CHAPTER

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Carotenoids in Vegetables: Biosynthesis, Occurrence, Impacts on Human Health, and Potential for Manipulation

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1. INTRODUCTION

Mounting scientific evidence provides the association between dietary choices and chronic disease expression. Dietary guidelines, now in place, are designed to prevent the onset of such chronic diseases as tissue-specific cancers, cardiovascular diseases, and osteoporosis. The cornerstone of recommended dietary guidelines is increased consumption of fruits and vegetables. Current United States Department of Agriculture (USDA) dietary guidelines recommend eating 7–9 servings of fruits and vegetables per day. However, average adult consumption in the USA is only 4.4 servings per day, with an estimated 42% of Americans eating <2 daily servings for fruits and vegetables (see: http://www.healthierus.gov/dietaryguidelines). Consumption of vegetables provides the human diet with many essential vitamins and minerals important for health maintenance. Vegetables also contain secondary metabolite phytochemicals, which provide benefits beyond normal health maintenance and nutrition and play active roles in chronic disease reductions. One important class of phytochemicals is the carotenoids. Carotenoids are lipid-soluble pigments found in all photosynthetic organisms. Among the naturally occurring plant pigments, carotenoids are widely distributed, demonstrate a high degree of structural diversity, and possess large variations in biological functions [1,2]. There are over 600 carotenoids found in nature, with 40 dietary carotenoids regularly consumed as part of a typical human diet [3]. The many health benefits attributed to carotenoid intake include prevention of certain cancers [4–6], cardiovascular diseases [7], and aging eye diseases

[8,9], as well as enhanced immune system functions [10,11]. Pro-vitamin A activity is the classical biological function of carotenoids in mammalian systems.

Research into carotenoid enhancement of vegetable crops to benefit human health has paralleled efforts to increase consumption of fruits and vegetables in the diet. Release of carotenoid compounds from the membranes of plant tissues facilitates intestinal absorption; however, changes in carotenoid chemistry by biotic and abiotic factors can influence bioavailability. Current methods to assess bioavailability include serum measurements and *in vitro* digestion models. Programs designed to improve carotenoid levels in vegetable crop tissues must successfully link plant physiology with accurate bioavailability assessments in human subjects. The focus of this chapter will be discussions of the current knowledge of carotenoid chemistry, bioavailability assessments, the impacts of certain carotenoids on human health and disease suppression, how pre- and post-harvest cultural practices can alter carotenoid levels and influence potential bioavailability, and current vegetable carotenoid enhancement efforts.

2. STRUCTURAL CHEMISTRY AND THE PLANT CAROTENOID BIOSYNTHETIC PATHWAY

Carotenoids are C_{40} isoprenoid polyene compounds that form lipid-soluble yellow, orange, and red pigments [12,13]. They are considered secondary plant metabolites and are divided into two main structural groups: the oxygenated xanthophylls such as lutein (3R,3′R,6′R β,ε-carotene-3,3′diol), zeaxanthin (3,3′R-β,β-carotene-3,3′diol), and violaxanthin (3S,5R,6S,3′S,5′R,6′S-5,5,5′,6′-depoxy-5,6,5′,6′-tetrahydro-β, β-carotene-3,3′ diol), and the hydrocarbon carotenes such as β-carotene (β,β-carotene), α-carotene (6′R, β,ε-carotene), and lycopene (ψ,ψ-carotene) [12]. In higher plants, carotenoid compounds are synthesized and localized in cellular plastids and are associated with light-harvesting complexes in the thylakoid membranes, or present as semicrystalline structures derived from the plastids [14,15].

