



Review

Carotenoid actions and their relation to health and disease

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Abstract

Based on extensive epidemiological observation, fruits and vegetables that are a rich source of carotenoids are thought to provide health benefits by decreasing the risk of various diseases, particularly certain cancers and eye diseases. The carotenoids that have been most studied in this regard are β -carotene, lycopene, lutein and zeaxanthin. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidants. β -Carotene may have added benefits due its ability to be converted to vitamin A. Additionally, lutein and zeaxanthin may be protective in eye disease because they absorb damaging blue light that enters the eye. Food sources of these compounds include a variety of fruits and vegetables, although the primary sources of lycopene are tomato and tomato products. Additionally, egg yolk is a highly bioavailable source of lutein and zeaxanthin. These carotenoids are available in supplement form. However, intervention trials with large doses of β -carotene found an adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos. Until

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the efficacy and safety of taking supplements containing these nutrients can be determined, current dietary recommendations of diets high in fruits and vegetables are advised.

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1. Introduction

Carotenoids are a family of compounds of over 600 fat-soluble plant pigments that provide much of the color we see in nature. For example, carotenoids are responsible for the red color of tomatoes and the orange color of carrots, and are partially responsible for fall coloration after the leaf chlorophyll has been destroyed. Apart from their aesthetic role, dietary carotenoids, or foods rich in these colorful pigments, are considered to be beneficial in the prevention of a variety of major diseases, including certain cancers and eye diseases. The beneficial effects of carotenoids are attributed to a small portion of the hundreds of carotenoids found in nature, given that only about two dozen are found in human blood and tissue, and only two in the retina and lens of the eye. This latter statement does not include the geometric isomers of the carotenoids, the so-called *cis-trans* or *E/Z* isomers, as well as various oxidation products. It is still not completely understood whether *E/Z* isomerization is an accidental event within the body, or whether it may serve a purpose. It is certainly clear that this type of isomerization is absolutely essential for the visual process (Wald, 1968) but as yet, we do not know what role it may play in human biology. The carotenoids that have been most studied in this regard are β -carotene, lycopene, lutein and zeaxanthin. β -Carotene and lycopene are hydrocarbons and belong to a class of carotenoids called carotenes that are very fat-soluble. Lutein and zeaxanthin belong to a class of carotenoids called xanthophylls. Because xanthophylls contain at least one hydroxyl group, they are more polar than carotenes. Thus, β -carotene and lycopene tend to be localized predominately in the low-density lipoproteins (LDL) in the circulation, whereas lutein and zeaxanthin are more evenly distributed among both LDL and high-density lipoprotein (HDL) (Clevidence and Bieri, 1993). In part, the protection by carotenoids is thought to be through their antioxidant activity, but other mechanisms of protection may exist. Lutein and zeaxanthin are thought to have an additional role of absorbing damaging blue light that enters the eye, thus preventing light-associated damage, such as the development of age-related macular degeneration and cataracts.

2. Sources and absorption of dietary carotenoids

Major dietary carotenoids include the hydrocarbons, β -carotene, α -carotene and lycopene and the xanthophylls, or oxygen-containing carotenoids, β -cryptoxanthin, lutein and zeaxanthin (Fig. 1). The estimation of carotenoid intakes has been made possible through the publications of the qualitative and quantitative carotenoid content of commonly consumed foods (Block et al., 1990; Mangels et al., 1993; Ritenbaugh et al., 1996).

2.1. β -Carotene

β -carotene is the most widely studied carotenoid and is one of the major carotenoids in our diet and in human blood and tissues (Schmitz et al., 1991; Enger et al.,

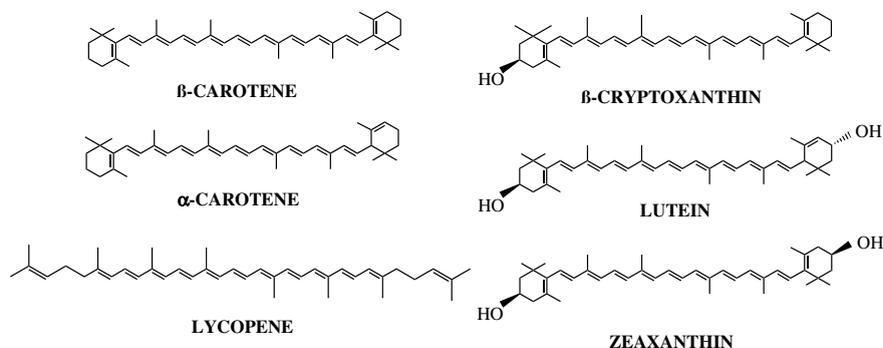


Fig. 1. The structures of the predominant carotenoids found in human plasma.

1996). Major sources of dietary β -carotene include green leafy vegetables as well as orange and yellow fruits and vegetables (Table 1). However, the bioavailability of β -carotene from green leafy vegetables such as spinach is thought to be low (Castenmiller et al., 1999a). Factors other than the food vehicle are thought to be important in the bioavailability of β -carotene. These include, cooking, chopping and the presence of dietary fat, all of which improve the bioavailability (Rock et al., 1998; van het Hof et al., 1998). Of the 50 different carotenoids that can be metabolized into vitamin A, β -carotene has the highest provitamin A activity. However, this bioconversion is highly variable among individuals and perhaps food sources. Tang et al. reported that spinach β -carotene conversion to retinal averaged 21 to 1 (range: 10–47 to 1) and carrot β -carotene conversion to retinol was 15 to 1

Table 1
 β -Carotene content of foods (Adapted from Database, 1998)

Food	Content (mg/100 g wet wt) ^a
Carrots, raw	18.3
Mangos, canned	13.1
Sweet potato, cooked	9.5
Carrots, cooked	8.0
Pumpkin, canned	6.9
Kale, cooked	6.2
Spinach, raw	5.6
Spinach, cooked	5.2
Winter butternut squash	4.6
Swiss chard, raw	3.9
Apricots, raw	2.6
Pepper, red, raw	2.4
Pepper, red, cooked	2.2
Cantaloupe, raw	1.6
Lettuce, romaine, raw	1.3
Tomato paste	1.2

^a Edible portion.

(range: 8–25 to 1) (Tang et al., 2005). The National Health and Nutrition Examination Survey, 1999–2000 for the US population reported dietary intakes of β -carotene to be 5.4 ± 0.3 (mean \pm SE, $n = 8604$) (Ervin et al., 2004). However, intakes much higher than this are possible through over-the-counter supplements that are commonly available in health food stores in doses of 3–20 mg/capsule.

2.2. Lycopene

Dietary lycopene is derived predominately from tomatoes and tomato products. In the United States, more than 85% of lycopene consumed is from tomato products although other dietary sources include dried apricots, guava, watermelon, papaya, and pink grapefruit (Table 2) (Database, 1998). Similar to the effect on β -carotene bioavailability, heating tomatoes in oil was found to be associated with an increase in lycopene absorption when compared to the absorption for unprocessed tomato juice (Stahl and Sies, 1992). Also, the lycopene bioavailability was greater from a single dose of tomato paste than it was from an equal lycopene dose from fresh tomatoes (Gärtner et al., 1997). Interestingly, these studies support the findings of Giovannucci et al., that the association between consumption of various tomato products and the risk of prostate cancer depends on the bioavailability of lycopene (Giovannucci et al., 1995). That is, an association was found with the consumption of tomato paste or sauce and not with consumption of minimally processed tomato juice. Typical dietary intakes of lycopene in the United States are about 2–5 mg/d, which probably reflects a diet high in tomato and tomato products e.g., pizza (Witschi et al., 1970; Henderson et al., 1989; Yeum et al., 1996). Supplemental lycopene is also available, usually in amounts of 5–10 mg/capsule.

2.3. Lutein and zeaxanthin

The two foods that were found to have the highest amount of lutein and zeaxanthin are spinach and kale (Table 3) (Database, 1998; During et al., 2002). Other

Table 2
Lycopene content of foods (Adapted from Database, 1998)

Food	Content (mg/100 g wet wt) ^a
Tomato paste	29.3
Catsup	17.0
Tomato puree	16.7
Pasta sauce	16.0
Tomato sauce	15.9
Tomato soup	10.9
Tomato, canned, whole	9.7
Tomato juice	9.3
Watermelon, raw	4.9
Tomato, cooked	4.4
Tomato, raw	3.0

^a Edible portion.

Table 3
Lutein and zeaxanthin content of foods (Adapted from Database, 1998)

Food	Content (mg/100 g wet wt) ^a
Kale, cooked	15.8
Spinach, raw	11.9
Spinach, cooked	7.0
Lettuce, romaine, raw	2.6
Broccoli, raw	2.4
Broccoli, cooked	2.2
Summer squash, zucchini	2.1
Corn, sweet, cooked	1.8
Peas, green, canned	1.4
Brussels sprouts, cooked	1.3
Corn, sweet, canned	0.9
Beans, green, cooked	0.7
Beans, green, canned	0.7
Beans, gree, raw	0.6
Okra, cooked	0.4
Cabbage, white, raw	0.3
Egg yolk, medium ^b	0.3
Celery, raw	0.2
Orange, raw	0.2
Tomato past	0.2

^a Edible portion.

^b Handelman et al. (1999), Surai et al. (2000).

major sources include broccoli, peas, and brussel sprouts. Another good source of lutein and zeaxanthin to consider is egg yolk. Though the values are relatively low in eggs, recent data suggest that lutein and zeaxanthin from this food source are highly bioavailable (Handelman et al., 1999; Surai et al., 2000; Chung et al., 2004). Data on the lutein content of foods include zeaxanthin, i.e. lutein + zeaxanthin, making examination of specific effects of dietary lutein difficult. However, in terms of food sources, human metabolism, and tissue storage, lutein and zeaxanthin are similar. Intakes of lutein and zeaxanthin in the US are generally lower than that of β -carotene or lycopene, but levels of ~ 3 mg/d can be easily achieved with a high fruit and vegetable diet (Yeum et al., 1996). Although lutein and zeaxanthin are considered to be major carotenoids in the US diet, data from the 1987 and 1992 National Health Interview Surveys suggest that there was a decline in lutein intake (from dark green leafy vegetables) (Nebeling et al., 1997). Currently, there are a variety of supplement products available in health food stores that contain lutein in amounts of 6–25 mg/capsule. At this point, lutein is found in many multi-vitamin products in much smaller amounts (0.25 mg/capsule).

2.4. Absorption

Carotenoids, being fat-soluble, follow the same intestinal absorption path as dietary fat. Release from the food matrix and dissolution in the lipid phase appears to

be important initial steps in the absorption process. Carotenoids are thought to be absorbed by the small intestinal mucosa via a passive, diffusion process, although recent studies in Caco-2 cell monolayers indicate that intestinal absorption is a facilitate process (During et al., 2002; During et al., 2005). Fatty acid esters of xanthophylls are cleaved in the lumen of the small intestine prior to uptake by the mucosa. Carotenoids are taken up by the mucosa of the small intestine and packaged into triacylglycerol-rich chylomicrons. β -Carotene and other provitamin A carotenoids are partly converted to vitamin A, primarily retinyl esters, in the intestinal mucosa, and both carotenoids and retinyl esters are incorporated into chylomicrons and secreted into lymph for transport to the liver (Parker, 1996).

2.5. Transport

In fasting serum, hydrocarbon carotenes are found primarily in low-density lipoprotein (LDL), while xanthophylls (containing one or more polar functional groups) are more evenly distributed between LDL and high-density lipoprotein (HDL) (Cleveland and Bieri, 1993). The carotenes, being lipophilic, are located in the core of lipoprotein, which may explain why they do not transfer between lipoproteins at an appreciable rate (Massey, 1984). The xanthophylls, being more polar, are probably located on the surface of lipoproteins, and are likely to undergo more rapid surface transfer, resulting in the observed apparent equilibration between LDL and HDL.

3. Antioxidant and prooxidant activity

In the last few years, many reviews have appeared describing either the antioxidant (Krinsky, 1998; Paiva and Russell, 1999; Stahl et al., 2002; Stahl and Sies, 2002; Krinsky, 2003) or prooxidant (Edge and Truscott, 1997; Palozza, 1998) effects of carotenoids, and some have appeared describing both actions (Young and Lowe, 2001). In fact, some skepticism has appeared as to whether carotenoids have any antioxidant action in vivo (Rice-Evans et al., 1997; Halliwell, 1999; Briviba et al., 2004). Why do such ambiguous results exist with respect to carotenoids? The reasons for this discrepancy may be attributed to the use of different methodologies (a) in dissolving the carotenoid to be evaluated, (b) in initiating oxidant stress, (c) in the presence of other antioxidants, (d) in the type of animal used for in vivo studies, and (e) in the evaluation technique used to determine the efficacy of the various carotenoids. There is probably no ideal system for evaluating antioxidant efficacy, but some recent methodological investigations are detailed below, as well as studies involving in vitro, ex vivo, or in vivo systems. In addition, this section will also cover the evidence for a prooxidant effect of carotenoids.

