carotenoids besides β-carotene. The percentages of predominant carotenoids in serum are: β-carotene, 15–30; lycopene, 20–40; cryptoxanthin, 13–20; lutein, 10–20; zeaxanthin, 1–5; and α-carotene, 5–10. Lutein predominates in both red blood cells and peripheral blood mononuclear cells. As many important steps in atherosclerotic lesion development involve the action of white cells, the levels of carotenoids in these cells are particularly important. Kohlmeier *et al*. (57) found that adipose levels of lycopene and α- and β-carotene were all inversely associated with incidence of nonfatal myocardial infarction, but when modeled simultaneously, only lycopene remained an independent predictor of the outcome. Sahyoun *et al.* (58) studied dietary carotenoid intake in a cohort study of 747 Massachusetts residents ages 60 yr or older for a period of 12 yr. The dietary intakes of carotenoids included α-carotene, β-carotene, lutein, zeaxanthin, lycopene, and cryptoxanthin. Those having the highest 20% of intake had an adjusted relative risk of death of 0.64 compared to those with the lowest 20% of intake. The relative risk for subjects with a high intake of dark green/orange vegetables was 0.61, and the relative risk for those with a high intake of all vegetables was 0.49. High-carotenoid foods also contain folate, and it is possible that folate plays an important role in the prevention of heart disease through its role in homocysteine metabolism. The risk of CVD and the relation between folate intake and serum homocysteine concentration have drawn considerable attention in recent years. A specific relation between homocysteine concentration and CVD risk has not been firmly established (59). According to Lewis *et al*. (60), there is evidence that the relation between dietary folate intakes and mean blood homocysteine concentrations is not linear but apparently reaches a plateau at total folate intakes of >300 μg/d on the basis of data from several studies. A study on the incidence of myocardial infarction and CVD in women suggested that the risk reduction was greatest at median intakes of folate between 158 and 217 μg/d (60).

Kritchevsky (56) also pointed out that two physiologic factors may confound the epidemiological studies. One is the presence of serum carotenoid levels, and the other is the presence of inflammation markers such as C-reactive protein. Goulinet and Chapman (61) reported that a decrease in carotenoid content of LDL causes an increase in LDL density. Dense LDL is more readily oxidized than less dense LDL and is thought to have more atherogenic potential (56). Thus, low carotenoid levels may reflect differences in the lipoprotein density profile and may predict the risk of coronary heart disease. The role of inflammatory markers has not been explored in the context of the β-carotene–coronary heart disease relationship**,** although studies have shown a link between inflammations and reduced β-carotene level. Several workers found a possible link between C-reactive protein levels and carotenoids and heart disease. According to Talwar *et al.* (62), although clinical trials did not find any preventive role of supplemental β-carotene in coronary heart disease, other carotenoids might play an important role. The epidemiological evidence is generally supportive of the role of a diet rich in vegetables in the lowering the incidence of CVD. In one study, it was shown that β-carotene act as a prooxidant in the oxidative degradation of LDL and that elevated LDL concentrations might cancel the protective effect of α-tocopherol (59). Kushi *et al*. (63) found that supplemental vitamin A was not associated with a lower risk of dying from coronary disease. Cartmel *et al*. (64) evaluated the adverse effects of long-term systemic retinol intake. The median follow-up time was 3.8 yr. Participants, 2,297 men and women, were randomly assigned to receive retinal (7,576 retinol equivalents [RE] or 25,000 IU) or a placebo daily. After 49 mon of follow-up, triacylglycerol was 11% higher, cholesterol was 3% higher, and HDL was 1% lower in the retinal group than in the placebo group. Because a 1% increase in cholesterol concentration has been reported

to be associated with a 2% increase in coronary heart disease (64), the interpretation of the effects of long-term ingestion of 7,576 RE vitamin A/d should be approached cautiously. Further studies are needed to confirm this finding. Recently, Dugas *et al.* (65) reported that the formation of lipid hydroperoxides by LDL was inhibited when LDL was incubated with human endothelial cells in a β-carotene-enriched culture. The same effect was not observed with either lutein or lycopene.