The carotenoid biosynthetic pathway in plants was elucidated in the mid-1960s [15]. Carotenoids are produced in the plastids and are derived via the isopentenyl diphosphate biochemical pathway. In the first step in biosynthesis, isopentenyl diphosphate is isomerized to dimethylallyl diphosphate, which becomes the substrate for the C_{20} geranylgeranyl diphosphate. The enzyme geranylgeranyl diphosphate synthase catalyzes the formation of geranylgeranyl diphosphate from isopentenyl diphosphate and dimethylallyl diphosphate [16,17]. The first step unique to carotenoid biosynthesis is the condensation of two molecules of geranylgeranyl diphosphate to form the first C_{40} carotenoid, the colorless phytoene pigment, via phytoene synthase [1]. Two structurally similar enzymes, phytoene desaturase and ξ-carotene desaturase, make the conversions of phytoene to lycopene via several important intermediates [2,16,17]. These desaturase enzymes create the chromophore present in the carotenoid pigments, and change the colorless phytoene into the pink-colored lycopene. The carotenoid pathway then branches at the cyclization reactions of lycopene to produce carotenoids with either two β-rings (e.g. β-carotene, zeaxanthin, anteraxanthin, violaxanthin, and neoxanthin) or carotenoids with one β-ring and one ε-ring (e.g. α-carotene and lutein) [16,18]. The pathway advances with the additions of oxygen moieties, which convert the hydrocarbons, α-carotene and β-carotene, into the oxygenated subgroup referred to as the xanthophylls. Further steps in xanthophyll synthesis include epoxidation reactions. The reversible epoxidation/de-epoxidation reaction converting violaxanthin back to zeaxanthin via the intermediate antherxanthin is collectively referred to as the violaxanthin cycle and is vital for energy dissipation from incoming solar radiation [15,17]

(Fig. 40.1). Currently, genes and cDNAs for the major enzymes functioning in carotenoid biosynthesis have been cloned from plant, algal, and microbial sources [16].

Within the plant thylakoid membranes of chlorophyll organelles, carotenoids are found in close association with the specific protein complexes of photosystem I and photosystem II. Carotenoids function to help harvest light energy, mostly in the blue-green wavelength range, which is transferred to the photosynthetic reaction centers. In the photosystem II complex, β-carotene is highly concentrated close to the reaction center, while lutein is present in several light-harvesting antennae components [19,20]. When the absorption of light radiation exceeds the capacity of photosynthesis, excess excitation energy can result in the formation of triplet excited chlorophyll (^{3}Chl) and reactive singlet oxygen ($^1O_2$). Carotenoid pigments protect photosynthetic structures by quenching excited ^{3}Chl to dissipate excess energy, and by binding $^1O_2$ to inhibit oxidative damage [20–23]. The antioxidant capacity attributed to the carotenoid chemical structure conveys the ability of these compounds to protect plant photosynthetic machinery in time of excess excitation, and is also responsible for the antioxidant ability of carotenoids in the prevention of chronic diseases in humans. Xanthophylls may also be involved in structural stabilization of light-harvesting complexes and reduction of lipid peroxidation [21].

The conjugated double-bond systems of the carotenoids create the light-absorbing chromophores which result in the distinctive colors associated with carotenoid plant pigments [16]. In the pigment–protein complexes of photosynthetic organisms, the all-*trans* configurations of carotenoids are the major component of the light-harvesting complexes, while the photosynthetic reaction centers contain 15-*cis* carotenoid configurations [24–26]. The all-*trans* carotenoids in the light-harvesting complexes provide efficient singlet-energy transfer to chlorophyll molecules, and thus participate mainly in light-harvesting. The 15-*cis* carotenoids show a preference for isomerization toward the all-*trans* configurations upon excitation, and thus are better suited for photoprotective functions in the reaction centers [24]. Therefore, there is a physiological basis for carotenoid isomerization in plant tissues.