3.1. Antioxidant methodologies

For many years, investigators have reported on the “total antioxidant capacity” of human tissues, using a variety of techniques. One of the most common is to use an

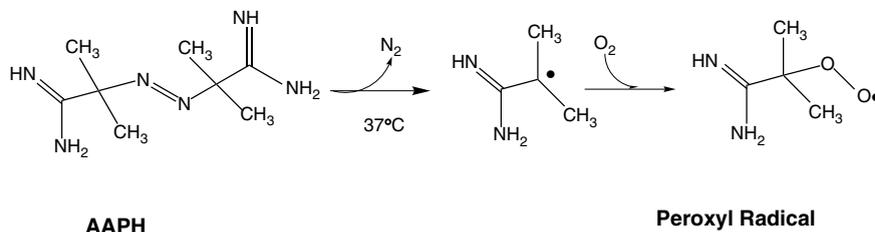
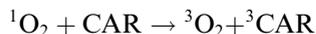


Fig. 2. The thermal decomposition of 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH), generating peroxy radicals at a fixed rate in the presence of oxygen.

azo compound that can decompose to yield an alkyl radical, and in the presence of oxygen, form a peroxy radical to initiate tissue oxidations. A compound such as 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) has been used extensively for such experiments, and the decomposition reaction is shown in Fig. 2. However, AAPH is a hydrophilic azo-initiator, and as such would not be expected to readily detect the antioxidant capacity of carotenoids buried deeply with the lipid matrix of serum or tissues. In fact, several methods utilizing AAPH as the radical initiator have reported that diets enriched with carotenoids do not alter the “total antioxidant capacity” of plasma (Cao et al., 1998; Pellegrini et al., 2000). However, a recent publication suggests that plasma lipid oxidizability can be evaluated using a hydrophobic azo-initiator, 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN), and a hydrophobic fluorescent reporter dye, and that micromolar addition of β -carotene prevents lipid oxidation in this system (Aldini et al., 2001). Subsequent studies have indicated that there is a direct association between plasma carotenoid levels and the extent of lipid oxidizability (Yeum et al., 2005), therefore showing that carotenoids do have an antioxidant action in plasma.

3.2. *In vitro* antioxidant studies

The best example of an antioxidant action of carotenoids involves the ability of these pigments to quench or inactivate singlet excited oxygen ($^1\text{O}_2$). The original studies, dating back to 1968 (Foote and Denny, 1968), provided a sound basis for the protective role of carotenoids in photosensitized oxidations. Since then, we have begun to understand the mechanism of photoprotection, which involves an electron exchange energy transfer between $^1\text{O}_2$ and a carotenoid to generate the triplet state of the carotenoid (^3CAR) and ground state oxygen ($^3\text{O}_2$), as seen below:



The ^3CAR formed can then return to its ground state by dissipating its energy through rotational and vibrational interactions with the solvent system, as shown below. In this way, carotenoids



can essentially act as catalysts to inactivate the highly dangerous and reactive $^1\text{O}_2$. However, carotenoids are not perfect catalysts for the above reactions inasmuch as chemical reactions between $^1\text{O}_2$ and carotenoids can also occur, yielding a variety of oxidized products (Stratton and Liebler, 1997). This action of carotenoids in inhibiting the damaging effects of $^1\text{O}_2$ has been demonstrated in many systems described including the work of Schafer et al. (2002).

One of the best, and oldest, model systems to study the antioxidant action of carotenoids involves the use of liposomes (Anderson and Krinsky, 1973; Anderson et al., 1974). It is very clear that the nature of the interaction between the carotenoids and the matrix in which they are studied dictates their effect. This is demonstrated clearly in the study of Liebler et al. (1997) who reported that when β -carotene was incorporated into liposomes, it was an effective antioxidant against AAPH-induced lipid peroxidation, but its effectiveness was lost when it was added to pre-formed liposomes. Many other studies have demonstrated an antioxidant effect when carotenoids are added to liposomes. Albrecht et al., using combinatorial biosynthetic pathways in *E. coli*, have produced several new hydroxycarotenoids and tested their antioxidant activity in liposomes (Albrecht et al., 2000). As had been shown many years earlier, the ability to protect against both photooxidation and radical-mediated peroxidation was related to the length of the conjugated double bond system in these novel carotenoids. Astaxanthin and canthaxanthin, two relatively minor dietary carotenoids, can protect liposomes against Cu^+ -initiated lipid peroxidation (Rengel et al., 2000). Zeaxanthin can react with peroxyxynitrous acid (HOONO) in liposomes, and presumably protect them (Scheidegger et al., 1998). On the basis of that observation, these authors suggested that zeaxanthin might protect the macular region of the retina from peroxyxynitrite attack. The activity of zeaxanthin in protecting liposomes against AAPH attack was reported to be equivalent to that seen with α -tocopherol, while β -carotene, canthaxanthin, and astaxanthin were somewhat weaker and lycopene was least effective (Woodall et al., 1997). In fact, these authors reported that after 60 min of incubation, lycopene became a pro-oxidant.

However, not all liposome studies have reported an antioxidant action of carotenoids. Chen and Djuric reported that carotenoids in unilamellar liposomes were destroyed by the free radicals generated from iron and AAPH, and were not effective in preventing lipid peroxidation (Chen and Djuric, 2001).

Many of the earlier investigation used the development of thiobarbituric acid-related substances (TBARS) as an index of lipid peroxidation (reviewed in Palozza and Krinsky, 1994), but this assay is quite non-specific, and as Kikugawa et al. have demonstrated (Kikugawa et al., 1999). The oxidation of β -carotene by either nitrogen dioxide or oxygen itself results in measurable TBARS activity (Kikugawa et al., 1999).

The order of effectiveness of carotenoids in preventing radical- or oxidative stress-initiated damage has been studied by a number of authors either in liposomes (Naguib, 2000) or in homogeneous solution (Mortensen and Skibsted, 1997a; Jiménez-Escrig et al., 2000; Miller et al., 1996; Bohm et al., 2002) but at this time the results are so variable that it does not appear to be useful with respect to the possible effectiveness of different carotenoids in humans.

Zhang and Omaye have published a series of articles detailing both antioxidant and prooxidant actions of carotenoids in both in vitro and ex vivo systems. They measured the AAPH-induced production of protein carbonyls when human serum albumin was incubated in the presence of β -carotene, α -tocopherol or ascorbic acid (Zhang and Omaye, 2000). At oxygen tensions up to 150 Torr, β -carotene inhibited carbonyl formation, but at 760 Torr, the addition of 1.6 μ M β -carotene resulted in a 26% increase in carbonyl formation, suggesting a prooxidant action of β -carotene at high oxygen tension. However, a mixture of β -carotene, α -tocopherol and ascorbic acid still showed antioxidant action at 760 Torr.

In addition, they studied the effectiveness of β -carotene, α -tocopherol or ascorbic acid in inhibiting the AAPH-induced strand breakage of supercoiled DNA at various oxygen tensions (Zhang and Omaye, 2001b). At 15 Torr, β -carotene was an antioxidant, but at β -carotene concentrations $>0.8 \mu$ M, a prooxidant effect was observed at 150 Torr, and this became significant at 760 Torr. At this high oxygen tension (100 % oxygen) even the ability of α -tocopherol and ascorbic acid were limited in overcoming the prooxidant action of β -carotene in this system (Zhang and Omaye, 2001b). Their other papers will be described in the following section.

Nagler et al. (2003) studied the effectiveness of β -carotene as an inhibitor of AAPH-induced oxidation of various unsaturated fatty acids and reported that 5 μ M β -carotene effectively inhibited the oxidation of linoleic acid, but not that of either α - or γ -linoleic acid (Nagler et al., 2003).

3.3. *Ex vivo antioxidant studies in LDL*

Most of the ex vivo studies involving carotenoids have used either low-density lipoproteins (LDL) or microsomal fractions from various tissues. The LDL investigations consisted of evaluating the antioxidant ability of carotenoids added directly to LDL or introduced into LDL by oral ingestion of supplements or of fruits and vegetables. In the last few years, more and more investigators are using this latter approach, which presumably inserts the carotenoids “appropriately” in the LDL particle.

In addition, isolated lymphocytes or neutrophils have also been investigated with respect to the effect of added carotenoids on their susceptibility to oxidative stress.

Several studies in which carotenoids have been added to either plasma or isolated LDL fractions demonstrate that β -carotene addition is protective (Carpenter et al., 1997; Romanchik et al., 1997; Dugas et al., 1998; Panasencko et al., 2000), although one study reports that β -carotene addition results in a pro-oxidative action, as demonstrated by an increase in TBARS (Bowen and Omaye, 1998). However, in this latter study there was no change in the lag period or rate of LDL oxidation upon addition of β -carotene. In the studies that looked at carotenoids other than β -carotene, mixed results were reported. In some cases carotenoids such as canthaxanthin and zeaxanthin were effective antioxidants (Carpenter et al., 1997) as were lycopene, α -carotene, β -cryptoxanthin, zeaxanthin and lutein (Panasencko et al., 2000). However, in some studies in which β -carotene was effective, the addition of either lutein or lycopene actually increased LDL oxidation (Dugas et al., 1998).

It would seem that the final answer to the efficacy of carotenoids added to either plasma or LDL to act as effective antioxidants remains to be answered. However, a report by Aviram and his associates indicates that lycopene has a strong synergistic action when combined with other dietary antioxidants in inhibiting Cu-initiated oxidation of LDL, either when the antioxidants are added to isolated LDL or when they are supplemented in the diet (Fuhrman et al., 2000). They added either pure lycopene or a tomato oleoresin, a lipid extract of tomatoes that contains 6% lycopene, 0.1% β -carotene, 1% vitamin E and polyphenols, to isolated LDL and oxidation was followed either by TBARS formation or the appearance of peroxides in the LDL preparation (El-Saadani et al., 1989). The tomato oleoresin was 4–5-fold more effective in inhibiting Cu-initiated LDL oxidation (Fuhrman et al., 2000). Similar synergistic results were observed when they combined lycopene with vitamin E, the flavonoid, glabridin from licorice, the phenolics, rosmarinic or carnosic acids from rosemary, or garlic containing a mixture of antioxidants. Thus, it may be that some of the differences observed in earlier studies did not take advantage of the synergism of lycopene (and possibly other carotenoids) and other antioxidants.

In addition, this same group enriched LDL with lycopene by feeding tomato oleoresin containing 30 mg lycopene to volunteers, and then evaluating lycopene uptake and oxidation of post-prandial LDL (Fuhrman et al., 2000). There was a significant increase in lycopene and a significant decrease in LDL oxidation (TBARS) 5 h after ingestion of tomato oleoresin. These results should be contrasted with other studies where LDL particles were enriched by either supplementation with carotenoids or by dietary intervention with fruits and vegetables. Two different patterns have been reported. In one case, supplementation with either β -carotene (Levy et al., 1996; Dugas et al., 1999) or mixed carotenoids to a population depleted of dietary carotenoids (Lin et al., 1998) resulted in protection of the isolated LDL. Similar results were reported by Hininger et al. (1997). Green vegetable supplementation does not protect LDL in either smokers or non-smokers, whereas red vegetable supplementation was protective only in non-smokers, and not in smokers (Chopra et al., 2000). Lycopene from tomato-based products was reported to be effective (Agarwal and Rao, 1998) whereas pure lycopene supplementation was ineffective (Dugas et al., 1999). In addition, two other studies have not been able to verify that fruit and vegetable supplementation alters LDL resistance to oxidation (Chopra et al., 1996; van het Hof et al., 1999).

Not all studies of carotenoid supplementation have resulted in a change in LDL oxidizability. A 12 week period of daily supplementation with either 13 mg lycopene or 112 mg β -carotene resulted in an increase in LDL carotenoids, but no change in LDL oxidizability (Carroll et al., 2000). Similar results were seen with lutein supplementation for 1–2 weeks that resulted in a 4–6-fold increase in serum lutein but was without effect on LDL lag time (Chopra et al., 1996). Also, when tomato juice was used as a supplement in a volunteer, there was a decrease in the rate of accumulation of cholesterol ester hydroperoxides when the isolated LDL particles were subjected to singlet oxygen oxidation, but no difference was observed when the LDL was treated with the radical initiator, AAPH (Oshima et al., 1996). Finally, a 3 month course of daily ingestion of 15 mg of β -carotene, lutein or lycopene to healthy volunteers

resulted in a significant increase in LDL carotenoids, but no difference in the effects of Cu-initiated LDL oxidation (Hininger et al., 2001).

The above observations would suggest that carotenoids are not effective as antioxidants in isolated LDL particles, but this conclusion may be a simplification of a very complex situation. In a recent study evaluating the resistance of LDL to Cu-initiated oxidation, using serum from six different European countries, some rather remarkable observations were reported (Wright et al., 2002). This group reported wide variations in both tocopherol content as well as in carotenoid content, and concluded “LDL composition did not predict resistance to Cu-stimulated oxidation, nor is there evidence that LDL from volunteers in countries with lower rates of CVD have greater resistance to oxidation.” This conclusion puts in question that entire body of literature suggesting that altering the carotenoid content of LDL by itself can have an impact on resistance to oxidation.

When fruits and vegetable are added to the diet, not only do plasma carotenoids increase, but vitamin C and other potential antioxidants such as polyphenols and flavonoids may also increase. Therefore, it is very difficult to interpret whether the changes observed in LDL oxidizability are due to an increase in carotenoids or to other components of the fruit and vegetables. These variable results might be attributed to different lengths of time on the diets, different degrees of changes in the plasma carotenoid levels, different study populations, and to factors that still remain elusive (Wright et al., 2002).

3.4. *Ex vivo antioxidant studies in other tissues*

Rat liver microsomes have been used in the past to demonstrate an antioxidant action of added carotenoids, but a basic problem exists as to the exact location of the carotenoid molecules in the microsome fraction (reviewed by (Palozza and Krinsky, 1992)). More recently, it has been reported that β -carotene added to rat liver microsomes was an effective antioxidant at 15 Torr, but a prooxidant at 150 Torr, when the lipid-soluble azo initiator, 1,1'-azobis (cyclohexane-carbonitrile) was used to initiate lipid oxidation, as evaluated by MDA formation (Zhang and Omaye, 2001c).