ASSOCIATION BETWEEN ALCOHOL, β-CAROTENE CONCENTRATION, AND LIVER DISEASE

When carotenoids are consumed in large amounts, no toxic effects are observed (66). In humans who consume alcohol, a correlation exists between β-carotene intake and plasma β-carotene concentrations (67). Alcohol intake causes an increase in β-carotene concentration in blood. This phenomenon has also been observed in nonhuman primates (66). Concentrations of β-carotene in baboons fed ethanol chronically show increase in both liver and plasma, with a depletion of vitamin A in the liver. β-Carotene administration in control animals increases hepatic vitamin A level in contrast with alcohol-fed animals. Thus a corresponding rise in β-carotene concentration with a corresponding decrease in liver vitamin A suggests a blockage of conversion of β-carotene to vitamin A. In most human patients with liver disease, absolute concentrations of hepatic α- and β-carotene and retinoids are very low, even in the presence of normal serum concentrations of lycopene, α- and β-carotene or both. In patients with cirrhosis, hepatic concentrations are particularly low (66). However, even in patients with very low liver α- and β-carotene concentrations, more than half had blood concentrations in the normal range, suggesting that liver disease interferes with the uptake, excretion, and perhaps metabolism of α- and β-carotene. In baboons, consumption of ethanol together with β-carotene resulted in a more striking hepatic injury than did consumption of either compound alone (66). This toxic interaction occurred at a total dose of 7.7–10.8 mg β-carotene/J diet (30–45 mg/1000 kcal diet), which is common in subjects taking supplements, i.e., 30 mg supplemental β-carotene/d. The dose of alcohol administered to the baboons (50% of total dietary energy) was equivalent to that consumed by the average alcoholic. The hepatotoxicity of ethanol was potentiated by large amounts of β-carotene, and the administration of both β-carotene and alcohol resulted in striking liver lesions (66). Ethanol, while promoting a deficiency of vitamin A, also enhances its toxicity as well as that of β-carotene. These facts should be considered when formulating diets for correcting the vitamin A deficiency of alcohol-consuming populations.

When β-carotene was administered as beadlets, the bioavailability of β-carotene was enhanced. To determine the effect of beadlet carrier and its toxicity, experiments were carried out with rats for a period of 2 mon using vitamin A, β-carotene (with or without beadlets), and corresponding amounts of beadlet carriers without β-carotene. The diets contained either carbohydrate or equivalent amounts of ethanol. Beadlets resulted in the proliferation of the smooth endoplasmic reticulum and in leakage of mitochondrial glutamate dehydrogenase into the plasma reflecting mitochondrial injury (66). The ingredients of the beadlets have not been identified as toxic; therefore the reason for this toxicity is not known.

β-CAROTENE AND ITS EFFECT ON CANCER

The hypothesis that β-carotene and/or its analogs prevent human cancer has been confirmed by animal studies and epidemiological studies. Chemopreventive trials have shown, however, that β-carotene alone or in combination with vitamin A or vitamin E actually increases lung cancer incidence and mortality in heavy smokers and asbestos workers (53,54). Thus the results of the ATBC Cancer Prevention Study and CARET were almost the same. The exact mechanisms of the action of β-carotene are still unknown. However, β-carotene has been shown to be an antioxidant and a precursor of retinoic acid, to enhance gap junction intercellular communication (GJIC), and to increase immunological function. It may also activate detoxifying carcinogen enzymes. The failure of these trials could be explained by the fact that in the oxidative environment in the lungs of smokers, oxidative metabolites of β-carotene are formed. These may enhance DNA damage and/or promote an environment for carcinogenesis. The ATBC and CARET studies suggested that heavy smokers should avoid high-dose β-carotene supplements because of a high risk of lung cancer. The ATBC (29,133 participants) study recorded an 18% higher incidence of lung cancers and 8% more overall deaths in smokers taking the β-carotene supplement. In the CARET study (18,314 participants) with smokers and asbestos workers who were taking β-carotene plus vitamin A supplements, 28% more lung cancers and 17% more deaths were observed. It is possible that instead of initiating new tumors, β-carotene might act as a co-carcinogen on latent ones (68). Studies were performed to elucidate the co-carcinogenic effect of β-carotene. Paolini *et al*. (68) investigated whether β-carotene was acting by an epigenetic mechanism that involved cytochrome P-450 (CYP) systems. They found a significant increase in the carcinogenic metabolizing enzymes in the lungs of rats supplemented with high doses of β-carotene. Although β-carotene is known to increase the levels of phase II detoxifying enzymes such as glutathione S-transferase and glutathione peroxidase enzymes, smokers with the CYP enzymes have significantly higher polycyclic aromatic hydrocarbon-DNA adduct levels that are not significantly reduced by β-carotene. By using electron paramagnetic resonance, the superoxide production by the CYP system induced by β-carotene was evaluated. Paolini *et al*. (68) found a significant association between the induction of CYP content and the overgeneration of superoxide yield in subcellular lung preparations that could act synergistically with peroxy radicals, nitrogen dioxide, and hydroquinones that are present in cigarette smoke to enhance the carcinogenic effects. In a study by Perocco *et al*. (69) with