3. EPOXIDATION AND ISOMERIZATION OF CAROTENOID STRUCTURES

In the human body, oxidants produced during normal metabolism and immune defense against infectious and/or chemical agents are responsible for damage to cellular tissues, DNA structures, and proteins [27,28]. This harmful oxidative damage is considered the major cause of aging and degenerative diseases such as cancer, cardiovascular disease, immune-system decline, and cataract. Compounds such as ascorbate, α-tocopherol, and carotenoids are examples of antioxidants possessing the ability to quench reactive oxygen species [28]. The physical properties of carotenoid molecules, especially the conjugated carbon–carbon double-bond system, permit the quenching of $^1O_2$. The localization of carotenoid molecules in biological tissues will also influence their ability to encounter and scavenge free radicals. *In vivo* antioxidant activity is determined by carotenoid structure and concentration, as well as the nature and concentration of the reactive oxygen species present. In fact, carotenoids are one of the most potent biological quenchers of reactive oxygen species [29].

The antioxidant activity of carotenoids comes from the susceptibility of 5,6 and 5′,6′ double bonds in their cyclic end groups to undergo epoxidation with $^1O_2$ [30,31]. Based on their chemistry, epoxide isomers would lack antioxidant activity because they are unable to bind more $^1O_2$, and some may even have pro-oxidant