Various immunological cells have also been supplemented with carotenoids, and in some circumstances, protection against oxidation have been reported. When human neutrophils are treated with opsonized zymosan, reactive oxygen species are released that can be detected by a burst of chemiluminescence if a suitable reporter dye such as lucigenin is used. Both all-*trans*- β -carotene and the 9-*cis*-isomer are equally effective in decreasing lucigenin-dependent chemiluminescence in these neutrophils (Liu et al., 2000). Other inflammatory cells such as mouse macrophage RAW 264.7 cells and human Promyelocytic HL-60 cells can be stimulated to release reactive oxygen species, and the addition of a wide variety of carotenoids have been reported to inhibit this process (Murakami et al., 2000).

Cells can also be oxidized by a variety of oxidants and there are reports that added carotenoids can inhibit this process. Human lymphoid cells, treated with water dispersible preparations of a variety of carotenoids, are protected against

dye-sensitized $^1\text{O}_2$ -generated damage as evaluated by lethal membrane lesions (Tinkler et al., 1994). This group has also found that lymphocytes from subjects supplemented with β -carotene are also protected from $^1\text{O}_2$ damage.

When CV1-P monkey cells were treated with ferric nitrilotriacetate as the oxidant, lipid peroxidation could be measured as an increase in TBARS formation (Matos et al., 2000). Cells supplemented with lycopene (20 pmol/ 10^6 cells) significantly decreased TBARS formation, and also decreased the amount of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) that was formed.

There have been many other studies monitoring the effectiveness of added carotenoids with respect to inhibition of DNA oxidation, and many of these involve using lymphocytes stimulated with an external agent to initiate DNA oxidation. So for example, the addition of phorbol 12-myristate 13-acetate to monocytes results in the release of reactive oxygen species, and when these activated monocytes are incubated with lymphocytes, DNA can be damaged. By assessing DNA damage using the single-cell gel electrophoresis (comet) assay, it has been demonstrated that the addition of a variety of antioxidants, including β -carotene, can result in a very significant decrease in lymphocyte DNA damage (Fabiani et al., 2001). Another oxidant that has been used to initiate DNA damage in lymphocytes is H_2O_2 . Under these circumstances, there is an increase in DNA damage as measured by the comet assay. Volunteers were supplemented with 15 mg/day of β -carotene, lycopene or lutein, and DNA damage was evaluated following H_2O_2 treatment. Although DNA strand breaks are usually only slowly rejoined in lymphocytes, the rate was increased in lymphocytes from β -carotene supplemented subjects, as well as those in which there had been a substantial increase in plasma lycopene levels. No such effect was observed in the lutein-supplemented subjects (Torbergson and Collins, 2000).

Food rich in carotenoids have also been used to elevate carotenoid levels, and lymphocyte DNA damage has been measured. Much of that work has been reviewed recently (Collins, 2001), and the general conclusions are that oxidative damage to lymphocytes correlates inversely with plasma carotenoid concentrations, and the extent of DNA damage is susceptible to reduction by carotenoid-containing foods. Under these circumstances, the conclusion has been drawn that carotenoids act as antioxidants in vivo (Collins, 2001). However, these foods contain many other compounds, in addition to carotenoids, and the relation between carotenoid intake and prevention of DNA damage must still be considered to be associative, and not necessarily causal.

In order to evaluate whether the effect on DNA damage is due to the carotenoid component of foods, Yeum and her associates supplemented volunteers with mixed carotenoids (β -carotene, lutein and lycopene, at 4 mg/d of each) for 8 weeks. At the end of that period, DNA damage was significantly decreased in the carotenoid-supplemented group, as determined by the comet assay (Zhao et al., in press).

Another example of an ex vivo effect deals with the ability of either β -carotene or astaxanthin to inhibit the invasion of rat mesentery derived hepatoma cells, AH109A, by rat ascites hepatoma cells (Kozuki et al., 2000). This process is markedly increased by pre-treating the AH109A cells with hypoxanthine and xanthine

oxidase to generate reactive oxygen species, and this enhanced effect is also inhibited by these carotenoids, suggesting that it is the antioxidative properties of the carotenoids that is involved in their anti-invasive property (Kozuki et al., 2000).

3.5. *In vivo* antioxidant studies

Various animal species have been used for many years in attempts to evaluate the *in vivo* antioxidant effect of carotenoids. However, these studies are marred by the fact that most experimental animals are very poor absorbers of carotenoids, and only large, pharmacological doses permit absorption of carotenoids into these animals. Some animals that can absorb dietary carotenoids, such as the ferret, gerbils and pre-ruminant calves, have been used to study carotenoid absorption, but virtually nothing has been done with respect to antioxidant effectiveness in these species (Lee et al., 1999). Nevertheless, there are some interesting observations made in animals that relate to potential antioxidant efficacy of carotenoids.

In mice treated for 15 days with canthaxanthin (4,4'-diketo- β -carotene), a direct antioxidant effect was not observed, inasmuch as the endogenous levels of MDA were not changed, but indirectly, a significant over-expression of the MnSOD gene was observed, strongly suggesting that this carotenoid was capable of altering the antioxidant protection in this species (Palozza et al., 2000).

Lycopene has been demonstrated to overcome the prooxidant effect of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced hamster buccal pouch carcinogenesis. Treatment with DMBA increases plasma TBARS, and decreases plasma levels of ascorbic acid, vitamin E, reduced glutathione (GSH) and GSH-dependent enzymes glutathione peroxidase, glutathione-S-transferase and glutathione reductase. The administration of lycopene (2.5 mg/kg body weight) significantly reversed all of these prooxidant responses, as well as decreasing the DMBA-induced carcinomas (Bhuvanewari et al., 2001a). Another study by this group was carried out in rats treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and saturated sodium chloride to induce gastric carcinogenesis. Increased liver TBARS and decreased levels of GSH and the GSH-dependent enzymes, caused by the carcinogen treatment, were all reversed by lycopene feeding, suggesting that the carotenoid had up-regulated the GSH-dependent hepatic detoxification system, thus preventing carcinogen-induced oxidative damage (Bhuvanewari et al., 2001b).

A direct attempt to demonstrate an antioxidant action was carried out by inducing oxidative damage in rat liver following intraperitoneal injection of the oxidant, ferric nitrilotriacetate, which resulted in significant increases in both MDA and 8-oxodGuo levels. Pre-treatment with lycopene almost completely prevented these oxidative effects (Matos et al., 2001).

The definitive studies therefore must deal with humans, who have an almost unlimited capacity to absorb dietary carotenoids (Parker et al., 1999) but have a somewhat limited ability to indicate their oxidative stress status. It has been suggested that the evidence for an antioxidant role for carotenoids *in vivo* is not very strong (Rice-Evans et al., 1997). Nevertheless, there are some studies that are worth discussing.

The key issue in determining whether dietary carotenoids alter the oxidative stress status in humans is the selection of appropriate biomarkers. For many years, determination of thiobarbituric acid-reactive substances (TBARS), such as MDA, was assumed to be a valid measure of lipid peroxidation, but we now know that this is a somewhat non-specific biomarker. Nevertheless, using TBARS or MDA, investigators have evaluated the effect of added carotenoids in several instances where an oxidative stress might arise.

Dixon and her associates (Dixon et al., 1994; Dixon et al., 1998) put women on carotenoid-deficient diets, and observed an increase in plasma MDA levels. This effect could be reversed when the diets were supplemented with a mixture of carotenoids, strongly supporting the idea that dietary carotenoids can serve to decrease oxidative stress in humans.

Another group that exhibits oxidative stress are patients suffering from cystic fibrosis (CF), which by preventing pancreatic enzyme secretion prevents appropriate absorption of fat-soluble vitamins such as vitamin E. Children with CF are routinely supplemented with vitamin E, but even so, their plasma MDA levels may be above control subjects. When treated with β -carotene (0.5 mg/kg) for 3 months, not only does the elevated MDA level fall, but there was also a prolongation in the lag time of LDL oxidation (Winkhofer-Roob et al., 1995). Other groups have also demonstrated normalization of MDA levels in CF children treated with either 13 mg β -carotene/d for 2 months (Lepage et al., 1996) or 50 mg/d for 10 weeks (Rust et al., 1998).

Another study reported in a group of Iranian men that had high MDA levels, supplementation with 30 mg/d β -carotene for 10 weeks could significantly reduce the MDA levels (Meraji et al., 1997).

In addition to lipid products, damage to DNA has also been used as a biomarker of oxidative damage. The most common product measured has been 8-hydroxy-2'-deoxyguanosine (8OHdG), even though there is still some question as to the relative importance of this marker in terms of evaluating DNA damage (Halliwell, 2000). In addition, levels of this marker, either in urine or lymphocytes, have not decreased when diets were supplemented with β -carotene (van Poppel, 1995; Collins et al., 1998), lutein, or lycopene (Collins et al., 1998). In contrast to these observations have been the reports of significant decreases in 8OHdG levels following supplementation with carrot juice (Pool-Zobel et al., 1997) or increased fruit and vegetable consumption (Haeghele et al., 2000). Another marker of DNA damage is the number of strand breaks observed in lymphocytic DNA, and increased fruit and vegetable intake can decrease that biomarker (Haeghele et al., 2000). These strand breaks can also be induced by treating lymphocytes with hydrogen peroxide (H_2O_2), and pre-treatment of the donors of these lymphocytes with a tomato puree supplement also significantly decreases strand breakage (Porrini and Riso, 2000).

In the work of Astley et al. (2004) examining the effects of carotenoids on DNA damage and susceptibility to oxidative damage in lymphocytes *in vitro* and *in vivo* it was reported that carotenoids (lycopene, lutein, β -carotene) were capable of exerting antioxidant protection by scavenging DNA-damaging free radicals and modulating DNA repair mechanisms. Zhao et al. (*in press*) have reported similar protection of

DNA damage by supplementation with a mixed carotenoid preparation containing β -carotene, lutein and lycopene (Zhao et al., in press).

Other markers of oxidative stress that have been evaluated with respect to carotenoid plasma levels, and intake from fruits and vegetables or supplements have included the ferric reducing ability assay (FRAP) (Benzie and Strain, 1996) as well as a total antioxidant capacity assay (Miller et al., 1993). It has been reported that supplementation with either β -carotene or spinach (whole-leaf, minced or liquefied) did not result in any change in the plasma FRAP level (Castenmiller et al., 1999b). Lee et al. added tomato products (tomato soup and canned tomatoes) to the diet with either olive oil or sunflower oil, and determined FRAP activity (Lee et al., 2000). Using either oil, the plasma lycopene level increased significantly, but only the olive oil arm resulted in an increase in FRAP levels, whereas the sunflower oil did not improve antioxidant activity. This would suggest that it is not the carotenoid component of the tomato products that was associated with FRAP activity, but something else whose absorption was modified by the type of oil in the diet. It could not be the vitamin E content, for the sunflower oil contained 14 times that found in the olive oil.

Ben-Amotz and associates have used patients with exercise-induced asthma (EIA) to evaluate the effect of supplemental carotenoids on the extent of this effect. Using either a dry powder of the β -carotene enriched alga *Dunaliella bardawil* (Neuman et al., 1999) or a tomato oleoresin product containing 6% lycopene, but with other carotenoids and phytochemicals (Neuman et al., 2000), all subjects receiving the carotenoid supplement showed a significant protection, as evaluated by measuring their post-exercise forced expiratory volume in 1 s (FEV_1). These authors conclude that they were observing an in vivo antioxidant action of the carotenoids, although they cannot exclude the possibility that the other components of these extracts were involved in their results (Neuman et al., 1999; Neuman et al., 2000).

To summarize, the addition of carotenoids in animal systems can be shown to demonstrate an antioxidant action, but in humans, carotenoids added as either as supplements or from food sources, has not yet been clearly shown to act as an in vivo antioxidant.

3.6. Prooxidant activity

The concept that carotenoids might behave as pro-oxidants was originally postulated by Burton and Ingold who observed that at high, non-physiological, oxygen tensions (760 Torr; 100% oxygen), relatively high concentrations (>0.5 mM) of β -carotene behaved as a pro-oxidant (Burton and Ingold, 1984). However, close inspection of the data in that important paper strongly suggests that the phenomenon observed was actually a decrease in antioxidant activity under the above conditions, and not necessarily a pro-oxidant effect. Thus, at 150 Torr (20% oxygen), β -carotene was an effective antioxidant in inhibiting the oxidation of methyl linoleate initiated by the radical generator, AIBN (Burton and Ingold, 1984). At 760 Torr, and with prolonged time, there was a marked decrease in the antioxidant effect, suggesting an autocatalytic inactivation of the β -carotene. In a subsequent paper,

Burton concluded that at the low partial pressures of oxygen found in mammalian tissues, β -carotene has the potential to act as an antioxidant, complementing the role of vitamin E, which is effective at higher oxygen tensions (Burton, 1989). Nevertheless, many investigators confused the high oxygen tensions (760 Torr) used by Burton and Ingold (Burton and Ingold, 1984) with the oxygen tension in the lung, which would be 150 Torr for the inspired air, and then drop rapidly to 15 Torr or less in the tissues.

There have been additional reports that at either high oxygen tensions or in experiments using high concentrations of carotenoids, there can be some evidence of a pro-oxidant effect. Much of this material has been reviewed (Palozza, 1998) so only a few new articles will be discussed here.

With respect to oxygen tension, there are recent reports that indicate that β -carotene, at 150 Torr, loses only 4% of the effectiveness observed at 15 Torr in protecting human serum albumin from oxidation by AAPH. However, at 760 Torr, 1.6 μ M β -carotene increased protein oxidation by 26% (Zhang and Omaye, 2000). Human plasma contains about 1–2 μ M total carotenoids (Yong et al., 1994), so the concentration used in this study is in the physiological range. Zhang and Omaye were also able to demonstrate a specific prooxidant effect of β -carotene at 722 Torr oxygen in human lung cells exposed to AAPH, whereas it was an antioxidant at 143 Torr. A similar study was carried out using human lung cells exposed to AAPH, and at 143 Torr, β -carotene protected cellular lipid, protein and DNA damage (Zhang and Omaye, 2001a). However, at 722 Torr, 1.5 mM β -carotene promoted isoprostane formation, whereas the protective effect against protein oxidation and DNA damage was decreased, without demonstrating a prooxidant effect (Zhang and Omaye, 2001a). Thus, it would appear that even at a physiological concentration, β -carotene could exhibit a prooxidant effect at essentially 100% oxygen.