BALB/C3T3 cells, the authors showed that β-carotene enhanced *in vivo* conversion of the benzo(a)pyrene to the ultimate carcinogens. This co-carcinogenic property of β-carotene is in line with the boosting effect of β-carotene itself on activating enzymes during cell growth. Although cancer chemoprevention cannot rely on the antioxidant capability of β-carotene (and other carotenoids), there have been proposals to take advantage of the radical-trapping capability of β-carotene and other carotenoids to decrease the incidence of lung cancers in humans. Paolini *et al*. (68) postulated that the increased incidence of morbidity and mortality observed in clinical chemopreventation is probably due to co-carcinogenic properties of β-carotene and its ability to generate oxidative stress. Wang and Russell (70) proposed a mechanism by which high doses of β-carotene increase cancer risks in smokers, whereas low doses of β-carotene combined with vitamin C and vitamin E may provide modest protection against cancer risk in smokers. It is possible that chronic high doses of β-carotene alter normal β-carotene metabolism and result in a significantly higher amount of oxidized metabolites or oxidized forms that damage DNA directly and/or provide an environment for carcinogenesis. Classically, β-carotene is believed to be mainly split into two moles of retinal by mammalian tissues, mainly at the central bond (71), but Wang *et al.* (72) also described significant conversion through side-chain oxidation (eccentric clevage) to form retinoids and β-apo-carotenoids. Wang *et al*. (72) suggested that β-carotene in an oxidative environment might be cleaving eccentrically and thus producing retinoids such as β-apo-14′-, -12′-, -10′-, and 8′-carotenals. In ferrets, Wang *et al.* (72) noted a decrease of β-carotene level in both β-carotene-supplemented and -unsupplemented animals. In order to confirm the eccentric cleavage of β-carotene accompanying smoke exposure *in vitro,* experiments were carried out with a *trans*-β-carotene and with post-nuclear fractions of lung tissue from either smoke-exposed or nonsmoke-exposed ferrets. The results showed that the formation of β-apo-14′-, -12′-, -10′-, and -8′-carotenals was about three times higher in smoke-exposed ferrets than the nonexposed ferrets. From these data, the authors pointed out that the free radical-rich atmosphere in the lungs of cigarette smokers could modify β-carotene metabolism in the lung epithelia to form an abundance of oxidative metabolites. The hypothesis was made that these oxidative metabolites accelerate malignant transformation by down-regulating the retinoic acid receptor (RARβ) gene and up-regulating AP-1 (c-Jun and c-Fos) activity. Some CYP family members also may be involved in the biotransformation, i.e., the unusual eccentric breakdown of β-carotene in the lung in the absence of other antioxidants such as tocopherols and vitamin C. Adequate amounts of vitamins C and E must be present to prevent β-carotene from being oxidized and to have a chemopreventive effect. This hypothesis has been confirmed by *in vitro* studies and also with several human epidemiologic investigations (72). Recent studies have suggested that vitamin C is able to convert the β-carotene radical back to β-carotene and thus should be able to keep β-carotene in unoxidized form. There is also evidence that there is interaction between β-carotene and tocopherols with respect to pro- or antioxidant effects. The prooxidant effect of β-carotene has been attributed to its oxidation products (72), but tocopherol may limit the prooxidant effects of carotenoids by protecting them from oxidation in biological systems. At the same time β-carotene is capable of regenerating α-tocopherol from its radical. Lowe *et al.* (73) recently showed that low concentrations of β-carotene (1–3 μM) in HT29 cells protected against oxidative DNA damage induced by xanthenes/xanthene oxidase, whereas at higher doses (4–10 μM) the protective effect was lost.