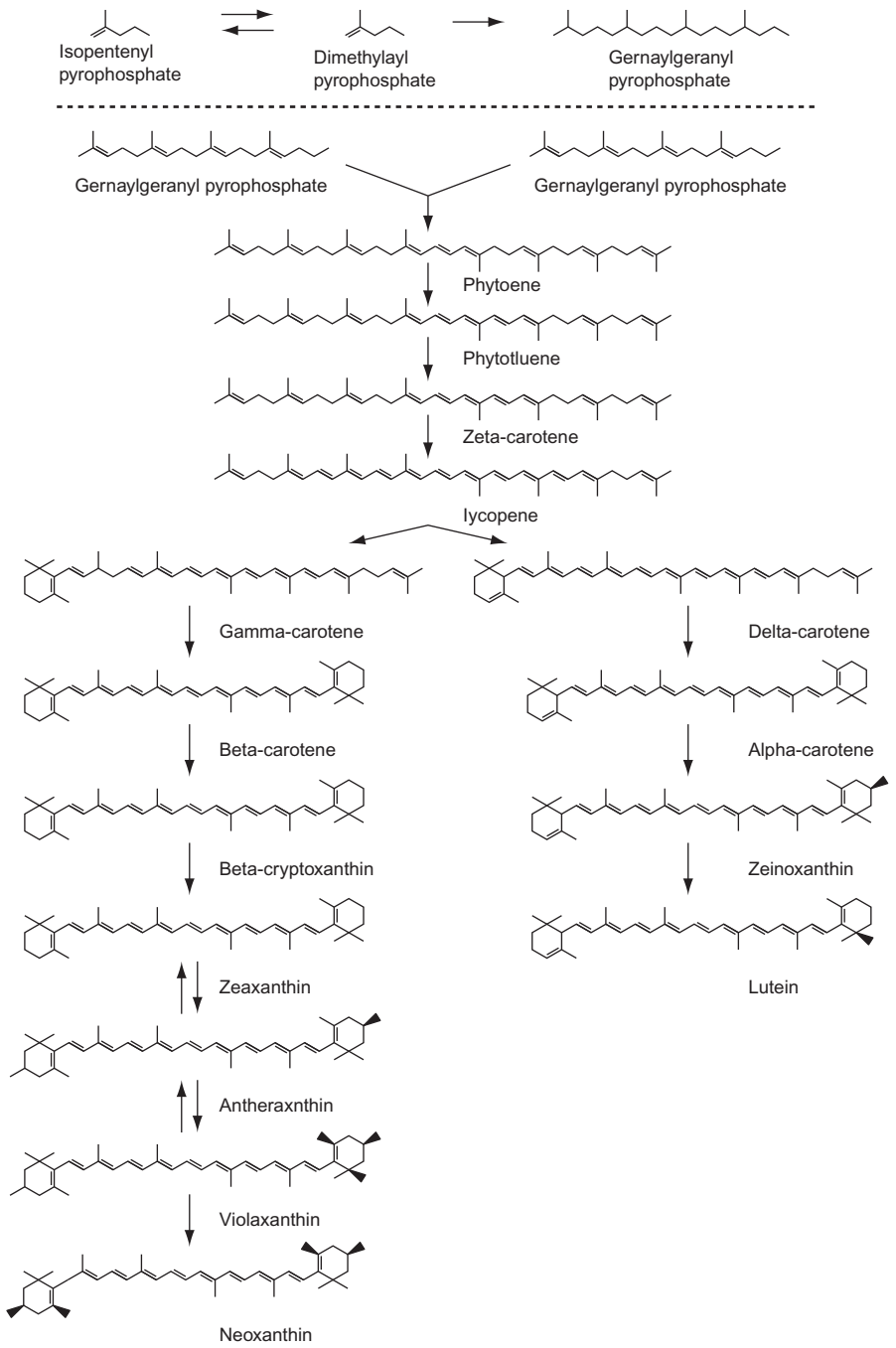

FIGURE 40.1 A simplified version of the carotenoid biosynthetic pathway in plants.

TABLE 40.1 Carotenoid Pigments Identified and Quantified in the Edible Tissues of Major Vegetable Crops

Commodity	Carotenoid Pigments Identified in Edible Tissues	Reference
Beans, green	all-*trans* β-carotene, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* lutein epoxide, 9′-*cis* neoxanthin, neolutein, all-*trans* violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, 13-*cis* zeaxanthin	[36,109]
Broccoli	all-*trans* β-carotene, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, 13-*cis* zeaxanthin	[36,109]
Cabbage	β-carotene, lutein, lutein epoxide, neoxanthin, violaxanthin, zeaxanthin	[36,48,80]
Carrot	all-*trans* α-carotene, all-*trans* β-carotene, lutein, lycopene	[47,93,96]
(Sweet) Corn	α-carotene, β-carotene, β-cryptoxanthin, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* zeaxanthin, 9-*cis* zeaxanthin	[36,56]
Kale/collards	all-*trans* β-carotene, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, 13-*cis* zeaxanthin	[36,48,49,109]
Lettuce	all-*trans* β-carotene, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, 13-*cis* zeaxanthin	[36,55,109]
Pepper	α-carotene, β-carotene, β-cryptoxanthin, capsanthin, lutein, zeaxanthin	[86]
Spinach	all-*trans* β-carotene, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, 13-*cis* zeaxanthin	[36,96,109]
Squash	α-carotene, β-carotene, β-cryptoxanthin, lutein, neurosporene, neoxanthin, phytofluene, violaxanthin	[108]
Tomato (raw)	all-*trans* β-carotene, all-*trans* γ-carotene, all-*trans* δ-carotene, ξ-carotene, all-*trans* lutein, all-*trans* lycopene, neurosporene, phytoene, phytofluene, lycopene-5,6 diol	[37,67,110]
Watermelon	β-carotene, phytofluene, all-*trans*-lycopene, *cis*-lycopene	[68]

Source: Table reprinted in part from *Trends in Plant Science*, Vol. 11/No. 10, D.A. Kopsell and D.E. Kopsell, Assessing bioavailability of carotenoids in vegetable crops, pp. 499–507, Copyright 2006, with permission from Elsevier.

activity. Epoxide forms of carotenoids, with a bound oxygen to the 5,6 or 5′,6′ position are present in the edible tissues of many different vegetable crops (Table 40.1). The ratio of lutein epoxide:all-*trans*-lutein was 1:1.5 in cabbage (*Brassica oleracea* L. var. *capitata*), 1:2 in broccoli (*B. oleracea* L. var. *botrytis*), 1:6 in spinach (*Spinacia oleracea* L.), and 1:23 in kale (*B. oleracea* L. var. Acephala) [32]. Low doses of lycopene from tomato (*Lycopersicon esculentum* Mill.) products significantly increased serum lycopene levels and reduced lipid peroxidation *in vivo* [33]. However, the complexity of measuring *in vivo* antioxidant behavior, the variability associated with carotenoid content of vegetables, and the nutritional status of subjects used in human dietary intervention studies will affect interpretation of results [14,31].