There have also been some examples of a prooxidant action of β -carotene at physiological oxygen tension (150 Torr). When supercoiled plasmid DNA was incubated with AAPH, β -carotene prevented strand breakage at concentrations below 8 μ M, but above that level, an increase in both single- and double-strand breaks was observed (Zhang and Omaye, 2001b). In addition, when β -carotene was added to rat liver microsomes at 160 nmol/mg protein, it increased MDA production at 15 Torr following treatment with AAPH (Zhang and Omaye, 2001c).

The effects of carotenoid concentration on antioxidant/pro-oxidant effectiveness has been studied by Lowe et al. (1999), who added either β -carotene or lycopene to HT29 colon carcinoma cells and used xanthine/xanthine oxidase to induce oxidative damage. They measured both the comet assay for DNA damage and changes in membrane integrity using ethidium bromide uptake. At physiological levels (1–3 μ M), both β -carotene and lycopene prevented cellular damage, but at higher doses (4–10 μ M), the ability to protect these cells was lost, and their data suggests that the membrane integrity was more sensitive to these high doses than was the DNA damage. Bestwick and Milne (Bestwick and Milne, 2000) treated Caco-2 cell cultures with varying levels of β -carotene (0.1–50 μ M) and reported that at 50 μ M β -carotene, there was a significant reduction in intracellular levels of reactive oxygen

species, but at the same time, there were indications of decreased resistance of a H₂O₂ challenge with respect to enhanced Trypan blue staining, indicating increased membrane lability.

In other cell systems, antioxidant, pro-oxidant, or no effect have been observed. The antioxidant effects were observed in human lung cells pre-treated with β -carotene and exposed to tobacco-specific nitrosamines (Weitberg and Corvese, 1997), whereas a pro-oxidant effect was reported for Hep62 cells treated with 10 μ M β -carotene and then exposed to H₂O₂, in which case the cells treated with β -carotene showed increased levels of DNA strand breaks (Woods et al., 1999). Adding β -carotene directly to human plasma and then exposing the LDL to Cu²⁺-mediated oxidation resulted in a large increase in MDA, but with only a modest increase in the rate of LDL oxidation (Dugas et al., 1999). HL-60 cells pre-loaded with β -carotene (up to 1.5 nmol/10⁶ cells) and treated with 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) did not show any effect on either cell viability or oxidation of *cis*-parinaric acid incorporated into a variety of membrane phospholipid classes (Day et al., 1998).

Cigarette smoke has been invoked as a prooxidant, and it has been suggested that in the presence of high concentrations of β -carotene, cigarette smoke might lead to oxidative destruction of β -carotene, resulting in the formation of oxidized metabolites that might facilitate carcinogenesis (Wang and Russell, 1999). This effect was not observed with high doses of lycopene (Liu et al., 2003). Rather, when high doses of lycopene were fed to ferrets, protective effects against smoke induced lung carcinogenesis were observed. These results have been reviewed recently (Wang, 2005). A direct attempt was made to see if an interaction of β -carotene and cigarette smoke would result in a prooxidant effect. Human bronchial epithelial cells were exposed to gas phase cigarette smoke in the absence or presence of β -carotene, but the incorporation of β -carotene into the cells did not lead to an increase in the rate of lipid peroxidation (Arora et al., 2001).

4. Reactions with radical species

It has been known for many years that carotenoids “bleach” i.e., lose their color, when exposed to radicals or to oxidizing species. This process involves interruption of the conjugated double bond system either by cleavage or by addition to one of the double bonds. Cleavage can be detected by characterizing the products that are formed, which frequently are carbonyls (Handelman et al., 1991) or epoxides (Kennedy and Liebler, 1991). Addition products such as 4-nitro- β -carotene have also been reported following treatment of carotenoids with smoke containing nitrogen oxides (Baker et al., 1999), as well as addition products between the radical generator and the carotenoid (Liebler and McClure, 1996).

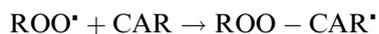
There are at least three possible mechanisms for the reaction of carotenoids with radical species (Fig. 3). They include (1) radical addition; (2) electron transfer to the radical; or (3) allylic hydrogen abstraction (Fig. 3). It has been proposed that a lipid peroxyl radical (ROO[•]) might add at any place across the polyene chain of

POSSIBLE REACTIONS OF CAROTENOIDS WITH RADICALS (R•)

1. **Adduct formation:** $\text{CAR} + \text{R}\bullet \text{-----} \rightarrow \text{R-CAR}\bullet$
2. **Electron transfer:** $\text{CAR} + \text{R}\bullet \text{-----} \rightarrow \text{CAR}\bullet^+ + \text{R}^-$
3. **Allylic H abstraction:** $\text{CAR} + \text{R}\bullet \text{-----} \rightarrow \text{CAR}\bullet + \text{RH}$

Fig. 3. Three possible reactions of carotenoids with radical species (R•).

a carotenoid, resulting in the formation of a resonance-stabilized, carbon-centered radical (ROO-CAR•). Since this radical should be quite stable, it would interfere with the propagating step in lipid peroxidation and would explain the many examples of the antioxidant effect of carotenoids in solution.



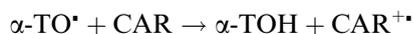
However, the subsequent reactions of ROO-CAR• are not well understood.

Electron transfer reactions have also been reported, resulting in the formation of the carotenoid cation radical, CAR⁺, most frequently detected by very fast spectroscopic techniques such as laser flash photolysis. This radical has been observed in studies involving photosystem II (Hanley et al., 1999; Tracewell et al., 2001a), and it has been proposed to play a role in photoprotection in photosystem II (Tracewell et al., 2001b).

When carotenoids react with soybean lipoxidase in the presence of unsaturated fatty acids, a co-oxidation occurs with the concomitant formation of a series of volatile carbonyls. Similar compounds are observed following either the autooxidation of β-carotene or when β-carotene is treated with radical initiator compounds. The structures of some of these carbonyl derivatives are shown in Fig. 4.

There are two laboratories that have studied carotenoid/radical interactions very extensively. These are the laboratories of Mortensen and Skibsted and the laboratory of Truscott.

The former group began their studies with the observation that when β-carotene reacted with a radical such as the phenoxyl radical, two products were formed, one an adduct of β-carotene and the radical and another was the carotenoid cation radical, CAR⁺ (Mortensen and Skibsted, 1996). Subsequent studies dealt with the interaction of carotenoids or their radicals with α-tocopherol or its radicals. This work was stimulated by the report that various carotenoids can protect α-tocopherol by electron transfer to the α-tocopherol radical, α-TO•, as seen in the following reaction (Böhm et al., 1997):



A number of investigators disagreed with this interpretation (Mortensen and Skibsted, 1997b; Valgimigli et al., 1997), with the latter group demonstrating that α-TOH reduces CAR⁺ to regenerate the intact carotenoid as seen below:

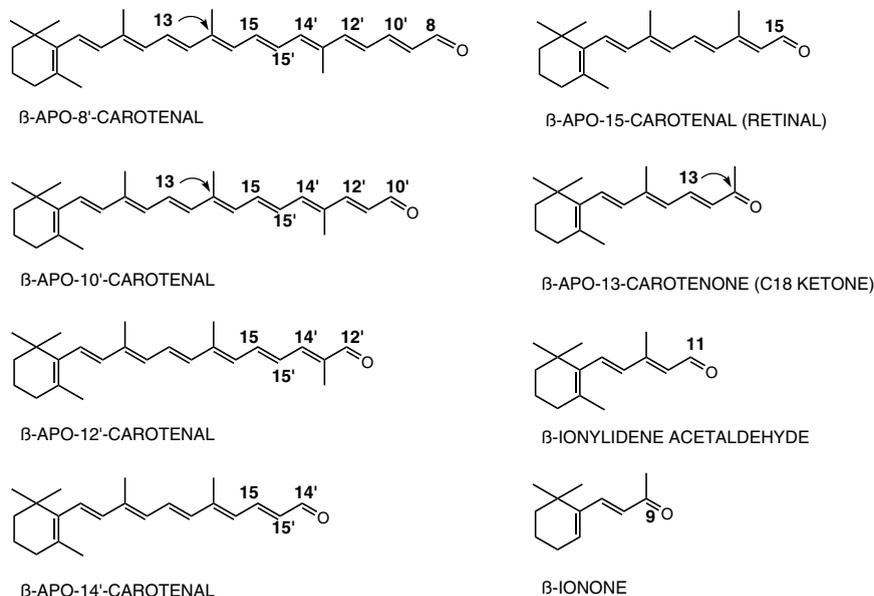
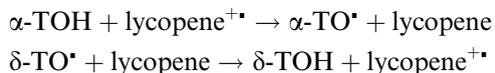


Fig. 4. The structure of a family of carbonyl breakdown products of β -carotene following autoxidation or treatment with either lipoxidase or radical initiators.



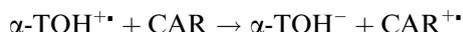
This is an important point, as will be discussed below, as Truscott and his associates try to demonstrate the role of carotenoids in electron transfer reactions between α -tocopherol and ascorbic acid.

Subsequent work of Mortensen and Skibsted demonstrated differing reactivities of $\text{CAR}^{+\bullet}$ and TOH^{\bullet} , such that the following reactions occur (Mortensen and Skibsted, 1997c):



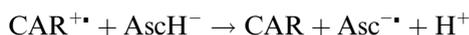
The differing stabilities of $\text{CAR}^{+\bullet}$ suggested that lycopene should be able to reduce other $\text{CAR}^{+\bullet}$ by electron transfer to produce lycopene⁺, and that α -tocopherol can reduce all of the $\text{CAR}^{+\bullet}$ (Mortensen et al., 1997d).

The controversial proposal from Truscott and his associates (Böhm et al., 1997) was clarified in a subsequent article (Edge et al., 1998) in which they suggested that in non-polar solvents, carotenoids react with $\alpha\text{-TOH}^{+\bullet}$, rather than with $\alpha\text{-TO}^{\bullet}$ as originally proposed and give the following reaction:



Thus, carotenoids can protect tocopherol, although the result is the formation of $\text{CAR}^{+\bullet}$. However, in polar environments, $\alpha\text{-TOH}^{+\bullet}$ rapidly deprotonates to $\alpha\text{-TO}^\bullet$ and no reaction has been observed between carotenoids and $\alpha\text{-TO}$ (Truscott, 2001).

Now the Truscott group had also reported a reaction between ascorbic acid and carotenoids, in which ascorbic acid increased the decay rate of $\text{CAR}^{+\bullet}$, presumably due to the following reaction:



Thus, they have presented a synergistic effect of β -carotene with both ascorbic acid and α -tocopherol, with the ultimate formation of the ascorbyl radical (Truscott, 2001). These studies of Mortensen, Skibsted and Truscott have been reviewed recently (Mortensen et al., 2001; Cantrell and Truscott, 2004).

Yeum, Aldini and their associates have been investigating the interaction of carotenoids with other antioxidants in both the lipophilic and hydrophilic compartments of human plasma by taking advantage of both water-soluble and lipid-soluble radical initiators (Aldini et al., 2001; Aldini et al., 2003; Yeum et al., 2003). Under these conditions, they have demonstrated the interaction of carotenoid radicals with both α -tocopherol and ascorbic acid, as shown in Fig. 5 (Yeum et al., 2004).

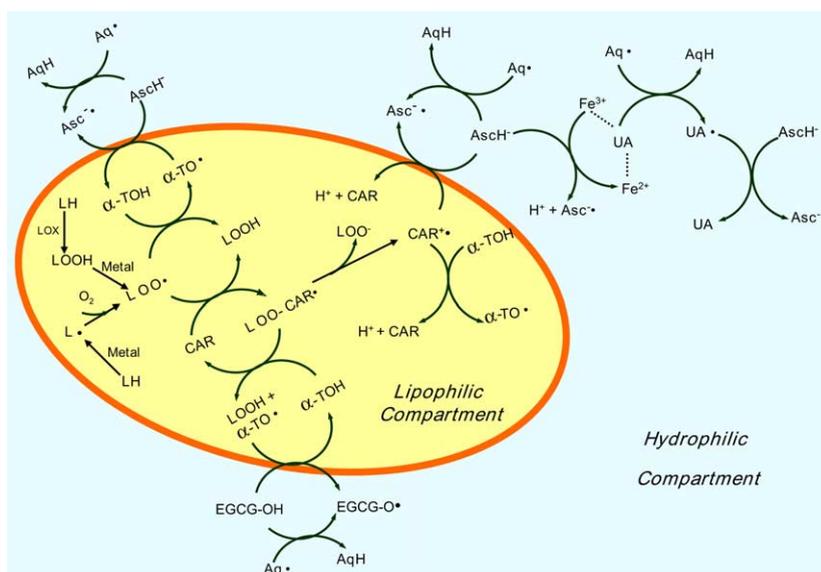


Fig. 5. A scheme of cooperative/synergistic interactions among antioxidants located in the hydrophilic and lipophilic compartments of plasma. AscH^- , ascorbic acid; $\text{Asc}^{\bullet-}$, ascorbyl radical; Aq^\bullet , aqueous radical; AqH , aqueous hydrogen donor; L^\bullet , lipid alkyl radical; LOO^\bullet , lipid peroxy radical; LOX , lipoxigenase; CAR , carotenoid; $\text{CAR}^{+\bullet}$, carotenoid radical cation; $\alpha\text{-TOH}$, α -tocopherol; $\alpha\text{-TO}^\bullet$, α -tocopheroxyl radical; EGCG-OH , (–)-epigallocatechin gallate; EGCG-O^\bullet , (–)-epigallocatechin gallate radical; UA^\bullet , uric acid radical; UA , uric acid. (From (Yeum et al., 2004)).