Epidemiological and experimental data have suggested that retinoids and carotenoids may have chemopreventive activity, causing regression of oral leukoplakia, a premalignant lesion, and preventing its progression to oral cancer (74). Papadimitrakopoulou *et al*. (75) reported on long-term follow-up studies of 59 patients with head and neck cancer in a maintenance study of chemoprevention of oral premalignancies. Their patients received 13-*cis*-retinoic acid, a widely recognized chemopreventive agent (0.5 mg/kg body weight/d) or β-carotene (3 mg/d). No control was used during this treatment. After 12 mon, oral premalignant lesion progression rate was significantly lower with 13-*cis* -retinoic acid than with β-carotene (8% vs. 53%), indicating that 13-*cis*-retinoic acid was far more effective in reducing cancer progression. In addition, patients in the β-carotene group developed more cancers than those taking 13-*cis*-retinoic acid (48% on β-carotene vs. 31% on 13-*cis*-retinoic acid).

In a small clinical trial in India, 160 fishermen with oral leukoplakias were randomized to receive oral vitamin A (300,000 IU retinyl acetate/wk), β-carotene (360 mg/wk), or placebo for 12 mon (76). In this study, complete regression was found in 7% of subjects taking placebo, 52% taking vitamin A, and 33% taking β-carotene. The authors concluded that there was evidence to support continuing intervention trials with the β-carotene supplement. The relationship between high serum concentrations (or dietary intake) of β-carotene and the reduced risk for oral leukoplakia or cancers of the head and neck has been established from three case control studies. An inverse association between serum β-carotene and oral leukoplakias was observed in a case control study of 50 cases and 50 controls in India, in which the control group included both tobacco chewers and nonchewers (76). Barbone *et al*. (77) conducted a follow-up study of 380 cases of head and neck cancers that had been evaluated in a previous case-control study and found that the risk for developing a second primary tumor decreased with increased dietary β-carotene intake.

Epidemiologic evidence has indicated that diets high in vegetables and fruits are associated with a reduced risk of several cancers, including cancers of the esophagus and stomach. The presence of vitamins and minerals in these foods may contribute to reduced cancer risk. The people of Linxian County, China, have the highest rates of esophageal and stomach cancers in the world, and they chronically consume low amounts of several nutrients. In a randomized nutrition intervention trial involving 3,318 patients for 5 yr and using nutri-

tional supplements including β-carotene, vitamin E, and selenium, there was a 13% reduction of cancer death and also a reversal of premalignant lesions (78).

TISSUE CULTURE STUDIES

To determine the effects of carotenoids on the culture of cells that have potential for neoplastic transformation, studies were carried out using mouse embryo fibroblasts of the 10T1/2 cell line. The early work of Merriman and Bertram (79) demonstrated that a wide range of retinoids was capable of completely suppressing the induction of neoplastic transformation. In their first study with carotenoids, they used β-carotene and canthaxanthin. These carotenoids were supplied as water-miscible suspensions, and the cell culture was treated with the carcinogen 3-methylcholanthrene. They observed the complete inhibition of neoplastic transformation when carotenoids were added at a concentration of 10^{-5} M 1 wk post-carcinogen and maintained for the 4-wk period of the experiments. After removal of carotenoids, the neoplastic transformed foci came back after 3–4 wk, indicating that the inhibition process was reversible. Canthaxanthin was more potent than β–carotene, and a higher concentration of canthaxanthin was observed in treated cells. These compounds were capable of inhibiting X-ray-induced transformation and were also active when they were added after irradiation. When added prior to or during irradiation, their activities were minimal, indicating that they were acting as scavengers of free radicals (80).