All carotenoids exhibit *cis-trans* isomerization, and both isomeric groups can be found in vegetable crops (Table 40.1) [32,34–36]. It is the presence of the conjugated double-bonds in the carotenoid structure that causes considerable isomerization to occur. The *trans* isomers are more stable and most commonly found in intact foods. Phytoene exists predominately as the 15-*cis* isomer; however, the predominate isomer of lycopene is the all-*trans* geometric form,

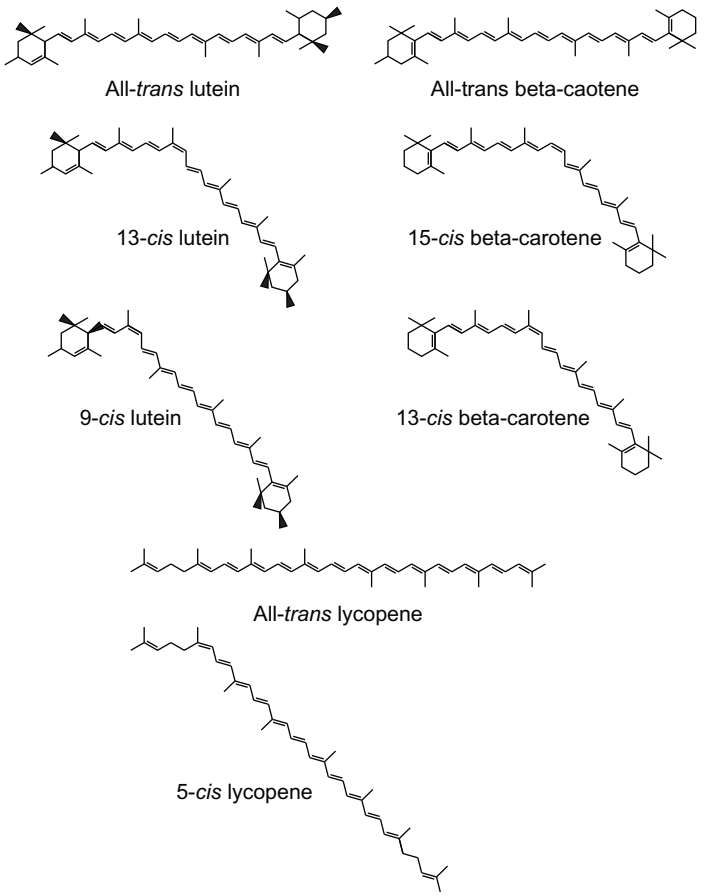

FIGURE 40.2 Structures of some common all-*trans* and *cis* carotenoid isomers found in vegetable crops.
The occurrence and properties of carotenoid isomers in food crops can influence intestinal absorption. Source: reprinted from *Trends in Plant Science*, Vol. 11/No. 10, D.A. Kopsell and D.E. Kopsell, Assessing bioavailability of carotenoids in vegetable crops, pp. 499–507, Copyright 2006, with permission from Elsevier.

indicating isomerase activity is present in higher plants to mediate *cis* to *trans* conversions [15]. All-*trans* carotenoids in plants are susceptible to photo, thermal, and chemical isomerization [24]. This can result in post-harvest cultural activities such as harvest, transport, and environmental storage conditions having a tremendous effect on the stability and preservation of carotenoid compounds in edible vegetable crop tissues.