Nishino and his associates have also observed a specific inhibition of tumor cell growth, using a variety of carotenoids. The brown algal carotenoid, fucoxanthin, when added to the human neuroblastoma cell line, GOTO, inhibits growth over a three day period, as well as inhibiting N-myc expression within 4 h of administration (Okuzumi et al., 1990). Using an emulsion of palm oil carotenoids, the same group also reported inhibition of a variety of tumor cell lines, including GOTO (neuroblastoma), PANC-1 (pancreatic cancer), HGC27 (stomach cancer) and HeLa (cervical cancer) (Nishino et al., 1992). A concentration of 15 μM palm oil carotenoids was found to produce 50% growth inhibition. With the GOTO cell line, α -carotene was the most active component of the palm oil carotenoids, and was found to be ten times as effective as β -carotene in inhibiting cell growth. At only 5 μM , α -carotene induced an 82% drop in N-myc mRNA expression and a transient arrest of the cell cycle in the G_0/G_1 phase, or quiescent state prior to the resumption of growth (Nishino et al., 1992).

Canthaxanthin, at a very high concentration (100 μM), inhibits the growth of three tumor cell lines, and stimulates the growth of 3T3 cells (a non-tumor cell line), with the authors concluding that canthaxanthin has a direct effect in inhibiting tumor cell growth (Huang et al., 1992). A possible explanation for this effect, as well as that observed by others with respect to inhibition of tumor cell growth comes from the report that a cytokine is secreted when carotenoids are added directly to human peripheral blood mononuclear cells (Abril et al., 1989). This cytokine has cytotoxic effects in four out of six human tumor cell lines, and had only minimal toxicity to a normal diploid fibroblast cell line. The maximum secretion occurred at β -carotene concentrations between 0.1 and 1 nM. This cytokine appears to be a novel compound, as it is distinct from tumor necrosis factor, interleukin-1, interleukin-2, γ -interferon or lymphotoxin.

Other studies of growth inhibition were reported by (Hazuka et al., 1990) who added β -carotene and other antioxidants to cultures of mouse B-16 melanoma cells and observed morphological differentiation in these cells, along with both an inhibition of growth and some cytotoxicity. This treatment also decreased basal and melanocyte hormone-stimulated adenylyl cyclase activity in the melanoma cells. Similar effects on growth inhibition were observed with α -carotene, retinol, and butylated hydroxyanisole, but the latter was not able to modulate the adenylyl cyclase activity.

Levy and Sharoni initiated an extensive series of experiments on the growth inhibitory actions of lycopene, the major carotenoid pigment of tomatoes. Using endometrial (Ishikawa), mammary (MCF-7) and lung (NCI-H226) human cancer cells, they reported that lycopene, with a half-maximal inhibitory concentration of 1–2 μM , is 4–10-fold more effective than either α -carotene or β -carotene in inhibiting growth, whereas these pigments were much less effective in human fibroblasts (Levy et al., 1995). Much of their work has utilized an oleoresin from tomatoes that is very rich in lycopene, and have reported that this preparation is much more effective than β -carotene in inhibiting DMBA-induced mammary tumors in rats (Sharoni et al., 1997). This group is now investigating the molecular mechanisms of growth inhibition, and found that lycopene inhibited cell cycle progression in the G_0/G_1 phase in HL-60 cells (Amir et al., 1999) and found that this effect was probably due to a

reduction in cyclin D levels and alterations in the cyclin E/cdk2 complexes (Nahum et al., 2001). In a related study, they have demonstrated that the growth stimulatory action of insulin-like growth factor 1 (IGF-1) in MCF-7 mammary cancer cells can be markedly reduced by as little as 0.75 μM lycopene, a concentration well within the normal levels found in humans (Karas et al., 2000). Another example of an effect on the molecular basis of growth inhibition is the observation that β -carotene inhibits cyclin D1-associated cdk4 kinase activity, along with a decrease in the levels of the hyperphosphorylated form of retinoblastoma protein (Stivala et al., 2000). Their work has been reviewed recently (Sharoni et al., 2004).

The effect of lycopene on prostate cell growth has become particularly intriguing with the appearance of the study by Giovannucci et al. (1995) reporting an inverse relation between the intake of tomatoes and tomato produces and the risk of prostate cancer. In the androgen-insensitive line, DU-145 and PC-3, lycopene alone was without much effect, but in combination with α -tocopherol (both at physiological concentrations of 1 μM and 50 μM), they exhibited a synergistic effect in inhibiting growth of these cell lines (Pastori et al., 1998). This is an important observation, as it indicates that single nutrient interventions might not be adequate as an effective therapeutic technique. The question as to what exactly lycopene does in inhibiting cell growth remains unanswered.

However, carotenoids other than those found in tomatoes have also been shown to inhibit prostate cancer cell lines. Using large concentrations of β -carotene (30 mM), growth inhibition of PC-3, DU 145 and LNCaP has been observed, but the formation of ^{14}C -labeled retinol from ^{14}C -labeled β -carotene suggested to these authors that the effects might be due to the formation of metabolites, rather than due to the intact carotenoid (Williams et al., 2000). Two 5,6-monoepoxy carotenoids, neoxanthin from spinach and fucoxanthin from brown algae, were both found to be very effective inhibitors of the growth of the prostate cancer cell lines, PC-3, DU 145 and LNCaP (Kotake-Nara et al., 2001). They also reported that several acyclic carotenoids found in tomatoes, phytofluene, ζ -carotene and lycopene, significantly reduced cell growth, whereas phytoene, canthaxanthin, β -cryptoxanthin and zeaxanthin did not affect growth (Kotake-Nara et al., 2001). Both neoxanthin and fucoxanthin apparently reduced cell viability by inducing apoptosis.

Other groups have reported on the ability of carotenoids to induce apoptosis as a means of regulating cell growth. In one of the earliest reports, β -carotene derived from *Dunaliella* was able to induce apoptosis in a preneoplastic cervical line, but not in a carcinoma-derived line (Muto et al., 1995; Toba et al., 1997). A differential effect of lutein on apoptotic pathways was reported for the MCF-7 mammary carcinoma cell line, SV40 transformed mammary cells, and normal human mammary cells. At 7 μM , lutein significantly decreased the viability of MCF-7 cells and induced apoptosis in transformed, but not normal mammary cells (Sumantran et al., 2000). On the other hand, lutein protected the normal, but not the transformed cells, from apoptosis induced by 2 chemotherapy agents, etoposide or cisplatin. This unusual property of lutein has not yet been investigated in other cell lines or using different carotenoids, but remains a potential therapeutic tool.

In the malignant T-lymphoblast cell line, Jurkat E6.1, β -carotene as well as several other carotenoids normally found in human plasma, was found to induce apoptosis, with β -carotene being the most effective at concentrations as low as 3 μ M (Müller et al., 2002). If the β -carotene was oxidized, it lost its efficacy. In contrast, the acyclic carotenoids, phytofluene and ζ -carotene, inhibited cell growth of the human promyelocytic leukemia cells, HL-60, but this activity was very markedly enhanced when these carotenoids, as well as lycopene, were oxidized, suggesting that the oxidation products were responsible for the apoptosis induction (Nara et al., 2001).

In many respects, the apoptotic-inducing effects have moved from the descriptive to the molecular level, and much of that has come from the laboratory of Palozza and her associates. Starting with the descriptive report that canthaxanthin can induce apoptosis in both WiDr colon adenocarcinoma and SK-MEL-2 melanoma cells (Palozza et al., 1998), they followed that with a report suggesting that the mechanism of β -carotene induced apoptosis involved a change in the intracellular redox potential, presumably brought about by the use of a relatively high concentration (50 μ M) of β -carotene (Palozza et al., 2001a). This group has reviewed the potential role of carotenoids in regulating cell growth through altering redox status, invoking an antioxidant or prooxidant effect that maybe related to the concentration of the carotenoid in the cellular system (Palozza et al., 2001b). Further support for the role of β -carotene in regulating cell growth via a redox mechanism was presented in a study of undifferentiated and dimethylsulfoxide-differentiated HL-60 cells exposed to β -carotene. At concentrations as low as 10 μ M, β -carotene modified cell cycle progression by decreasing cyclin A and Bcl-2 expression, thus inducing apoptosis (Palozza et al., 2002a,b). This inhibition of cell growth was accompanied by an increase in the level of both reactive oxygen species and oxidized glutathione, and the addition of 50 μ M α -tocopherol reversed the pro-apoptotic effects of β -carotene, suggesting that a redox effect was associated with the development of apoptosis (Palozza et al., 2002a,b). The pro-apoptotic activity of β -carotene was investigated in several human colon adenocarcinoma cell lines, and it was determined that efficacy was directly related to the degree of uptake of β -carotene by the cells. For example, as little as 1 μ M β -carotene was effective in inducing cell cycle arrest in the G2/M phase and apoptosis in the cell line COLO 320 HSR that accumulated significantly greater amounts of β -carotene than other lines such as LS-174, HT-29 or WiDr (Palozza et al., 2002a,b). These authors point out that this is a physiological level of β -carotene, and suggest that β -carotene might have a role in the treatment of colon cancer.

5.2. Antimutagenic action in bacteria

The development of strains of *Salmonella typhimurium* that could be mutated by compounds or factors associated with oxidant stress (Ames et al., 1973) has enabled investigators to study the protective, or antimutagenic effects of a wide variety of compounds. The ability of carotenoids to prevent bacterial mutagenesis in *S. typhimurium* has been reviewed earlier (Krinsky, 1993). These studies were initiated by Santamaria and his associates (Santamaria et al., 1984), who demonstrated the protective effects of β -carotene against the mutagenic potential of 8-methoxypsoralen

and ultraviolet light type A (UV-A, 340–400 nm). Cyclophosphamide-initiated mutagenesis can also be blocked by the addition of β -carotene to this organism (Belisario et al., 1985), and several other carotenoids have been evaluated using aflatoxin B_1 to induce mutagenesis. Cryptoxanthin was reported to be more effective than β -carotene or canthaxanthin, while lycopene had no effect (He and Campbell, 1990). When either 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) or benzo(a)pyrene (B(a)P) were used to initiate mutagenesis, β -carotene, canthaxanthin, β -apo-8'-carotenal and methyl- β -apo-8'-carotenoate all demonstrated a dose-dependent decrease in mutagenicity (Azuine et al., 1992). However, not all of these studies were supportive of a role for carotenoids as antimutagens. Terwell and van der Hoeven (1985) were unable to demonstrate any antimutagenicity for β -carotene when either B (Abiaka et al., 2001)P or cigarette smoke condensate was used as the mutagen. It has also been reported that solvent extracts of a variety of substances (coal dust, airborne particles, diesel emissions and tobacco snuff) were mutagenic in this assay but various antioxidants, including β -carotene, α -tocopherol and ascorbate (vitamin C) were without effect (Ong et al., 1989). A slight antimutagenic effect of β -carotene was reported by these authors when an aqueous extract of fried beef was tested as a mutagen. Finally, quinolones induce mutagenesis in sensitive strains of *S. typhimurium* and in the presence of the S9 fraction of rat liver, β -carotene inhibit this process, which has been attributed by the authors to a decrease in oxygen radical activity (Arriaga-Alba et al., 2000).

5.3. Effects on genotoxicity

Genotoxicity is not a very specific term, but can cover a variety of insults to the genome, which can be expressed as DNA damage, formation of micronucleated cells, sister chromatid exchanges (SCE), chromosomal aberrations, translocations, mutagenesis, or even death of the cell. In the following reports, these various aspects of genotoxicity have been modified by the addition of carotenoids.

When human lymphocytes are exposed to 2 Gy of γ -rays, extensive genotoxicity occurs, as evaluated by the formation of micronucleated cells. The addition of 1–5 $\mu\text{g/ml}$ β -carotene, either before or within 1 h of treatment results in a significant reduction in the number of micronuclei in the irradiated cells (Konopacka and Rzeszowska-Wolny, 2001).

Cells lacking mismatch repair exhibit elevated levels of spontaneous mutation. A strain of human colon carcinoma cells (HCT112) has been developed that is deficient in mismatch repair, and shows a high rate of spontaneous mutation. When such cells are grown in the presence of various antioxidants, a significant reduction in the spontaneous mutation is observed (Mure and Rossman, 2001). The most efficient antimutagenic compound was lycopene (at 5 μM), followed by α -tocopherol (at 50 μM) and then by ascorbate (at 284 μM). These authors suggested that a mixture of antioxidant might prove efficacious in people with a genetic defect in mismatch repair (Mure and Rossman, 2001).