Recently, carotenoids have been thought to stimulate GJIC. Gap junctions are water-filled pores, called connexons, that are cell-to-cell channels which allow passage of water-soluble molecules and ions. Because of the size-limiting pores, only molecules <1,000 Da can pass through. Molecules such as mRNA and protein are excluded, thus maintaining genetic identity of the cells (2). It has been shown that through increased expression of connexin protein 43, the only connexin protein responsible for carotenoids, carotenoids induce gap junction communication. There also is growing evidence that gap junction plays a role in the regulation of morphogenesis, cell differentiation, secretion of hormones, and growth (2). Banoub *et al*. (81), in an *in vitro* experiment with murine lung epithelial cells using β-carotene (1–10 μM; 1–5 d treatment duration), showed that it did not enhance GJIC and connexin 43 protein and that it did not have any effect on cell growth. When C3H10T1/2 murine fibroblast cells were treated with β-carotene for 5 d, dye coupling increased in a concentration-dependent manner, and growth was suppressed. Connexin 43 protein content was increased in these cells in a concentration-dependent manner. From these experiments, Banoub *et al*. (81) showed that β-carotene did not affect GJIC or connexin 43 expression in nontransformed and neoplastic epithelial lung cells. They concluded that the effects of β-carotene on GJIC and growth are cell-specific and that was the reason β-carotene was an ineffective chemopreventive agent. Stahl *et al*. (82) in their studies with natural and synthetic congeners of β-carotene such as retrohydro-β-carotene showed that they are efficient inducers of intercellular communication *via* gap junction. The presence of a six-membered ring was important for inducing GJIC (82), and five-membered ring-containing compounds were about half as active when compared with six-membered ring compounds. Experiments also showed that dialdehydes did not affect intercellular communications (82). Carotenoids such as β-carotene, canthaxanthin, and lutein are efficient inducers of GJIC, whereas α-carotene and lycopene are less active (83). All of these experimental results were obtained with murine embryo fibroblast cell line C3H/10T1/2. In a recent paper on carotenoids and intercellular communication *via* gap junction, Sies and Stahl (84) made some observations on biochemical mechanisms. The increase of intercellular communication induced by retinoids and carotenoids is accompanied by higher amounts of connexin mRNA. The biological effects of the non-provitamin A carotenoid, canthaxanthin, and its decomposition products on GJIC were studied. The decomposition products of canthaxanthin were identified as all-*trans*- and 13-*cis*-4-oxo-retinoic acids. Both enhanced the GJIC in murine fibroblasts, the expression of connexin 43 mRNA increased. Retinoid signaling occurs through nuclear receptors that appear to act as a transcription factor (85). Both retinoic acid receptors and retinoid X receptors are members of nuclear hormone receptor families of sequence-specific, ligand-activated transcription factors. The ligand-activated receptors regulate gene expression by binding as dimeric complexes to specific DNA sites known as the retinoic acid response element. 4-Oxo-retinoic acid was accepted as a ligand of the receptor (86). The cells were treated with 4-oxo-retinoic acid as a ligand receptor, which activated the transcription of β-glycosidase mRNA that leads to an increase in β-glycosidase enzyme activity. Thus the authors suggested that the biological effects of carotenoids were at least in part mediated *via* retinoids formed during oxidation or generated in metabolic pathways (84).

CONCLUSION

Studies on carotenoids with respect to dietary availability and/or bioavailability are complex, and much study is needed to unravel complexity. Although lutein interferes with β-carotene absorption and digestion, that it interferes with the cleavage of the central double bond in β-carotene has not been proven unequivocally. The effects of carotenoids on CVD and cancer are not very clear yet, but the carotenoids may play an important role in the prevention and/or treatment of these diseases in conjunction with other antioxidants such as vitamins E and C.

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