Polymorphonuclear leukocytes (PMNs) not only play an important defensive role elaborating reactive oxygen species to help destroy invading organisms, but can also

be harmful to tissues when there is an overproduction of these same reactive oxygen species (Bendich, 1994; Kehrer and Smith, 1994). Tumor promoters, such as phorbol myristate acetate, have a powerful effect in eliciting release of reactive oxygen species from PMNs. Weitzman and Stossel (1981) have studied the effects of adding activated PMNs to *S. typhimurium* (TA 102), and observed an increased number of histidine revertants, indicative of the occurrence of mutagenesis. In addition to inducing mutagenesis, activated PMNs can cause an increase in the frequency of SCE when they are placed on cultures of Chinese Ovary Hamster (CHO) cells (Weitzman and Stossel, 1982). Weitzman et al. (1985) used either phorbol myristate acetate-stimulated PMNs, or xanthine oxidase and hypoxanthine to generate reactive oxygen species directly to induce increased SCE in CHO cells. When 10–50 μM β -carotene was added to the cell culture system, a significant protection against the generation of SCE was observed. However, the significance of these findings may not be extended to in vivo situations. The total concentration of carotenoids in human serum is in the 1.5–3 μM level, with β -carotene representing about 30% (Krinsky et al., 1990), so the concentrations used above should be considered high by human physiological standards. Anderson and associates have determined the extent of SCE in PMNs from smokers, and did not find a significant correlation with serum concentrations of ascorbate, β -carotene, or α -tocopherol (van Rensburg et al., 1989).

When either methylmethanesulfonate or 4-nitroquinoline-1-oxide are added to CHO cells growing in culture, a variety of genotoxic symptoms appear, including chromosomal aberrations, translocations, or the development of micronuclei (Stich and Dunn, 1986). The addition of β -carotene to these cultures resulted in a dose-dependent inhibition of genotoxicity, and since the authors were not able to detect retinol in these cells, they concluded that the inhibitory effects of β -carotene were not associated with its conversion to retinol. The CHO cells were not protected by β -carotene against other genotoxic compounds such as gallic acid, tannic acid, an aqueous extract of the areca nut, or hydrogen peroxide. In a related study, (Stich et al., 1990) measured the chromosome instability of C127 cells transformed with bovine papillomavirus. A number of antioxidants, including β -carotene, canthaxanthin, retinoic acid, retinol, ascorbate, and ellagic acid, when added to the cultures at physiological concentrations (β -carotene and canthaxanthin at 0.5 μM), were able to decrease the papillomavirus-induced chromosome instability.

Banerjee and associates (Manoharan and Banerjee, 1985) have used mouse mammary cell organ cultures treated with chemical carcinogens such as dimethylbenzanthracene (DMBA) to induce SCE. When β -carotene was added to the medium during the 24 h initiation stage, there was a significant decrease in the number of SCE. They also noted a similar protective effect when two other carcinogens, *N*-nitrosodiethylamine and methylnitrosourea, were studied.

5.4. *Effects on malignant transformation*

The most profound effects of carotenoids on cells grown in culture have been reported with respect to the inhibition of malignant transformation. This

phenomenon involves the process whereby normal cells are converted to cells that have the ability to induce tumors when injected into animals. A much simpler assay can be carried out in cell culture, where the addition of a carcinogen such as 3-methylcholanthrene, or treatment with X-irradiation, permits the transformed cells to grow without contact inhibition, thus forming visible foci of cells that can be readily counted. It is then quite feasible to determine how much any added compound can inhibit the frequency of transformation. Much of this work comes from the laboratory of Bertram and his associates, who had demonstrated earlier the ability of various retinoids to inhibit malignant transformation in the fibroblast cell line, C3H/10T1/2 (Merriman and Bertram, 1979). These studies were followed with their report that both β -carotene and canthaxanthin can inhibit malignant transformation induced by 3-methylcholanthrene or X-ray treatment in this same cell line (Pung et al., 1988). The carotenoids appeared to act at the promotional phase of malignant transformation, as determined by their effectiveness when added 1 week after X-ray treatment, and their lack of effect when present prior to or during X-irradiation. In addition to β -carotene, canthaxanthin, α -carotene and lycopene (Fig. 1) were also effective in inhibiting 3-methylcholanthrene-induced malignant transformation (Bertram et al., 1991). An unexpected finding was that lutein was inhibitory at 10 μ M, but actually increased the number of transformants at lower concentrations. α -Tocopherol was also tested, and found to inhibit malignant transformation, but on a molar basis was only about 10% as active as lycopene. This group has also compared the efficacy of all-*trans*- β -carotene to 9-*cis*- β -carotene isolated from the algae, *Dunaliella salina*, and reported that the 9-*cis* isomer was consistently less active in suppressing neoplastic transformation in both murine 10T1/2 and human HaCaT keratinocytes (Hieber et al., 2000). This should be contrasted with the fact that the metabolite, 9-*cis*-retinoic acid, is much more active than the all-*trans*-retinoic acid, and the authors suggested that these cells do not convert the parent carotenoid to the respective retinoic acids (Hieber et al., 2000).

The inhibition of malignant transformation displayed by retinoids (Merriman and Bertram, 1979) and carotenoids (Pung et al., 1988; Bertram et al., 1991) and even by synthetic polyenes resembling carotenoids (Pung et al., 1993) raises the question as to whether carotenoids might show similar retinoid-like effects with respect to other cellular functions. For example, if the molecular basis for retinoid inhibition of malignant transformation were understood, it would be worthwhile to see if carotenoids functioned in a similar fashion. Unfortunately, even though we are learning a great deal about the molecular basis for gene control by retinoids with the demonstration of nuclear receptors that bind retinoic acid and lead to a transactivation of various genes (Mangelsdorf et al., 1990, 1991), it is not clear that malignant inhibition operates through such a mechanism.

5.5. Effects on cell–cell communication

One possibility for explaining the inhibition of transformation was proposed in the report by Bertram's group that retinoids could modify cell–cell communication (Mehta et al., 1989). Yamasaki has pointed out the importance of cell–cell

communication for regulating growth of cells, and described a system that involves contact between cells at so-called gap junctions (Yamasaki, 1990). These are composed of a pore-like structure (a connexon) made up of several proteins, or connexins. The entire structure allows molecules below about 1000 daltons to diffuse between connecting cells. Tumor cells are extremely deficient in these gap junctions (Yamasaki, 1990).

Following the observation that retinoids could increase gap junction communication between cells (Mehta et al., 1989), Bertram and his associates reported that carotenoids could also enhance gap junctional communication in C3H/10T1/2 cells (Zhang et al., 1991). To see if there was a relationship between the ability of these carotenoids to inhibit gap junction communication and their antioxidant capacity, TBARS were measured in these cells in the presence and absence of carotenoids (Zhang et al., 1991). No correlation was observed between the ability of carotenoids to increase gap junction communication and TBARS production, indicating that an antioxidant action of carotenoids was not related to the cellular effect. This group has been able to offer even more insight into possible molecular mechanisms of carotenoid action, by reporting that β -carotene, canthaxanthin, and lycopene up-regulate the expression of the connexin 43 gene, the gene responsible for the production of one of the important components of the gap junction (Zhang et al., 1992). As with gap junction communication, this process was not related to the antioxidant capacities of these carotenoids, and added α -tocopherol had no effect. To determine if a metabolite of lycopene could be responsible for stimulating gap junction communication, experiments were carried out comparing lycopene to acyclo-retinoic acid, the C20 product that would form if lycopene underwent central cleavage. In human fetal skin fibroblasts, lycopene was at least 10-fold more potent than acyclo-retinoic acid, strongly suggesting that the effect was due to the intact lycopene molecule, and not to a central cleavage metabolite (Stahl et al., 2000). Many of the effects of carotenoids on gap junction communication have been summarized in a recent review (Stahl and Sies, 2001).

5.6. Other cellular effects of carotenoids

One of the most intriguing actions of carotenoids (Tang et al., 2005), as well as some of their oxidative breakdown products, is their ability to induce xenobiotic metabolizing enzymes in rodents. This topic has been reviewed recently (Stahl et al., 2002; Stahl and Sies, 2002) and it has been suggested that modulation of these enzymes might be relevant to humans (Breinholt et al., 2000). However, there is at the present no evidence that this process obtains in humans.

A report has observed that extensive DNA damage can occur when either calf thymus DNA or human fibroblasts are incubated with oxidized β -carotene or lycopene, without inducing TBARS (Yeh and Hu, 2001). Although the mechanism for this effect remains unknown, it does raise the question as to the purity of carotenoid supplements that are administered to humans.

The effects of lutein on two parameters of coronary vascular disease have been studied in both a mixed culture system as well as in mice. Pre-treatment of

monocytes with lutein resulted in a dose-dependent significant inhibition of artery wall cell modified LDL-induced monocyte migration in a mixed culture model of the human intima (Dwyer et al., 2001). Such an observation would suggest that an increased intake of dietary lutein, obtained primarily from dark green vegetables, might be protective against the development of early atherosclerosis. This group has also studied lutein supplementation in two strains of mice that are prone to develop atherosclerotic lesions, the apo-E null mouse and the LDL receptor-null mouse. They report that this treatment results in significant decrease in lesion size in both strains of genetically modified mice (Dwyer et al., 2001). It is not clear if this observation is applicable to humans. Another group has investigated the effect of several carotenoids on the expression of cell surface adhesion molecules and the binding of monocytes to human aortic endothelial cells. Although it is sometimes difficult to compare the different carotenoids because different concentrations were used and the human aortic endothelial cells took up very different amounts of the pigments, they reported that lycopene appeared to be the most effective in reducing human aortic endothelial cell adhesion to monocytes as well as the expression of adhesion molecules on the cell surface (Martin et al., 2000).

6. Carotenoids and cancer

6.1. *Observational epidemiology*

Although epidemiological studies cannot provide evidence of causal relationship, they have proven useful in evaluating the possible protective effects of foods or food components in disease prevention. Observational epidemiologic studies have been very consistent in showing that people who consume higher dietary levels of fruits and vegetables have a lower risk of certain types of cancer. The results of 10 of 17 case-control studies show that a high intake of fruits and vegetables that are rich in carotenoids has been associated with decreased risk of cancer at a number of common sites. This association appears to be most consistent for lung and stomach cancer (Block et al., 1992; Ziegler et al., 1996). Given the variability in study design and statistical analysis among the epidemiologic studies, the consistency of the relationship between fruit and vegetable intake and lung cancer risk strongly suggests a real effect of fruit and vegetables on cancer prevention. It has been suggested that carotenoids are the chemopreventive agents in fruits and vegetables. There are several mechanisms by which a carotenoid can function in cancer prevention. As a provitamin A, a carotenoid would have an effect on cellular differentiation and proliferation (DeLuca et al., 1972; Sporn and Roberts, 1983; DiGiovanni, 1990). Moreover, the antioxidant function could prevent free radical-induced damage to cellular DNA and other molecules (Burton, 1989). Immunomodulatory effects could enhance immune surveillance in tumorigenesis (Bendich, 1989) and enhanced cell–cell communication would restrict clonal expansion of initiated cells (Zhang and Bertram, 1994).

6.1.1. β -Carotene

The epidemiologic observations, along with what is known about carotenoid function, have led to further study of the effect of β -carotene on cancer risk. Although other types of cancers have been evaluated, e.g. breast cancer (Cho et al., 2003) with a reduced risk with increased dietary intake, the relationship between β -carotene and lung cancer has been most studied and the data have been more consistent (see (van Poppel, 1993) for review). Evidence for β -carotene as the chemoprotective agent in fruits and vegetables comes from prospective epidemiologic studies in which 11 of 15 have demonstrated a significant inverse relation of β -carotene intake and/or plasma level and risk of lung cancer (Table 4).

6.1.2. Lycopene

One of the earliest case-control studies examining the relationship between lycopene-rich foods and cancer risk reported that weekly tomato consumption associated with a 40% reduction in risk for esophageal cancer (Cook-Mozaffari et al., 1979). Several studies have reported protective effects for diets rich in tomato products against gastric cancer (Belke, 1974; Correa et al., 1985; Buiatti et al., 1989; Tsugane et al., 1992; Franceschi et al., 1994) whereas others have found no relationship (Tajima and Tominaga, 1985; Ramon et al., 1993). A study of prospectively collected serum samples from a cohort was used to compare serum micronutrients from those developing pancreatic cancer and matched controls (Burney et al., 1989). The greatest difference was observed for lycopene. However, because lycopene uptake and serum concentrations may be related to the digestibility of dietary lipids, diseases of the pancreas could significantly reduce absorption and perhaps confound such studies. In a single study examine the role of tomatoes, lycopene and bladder cancer risk (Helzlsouer et al., 1989), serum micronutrient profiles were quantitated, and only lycopene and selenium concentrations were found to be inversely related to risk. However, others have found no such relationship (Nomura et al., 1991; Riboli et al., 1991; Bruemmer et al., 1996).

Less consistent results have been observed for a relationship between estimated lycopene intake or serum lycopene concentrations and breast cancer risk (Potischman et al., 1991; London et al., 1992). A recent study of dietary intake interview of 4697 women in Finland showed no relationship between estimate intake of tomato products and breast cancer risk (Jarvinen et al., 1997). However, a case-control study from Boston examined the relationship between breast adipose tissue lycopene concentrations and breast cancer risk, and an inverse association between lycopene and breast cancer risk was observed (Zhang et al., 1997).

In a study conducted in a cohort of Seventh Day Adventists, dietary and lifestyle factors were evaluated and related to subsequent risk of prostate cancer in a follow-up of 6 years. Multivariate analysis indicated that increasing age and previous prostate problem were among the risk factors, whereas, unexpectedly, smoking and alcohol consumption were not. Among dietary factors, high tomato consumption of five or more per week was associated with a significantly decreased risk

Table 4
Prospective studies on dietary intake or plasma levels of carotenoids and lung cancer

Exposure measured	Association	Relative risk (high vs low)	Population	Reference
Carotenoid intake	Decrease	0.14	Men, US	Shekelle et al. (1981)
Green salad and fruit	Decrease	0.56	Men and women, China	Long-de and Hammond (1985)
Green, yellow vegetables	Decrease	0.79 (men); 1.35 (women)	Men and women, Japan	Hirayama (1986)
Carotenoid intake	n.s.	0.68	Men and women, The Netherlands	Kromhout (1987)
Carotenoid intake	Decrease	0.72 (men); 0.67 (women)	Men and women, US	Paganini-Hill et al. (1987)
Carotenoid intake	Decrease 0.93 (smokers)	0.40 (non-smokers)	Finland	Knekt et al. (1991)
Fruit and green salad	Decrease	0.26 (fruit); 0.65 (salad)	Men and women, California Adventists	Fraser et al. (1991)
Plasma total carotene	n.s.		Men and women, US	Willett et al. (1984)
Plasma β -carotene	Decrease	0.43	Men, US	Connett et al. (1989)
Plasma β -carotene	Decrease	0.29	Men, Japanese (Hawaii)	Nomura et al. (1985)
Plasma β -carotene	Decrease	0.41	Men, UK	Wald et al. (1988)
Plasma β -carotene	Decrease	0.56	Men and women, Swiss	Stähelin et al. (1991)
Plasma β -carotene	n.s.	1.00	Men and women, Finland	Knekt et al. (1990)
Plasma β -carotene	Decrease	0.45	Men and women, US	Comstock et al. (1991)
Plasma β -carotene	Decrease	0.33	Men and women, US	Orentreich et al. (1991)

(Mills et al., 1989). An inverse association between high intake of tomato products and prostate cancer risk was confirmed in an analysis of the US Health Professionals Follow-up Study (Giovannucci et al., 1995). A dietary frequency questionnaire was applied at baseline and related to the incidence of prostate cancer that was assessed biennially. It was shown that lycopene intake but not the overall intake of fruits and vegetables was inversely related to prostate cancer risk. Among tomato-derived products, this association was strongest for tomato sauces, followed by tomatoes and pizza. No relationship was found for tomato juice. This may be explained by the low bioavailability of lycopene from unprocessed juice (Stahl and Sies, 1992). A summary of the epidemiologic studies evaluating lycopene and cancer risk is found in Table 5.

The reports of lycopene concentrations being relatively high in the prostate compared to other tissue (Kaplan et al., 1990) suggests that this carotenoid may exert unique biological effects at this site which may be related to cancer prevention. Clinton et al. have observed 14–18 different isomers of lycopene in prostate (Clinton et al., 1996). Approximately 80% of total lycopene is found as *cis* isomers in prostate tissue, compared to 50% in blood (Yeum et al., 1996) and 5–10% in foods (Clinton et al., 1996). The biological significance of this is not known. *cis* Isomers differ in molecular shape from the all-*trans* form and, therefore, may differ in metabolism (as has been reported for β -carotene (Johnson et al., 1996)). A chemoprevention effect of lycopene remains to be determined.

6.2. Intervention trials

Based on the epidemiological evidence, long-term large randomized intervention trials were designed to test the efficacy of high doses of β -carotene (20–30 mg/d) in the prevention of cancer. Surprisingly, the results from two trials provide possible evidence of harm from β -carotene supplements in relation to cancer among high-risk individuals such as smokers and asbestos workers (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994; Omenn et al., 1996) and no effect (neither beneficial nor detrimental) in a generally well-nourished population (Hennekens et al., 1996). However, in the Linxian (Chinese) Cancer Prevention Study, it was found that supplementation with β -carotene, vitamin E and selenium led to a significant reduction in total mortality (9%), especially from cancer (13%), particularly stomach (21%) (Blot et al., 1993). The positive results of the Chinese study may reflect the correction of a nutrient deficiency in this study population. Therefore, the usefulness of high doses of β -carotene in high-risk individuals needs to be re-evaluated. It should be noted that, like the earlier epidemiologic studies that lead to these intervention studies, there are recent studies that report a similar relationship between diet and/or blood β -carotene levels and cancer prevention (van Poppel, 1995; Ziegler et al., 1996; Zhang et al., 1999; De Stefani et al., 2000; Abiaka et al., 2001; Toniolo et al., 2001). It is probable that β -carotene is serving as a marker of increase fruit and vegetable intake and therefore of all components that have cancer-prevention potential, e.g. vitamin C, folic acid, other carotenoids.

Table 5
Epidemiologic studies examining tomato or lycopene intake or plasma level and cancer

Exposure measure	Type of study	Cancer examined	RR (95% confidence interval)	Reference
Raw tomato intake ≥1/wk vs <1/mo	Case control	Esophagus	0.61 (0.43–0.86) men 1.08 (0.69–1.67) women	Cook-Mozaffari et al. (1979)
Tomato intake quartile 4 vs 1	Case control	Stomach	0.43 (0.33–0.55)	Franceschi et al. (1994)
Tomato intake “high vs low”	Case control	Stomach	0.82 (0.52–1.28) whites 0.56 (0.32–0.90) blacks	Correa et al. (1985)
Tomato intake tertile 3 vs 1	Case control	Stomach	0.70, <i>P</i> for trend < 0.001	Buiatti et al. (1989)
Plasma lycopene	Ecologic	Stomach	Regions high in lycopene have lowest cancer rates; low regions have highest rates	Tsugane et al. (1992)
Tomato intake tertile 3 vs 1	Case control	Stomach	1.24, n.s.	Tajima and Tominaga (1985)
Tomato intake tertile 3 vs 1	Case control	Stomach	1.02, n.s.	Ramon et al. (1993)
Serum lycopene high vs lowest 2 tertiles	Cohort	Pancreas	0.16 (0.04–0.57) <i>P</i> for trend < 0.02	Burney et al. (1989)
Serum lycopene tertile 3 vs 1	Cohort	Bladder	0.5, <i>P</i> for trend = 0.06	Helzlsouer et al. (1989)
Lycopene intake quintile 5 vs 1	Case control	Bladder	0.7, <i>P</i> for trend = 0.27 (men) 0.9, <i>P</i> for trend = 0.41 (women)	Nomura et al. (1991)
Tomato intake	Case control	Bladder	No association	Riboli et al. (1991)
Tomato intake >0.29 vs ≤0.7/day	Case control	Bladder	0.71 (0.39–1.29) <i>P</i> for trend = 0.3	Bruemmer et al. (1996)
Plasma lycopene quartile 4 vs 1	Case control	Breast	0.62 (0.19–2.0) <i>P</i> = 0.43	Potischman et al. (1990)
Serum lycopene quintile 5 vs 1	Case control	Breast	1.0 (0.7–1.7)	London et al. (1992)
Lycopene intake tertile 3 vs 1	Cohort	Breast	~1.0	Jarvinen et al. (1997)
Breast adipose lycopene (>0.51 vs ≤0.22 μmol/L)	Case control	Breast	0.32 (0.11–0.94)	Zhang et al. (1997)
Tomato intake ≥5 vs <1/wk	Cohort	Prostate	0.60 (0.37–0.97) <i>P</i> = 0.02	Mills et al. (1989)
Tomato product intake >10 vs <1.5 svgs//wk	Cohort	Prostate	0.65 (0.49–0.90) <i>P</i> = 0.01	Giovannucci et al. (1995)
Tomato sauce intake 2–4 vs 0/wk				
Plasma lycopene highest vs lowest quintile <65 years	Case control	Prostate	0.43 (0.18–0.98)	Wu et al. (2004)
Negative family history			0.43 (0.18–1.04)	

7. Carotenoids and coronary vascular disease

7.1. Observational epidemiology

Because of their role as antioxidants, carotenoids have been suggested to be protective against coronary vascular disease. One contributor to the development of coronary vascular disease is the oxidation of low-density lipoproteins (LDL). When LDL is oxidized it is readily taken up by foam cells in the vascular endothelium where it contributes to the development of atherosclerotic lesion (Clinton and Libby, 1992; Frei, 1995). The fact that LDL is a major transporter of β -carotene and lycopene in the circulation (Clevidence and Bieri, 1993) and that these carotenoids have the capacity to trap peroxy radicals and quench singlet oxygen lends support to this hypothesis.

This hypothesis is supported by the observational epidemiological studies that report that foods rich in carotenoids and antioxidant vitamins are associated with reduced risk of cardiovascular disease (Mayne, 1996). Carotenoids have been detected in lipid-rich atherosclerotic plaques; however, the levels of lycopene were very low (Suarna et al., 1995). A study conducted in men with myocardial infarction and matched controls found that lycopene concentrations in adipose tissue was a decreased risk factor, with an OR of 0.52 for the contrast between the 10th and the 90th percentiles, after correcting for age, body mass, socioeconomic status, smoking hypertension, and family history (Kohlmeier et al., 1997). However, another case-control study examining serum carotenoids and risk of myocardial infarction did not find a relationship (Street et al., 1994). Three of three prospective studies found an inverse association between serum β -carotene and the risk of CVD, but only one of three prospective studies found an inverse association between β -carotene intake and CVD (smokers only) (van de Vijver et al., 1997).

Three prospective epidemiologic trials found no associations between dietary intake or plasma concentrations of total carotenoids and subsequent risk of CVD. Kushi et al. reported that carotenoid intake was not associated with risk of fatal coronary heart disease in a study of 34,486 women (Kushi et al., 1996). Similarly, Sahyoun et al. reported that dietary intake and plasma levels of total carotenoids were not associated with a reduced risk of mortality from heart disease in a study of 747 elderly men and women (Sahyoun et al., 1996). In the Multiple Risk Factor Intervention Trial (MRFIT) it was reported that there was no significant associations between total serum carotenoid concentrations and subsequent risk of coronary disease death or non-fatal myocardial infarction in smokers and non-smokers among 734 men (Evans et al., 1998).

Results among case-control and cross-sectional studies have been inconsistent. Tavani et al. (1997) reported that the risk of acute myocardial infarction was lower among women in Northern Italy with the highest intake of β -carotene. In the Seven Countries Study, it was reported that the average population intake of β -carotene was unrelated to coronary heart disease mortality rates in a group of 12,763 men followed for 25 years. In two studies that examined the relationship between adipose carotenoid concentrations and myocardial infarction in men and women with first

acute myocardial infarctions, it was found that adipose α - and β -carotene were not associated with risk of first acute myocardial infarction but that adipose lycopene concentration was (Kohlmeier et al., 1997; Tavani et al., 1997).

In the Physicians Health Study, a prospective, nested case-control analysis among male physicians without diagnosed cardiovascular disease were followed up for up to 13 years. Baseline plasma α -carotene, β -carotene, and lycopene tended to be inversely related to ischemic stroke with an apparent threshold effect (Hak et al., 2004). However, these investigators also reported no evidence for a protective effect of increased plasma carotenoids against myocardial infarction (Hak et al., 2003).

In the Nurses Health Study, a prospective study examining the risk factors to coronary artery disease, modest but significant inverse associations were observed between the highest quintiles of intake of β -carotene and α -carotene and risk of coronary artery disease but no significant relation with intakes of lutein/zeaxanthin (Osganian et al., 2003). However, Sesso et al. reported that in women, dietary lycopene was not strongly associated with risk of cardiovascular disease but there was a possible inverse association noted for higher levels of tomato-based products, particularly tomato sauce and pizza, with cardiovascular disease, suggesting that dietary lycopene or other phytochemicals consumed as oil-based tomato products confer cardiovascular benefits (Sesso et al., 2003). In this same cohort, these investigators also found higher plasma lycopene concentrations to be associated with a lower risk of cardiovascular disease in women (Sesso et al., 2004).

Plasma levels of lutein, zeaxanthin, β -cryptoxanthin, lycopene, α - and β -carotene were compared in a small case-control study of 28 subjects with an acute ischemic stroke and age- and sex-matched controls (Polidori et al., 2002). Plasma levels of lutein, lycopene, α - and β -carotene were significantly lower in patients in comparison with controls. Lower levels of lutein were found in patients with a poor early-outcome (functional decline) after ischemic stroke as compared to patients who remained functionally stable. These investigators concluded that the majority of plasma carotenoids are lowered immediately after an ischemic stroke, perhaps as a result of increased oxidative stress, as indicated by a concomitant rise in plasma malondialdehyde levels.

In the Atherosclerosis Risk in Communities Study serum concentrations of carotenoids were compared with carotid intima-media thickness (IMT) between 231 cases (IMT exceeded the 90th percentile) and 231 matched controls (IMT less than the 75th percentile) (Iribarren et al., 1997). After adjustment for the influence of covariates an inverse association with IMT was maintained only for lutein + zeaxanthin (OR per one SD increase = 0.77, 95% CI = 0.57–1.03), but it was not significant (Kritchevsky et al., 1998).

Another example of potential carotenoid involvement with coronary disease has been reported, using the progression of common carotid IMT as an end point. A cohort of 480 men and women, age 40–60, had their IMT determined over an 18 month period, and the IMT progression declined with increasing quintile of plasma lutein (Dwyer et al., 2001). Since this is an association, it means that foods rich in lutein may have an important role in preventing the increase in carotid artery thickness, a precursor of coronary heart disease.

More recently carotid IMT was examined in relation to serum lycopene concentration in middle-aged men (Rissanen et al., 2002). The men in the lowest quarter of serum lycopene concentration had a significantly higher mean carotid IMT and maximal carotid IMT than did the other men. The mean and maximal IMT increased linearly across the quarters of serum lycopene concentration. This finding suggests that the serum lycopene concentrations may play a role in the early stages of atherosclerosis.

7.2. *Intervention trials*

Two clinical studies measuring intermediate biomarkers of CVD have not demonstrated a benefit from β -carotene. Tardif et al. reported that 30,000 IU/d β -carotene in combination with 500 mg/d vitamin C and 700 IU/d vitamin E did not reduce the rate of restenosis in patients who had undergone angioplasty (Tardif et al., 1997). In another study, platelet function was not affected by ingestion of 15 mg/d β -carotene in non-smoking men and women (Calzada et al., 1997).

Two major intervention trials, one in Linxian County, China, and the α -Tocopherol, β -Carotene Study (ATBC) in Finland, showed that supplements containing β -carotene alone or in combination with vitamin E and/or selenium did not reduce the risk of CVD (Blot et al., 1993; Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994). Results of the Physicians' Health Study found that 50 mg β -carotene every other day did not reduce the risk of myocardial infarction, stroke, or cardiovascular death over 12 years, in 2000 male physicians (Hennekens et al., 1996). The results from the β -carotene and Retinol Efficiency Trial (CARET) found that β -carotene (30 mg/d) and vitamin A (25,000 IU/d) combined caused a non-significant 26% increase in mortality from CVD in a group of smokers, former smokers, and asbestos-exposed individuals (Omenn et al., 1996).

8. Carotenoids and ocular diseases

Lutein and zeaxanthin have been suggested to be protective against certain eye diseases. Given that lutein and zeaxanthin, and their metabolites, are the only carotenoids found in the retina (Snodderly, 1995; Bernstein et al., 2001) and lens (Yeum et al., 1995) and that the retina and lens suffer oxidant damage, these two antioxidant nutrients may serve a unique role in the protection against eye disease.

8.1. *Age-related macular degeneration*

Lutein and zeaxanthin are concentrated in the retina at the macula lutea and are responsible for the yellow color that gives the macula its name. Although the term macula lutea, or macula, originally applied to the yellow pigment, it is now commonly used to refer to the corresponding region of the retina. This region includes the fovea, which is the region that is responsible for our highest visual acuity and which contains the highest density of cone photoreceptors. Toward the periphery

of the retina, the concentration of zeaxanthin declines rapidly whereas the concentration of lutein declines gradually (Bone et al., 1988; Handelman et al., 1988; Bone et al., 1997). By preventing light initiated oxidative damage to the retina and retinal pigment epithelium, the macular pigment (lutein and zeaxanthin) may protect against age-related deterioration.

Lutein and zeaxanthin are thought to protect the eye in two ways. One hypothesis is that the macular pigment filters blue light, which is particularly damaging to photoreceptors and to the retinal pigment epithelium (Ham, 1983; Ham et al., 1984), and lutein and zeaxanthin absorb blue light. The second hypothesis is that these carotenoids act as antioxidants to limit the oxidant stress of the tissue that results from metabolism and light (Ham, 1983; Schalch, 1992; Khachik et al., 1997). It has been shown that one of the ways light damages the retina is by generation of free radicals that lead to peroxidation of membrane lipids (Ham, 1983; Ham et al., 1984). Carotenoids have long been known as powerful antioxidants (Zhang et al., 1991; Schalch, 1992; Krinsky, 2001).

Evidence from human studies suggest that dietary intake of carotenoids can lead to their accumulation in the retina and, therefore, may provide protection against retinal degeneration. In a recent prospective study (Hammond et al., 1997), eleven subjects modified their usual daily diets by adding 60 g/d of spinach (containing 11 mg lutein and 0.3 mg zeaxanthin) for 15 weeks. Eight subjects had increases in serum lutein and macular pigment density, two subjects showed substantial increases in serum lutein but not macular pigment, and one subject showed no changes in serum lutein or macular pigment density. Although the results were varied, augmentation of macular pigment through dietary modification appears to be possible for many people. Similar to this study, Landrum et al. have found that supplementation with lutein (30 mg/d for 140 days) resulted in increased serum levels of lutein and corresponding increases in the concentration of lutein in the macula on the human eye (Landrum et al., 1997).

Population studies provide evidence to suggest that protection from AMD can be obtained from lutein (Table 6). Investigators from the Eye Disease Case-Control study (Eye Disease Case-Control Study Group, 1993) reported that patients in the group with the highest level of plasma lutein/zeaxanthin had a decreased for

Table 6
Studies of lutein and age-related macular degeneration

Exposure measured	Association	Relative risk (95% confidence interval)	Reference
Plasma lutein/zeaxanthin	Decrease	Risk reduced to 1/3 (tertile 3 vs 1)	Eye Disease Case-Control Study Group (1993)
Tertile 3 and 2 vs 1		Risk reduced to 1/2 (tertile 2 vs 1)	
Lutein/zeaxanthin intake	Decrease	0.4 (0.2–0.7)	Seddon et al. (1994)
Quintile 5 vs 1			
Serum lutein/zeaxanthin	n.s.	–	Mares-Perlman et al. (1995a,b)

AMD. In a subsequent study (Seddon et al., 1994), the investigators found that protection from AMD was associated with dietary intake of specific carotenoids. In this case-control study, AMD patients and matched control subjects (who had other eye problems) were divided into five groups on the basis on their intake of various nutrients from foods. The nutrient class that was found to have the strongest protective effect against AMD was carotenoids. Those in the highest quintile of carotenoid intake had a 43% lower risk of developing AMD compared to those in the lowest quintile. The authors then investigated which specific carotenoids were responsible for this effect. The strongest association with protection from AMD was found for lutein/zeaxanthin. Subjects who were in the highest quintile for their intake of lutein/zeaxanthin had a 57% lower risk of advanced AMD compared to those in the lowest quintile. This odds ratio was calculated from a multivariate model, indicating that the reduction in odds associated with consumption of lutein/zeaxanthin was independent of effects from other carotenoids. A final analysis was performed by arranging the subjects on the basis of consumption of specific foods. In a multivariate model that included consumption of broccoli, cabbage-related vegetables, carrots, spinach or collard greens, sweet potatoes, and winter squash, only consumption of spinach was associated with protection from AMD. Subjects in the highest quintile for consumption of spinach had an 86% lower odds ratio of advanced AMD.

It should be noted that not all studies have found an association between serum carotenoids and protection from AMD. For example, a case-control study that used the population for the Beaver Dam Eye Study found no such association for lutein or zeaxanthin, but did find a weak protective effect of serum lycopene (Table 6) (Mares-Perlman et al., 1995a,b). However, this study included many fewer subjects than did the Eye Disease Case-Control study.

Recent studies suggest that lutein supplementation may improve visual function in AMD patients. Falsini et al., evaluated the influence of short-term antioxidant supplementation on retinal function in age-related maculopathy patients and control subjects (54–84 years) by recording focal electroretinograms (FERG) (Falsini et al., 2003). The supplementation regimen included 15 mg/d lutein for 180 days. They reported a significant increase in amplitude change of FERG in patients and controls with antioxidant supplementation. However, the supplementation regimen included vitamin E and nicotinamide. Therefore, the specific effects of any one component could not be assessed. It was concluded that increasing the level of retinal antioxidants may influence macular function early in the disease process, as well as in normal aging. In another study, it was reported that AMD patients ($n = 59$) supplemented with various antioxidants including 10 mg lutein resulted in positive effects in visual function, including improved contrast sensitivity, glare recovery, and Snellen acuity (Richer et al., 2004).

8.2. Cataracts

Lutein and zeaxanthin are the only two carotenoids that have been identified in the human crystalline lens (Yeum et al., 1995). Like the antioxidant enzymes found

within the lens, the lipid-based lutein and zeaxanthin, are primarily found in the metabolically active epithelium/outer cortex of the lens (Yeum et al., 1999), and therefore, may have a preferential role in protecting against UV-induced oxidative damage. This function would be similar to the role that lutein and zeaxanthin play in the retina, where they are optimally located to reduce risk from blue light (Snodderly, 1995).

Few studies have specifically examined the relationship between lutein and zeaxanthin with cataract risk. In a recent report, Chasan-Taber et al. (1999) observed in women that those with the highest intake of lutein and zeaxanthin had a 22% decreased risk of cataract extraction compared with those in the lowest quintile. Brown et al. (1999) also observed that there was a lower risk of cataract extraction in men with higher intakes of lutein and zeaxanthin but not other carotenoids. In this study, men in the highest fifth of lutein and zeaxanthin intake have a 19% lower risk of cataract relative to men in the lowest fifth. Mares-Perlman and coworkers observed in women a significant inverse trend across quintiles of lutein intake (Mares-Perlman et al., 1995a,b). Women in the highest quintile of lutein intake (median 0.95 mg/d) had a 27% lower prevalence of nuclear cataract than women in the lowest lutein intake quintile (median 0.28 g/d). The trend was in the same direction in men, but did not reach significance. Hankinson et al. (1992) reported that the rate of cataract surgery was associated with lower intakes of lutein rich foods such as spinach and other green vegetables. These studies suggest that dietary lutein and zeaxanthin play a role in cataract prevention.

In a double blind study involving dietary supplementation with lutein (15 mg/d, 3 times/wk for up to 2 years, $n = 5$), α -tocopherol (100 mg/d, 3 times/wk ($n = 6$) or placebo ($n = 6$) in patients with cataracts visual performance (visual acuity and glare sensitivity) improved in the lutein supplemented group only (Olmedilla et al., 2003). In a recent study, it was reported that a high dose combination of antioxidants (vitamins C and E, β -carotene, and zinc) had no significant effect in the development or progression of cataract (Age-Related Eye Disease Study Research Group, 2001a). The Linxian trial (Sperduto et al., 1993) examined the role of antioxidants in prevention of cataract, and the effect was not clear. The intervention was a combination dose of 14 vitamins and 12 minerals. Therefore, a specific role of any one nutrient could not be accurately evaluated. The multivitamin component demonstrated that nutrition can modify the risk of nuclear cataract, but specific nutrients were not evaluated. Also, the population examined had suboptimal nutritional intakes at the study start and the effect may have been due to a correction of certain nutrient deficiencies.

The Roche European–American Anticataract Trial (REACT) was carried out to examine if a mixture of oral antioxidant micronutrients (β -carotene, 18 mg/d; vitamin C, 750 mg/d; vitamin E, 600 mg/d) would modify the progression of age-related cataract (Chylack et al., 2002). This was a multi-center prospective double masked randomized placebo-controlled 3 yr trial in 445 patients with early age-related cataract. REACT demonstrated a statistically significant positive treatment effect after 2 years for US patients and for both subgroups (US, UK) after 3 years, but no effect for the UK patients alone. The conclusion from this study was that daily

supplementation with these nutrients for 3 years produced a small deceleration in progression of age-related cataract.

In recent studies of the AREDS Group (*Age-Related Eye Disease Study Research Group, 2001a; Age-Related Eye Disease Study Research Group, 2001b*), it was reported that a high dose combination of antioxidants (including β -carotene and also vitamins C and E, and zinc) had no significant effect on the development or progression of cataract but significantly reduce the risk of age-related macular degeneration (AMD). It was found that people at high risk for developing advanced stages of AMD (people with intermediate AMD or advanced AMD in one eye but not the other eye) lowered their risk by about 25% when treated with the high dose combination. In the same high-risk group the nutrients reduced the risk of vision loss caused by advanced AMD by about 19%. For those subjects who had either no AMD or early AMD, the nutrients did not provide a measured benefit. Because single nutrients were not evaluated, specific effects could not be determined. However, it should be noted that β -carotene is not found in the lens (*Yeum et al., 1995*) or macula (*Snodderly, 1995*) of the eye and, therefore, the result may be due to the other nutrients tested.

9. Other diseases

Several studies have reported reduced concentrations of micronutrients in patients with human immunodeficiency virus (HIV) infection, despite adequate nutritional intakes (*Baum et al., 1992*). A recent study in HIV-infected women reported lower serum concentrations of lycopene, α -carotene, and β -carotene, especially in those with low counts of CD4 helper cells (*Coodley et al., 1995*). In children infected with HIV-1 who had normal dietary intakes, serum concentrations of lycopene, retinol and tocopherol were reduced with the severity of depletion being correlated with the reduction in CD4 helper counts (*Periquet et al., 1995*). A reduction in serum concentrations of lycopene and other antioxidants in HIV infection suggests increased oxidative processes, however, given that lipid malabsorption is common in the progression of HIV infection, these findings may be the result of a decreased absorption of lycopene. The clinical significance of reduced serum concentrations of lycopene requires further investigation.

10. Conclusions

Epidemiologic studies support for a protective role of carotenoids in prevention of certain major diseases, including certain cancers and eye disease. However, the results from such studies have not been entirely consistent. The reason for this may be explained by differences in the population studies and differences in the study design and methods used to define both nutrient exposures and disease outcome. The hypothesis that these antioxidant nutrients may protect against the disease is a plausible one given the role of oxidative damage in the etiology of these diseases. It is not

known at what stage the protective effect may be important. The research to date has not sufficiently evaluated the effectiveness vs safety of nutrient supplements. But advocating the use of nutrient supplementation must be done with a cautionary note given that there have been trials which have suggested that supplementation with β -carotene may have an adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos. Clearly further trials are warranted to address the usefulness nutrient supplementation in disease prevention.

It is likely that cancer and eye disease develops over many years and the etiology of these diseases is due to many factors. There are likely to be differences in the potential protective effect of antioxidant supplementation depending on the stage of the disease. Future research needs to take into account the stage at which oxidative damage, and therefore antioxidant supplementation, may be important. In the meantime, a healthy diet with a variety of fresh fruit and vegetables will have many benefits, will not do any harm, and will be a good source of the antioxidant nutrients implicated (but not proven) in the etiology of disease. There is no evidence that nutrient-dense diets high in fruits and vegetables, which provide known and unknown antioxidant components, are harmful. In fact, intake of fruits and vegetables is associated with reduced risk of death due to cancer, cardiovascular disease, and all causes. Thus, recommendations such as consuming a more nutrient-dense diet, i.e. lower in sweets and fats, and increasing levels of fruit and vegetable intake do not appear to be harmful and may have other benefits despite their unproven efficacy in prevention or slowing disease. Until the efficacy and safety of taking supplements containing nutrients can be determined, current dietary recommendations of diets high in fruits and vegetables (Anonymous, 1990) are advised.

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