Carotenoid *cis-trans* isomers differ in their intestinal absorption in humans (Fig. 40.2). Human blood plasma contains mostly all-*trans* carotenoids, but some plasma carotenoids can be found as high as 50% in the *cis* form [37]. As mentioned previously, the majority of lycopene found in fresh and processed tomatoes exists in the all-*trans* form [38,39]. However, lycopene in human and animal tissues exits predominantly as *cis*-isomers, indicating possible preference for *cis*-lycopene in intestinal absorption [40,41]. In contrast, greater excretions of *cis*-β-carotene and lower excretions of *trans*-β-carotene were measured in human subjects after ingestion of

both raw and processed carrots (*Daucus carota* L. var. *sativa*). Such data may indicate an absorption preference for all-*trans* β-carotene [42]. Differences in intestinal absorption among the carotenoid isomers have been established; however, very little is currently known about the biological significance of these different isomers in human health [43].

4. VEGETABLE CAROTENOIDS AND THEIR IMPACT ON HUMAN HEALTH

Epidemiological data clearly support the positive association between higher dietary intake of foods high in carotenoids and greater carotenoid tissue concentrations with lower risks of certain chronic diseases. Many of these disease suppressing abilities can be attributed to the antioxidant properties of carotenoids. One of the most important physiological functions of carotenoids in human nutrition is to act as vitamin A precursors. Pro-vitamin A carotenoid compounds support the maintenance of healthy epithelial cell differentiation, normal reproductive performance, and visual functions [44]. Both provitamin A carotenoids (β-carotene, α-carotene, and cryptoxanthins) and non-provitamin A carotenoids (lutein, zeaxanthin, lycopene) function as free radical scavengers, enhance the immune response, suppress cancer development, and protect eye tissues [45]. Humans cannot synthesize carotenoids, and therefore must rely on dietary sources to provide sufficient levels. Fruits and vegetables are primary sources of carotenoids in the human diet and their consumption has been associated with numerous health benefits [27,30]. Studies indicate that a high intake of a variety of vegetables, providing a mixture of carotenoids, was more strongly associated with reduced cancer and eye disease risk than intake of individual carotenoid supplements [9].

The colors visible in vegetable crop tissues come from the presence of anthocyanin, betalain, chlorophyll, and carotenoid pigments. Vegetable crop species differ in the composition and concentrations of these pigments present. Carotenoid pigments are responsible for the brilliant reds, yellows, and oranges that become visible as fruit tissues of vegetable crops ripen and mature. Carotenoids are still present in leafy tissues, although they are masked by the high concentrations of the green chlorophyll pigments. There is a wealth of scientific information which demonstrates that vegetable carotenoid concentrations are influenced by both genetic and environmental factors, both pre- and postharvest. In this respect, it would be misleading to list the reported carotenoid concentrations identified thus far in vegetable crops. For example, simply selecting one spinach cultivar vs. another may result in greater than a 2-fold difference in leaf tissue lutein concentrations, based solely on genetic propensity [46]. It is therefore advisable to consult current web data published by the USDA-Agricultural Research Service Nutrient Database Laboratory located in Beltsville, MD. The database is routinely updated and maintains a searchable menu of the nutritional content, including the major carotenoids, of the top foods and food products sold in the USA (see: http://www.nal.usda.gov/fnic/foodcomp/search/).

4.1 Vegetable Sources of β-*carotene and Its Impact on Human Health*

β-carotene participates as an accessory pigment in light absorption and energy dissipation in photosynthesis, as well as general antioxidant functions. Therefore, β-carotene can be found in leaf, fruit, and even root tissues of many vegetable crops (Table 40.1). The root crop carrot (*Daucus carrota* L.) has some of the highest concentrations of β-carotene. β-carotene levels in carrot can range from 3.2 to 6.1 mg/100 g fresh weight [47]. However, cruciferous leafy vegetable crops can have concentrations

equal to, or higher than carrot. Values in kale and collards are reported to range from 3.8 to 10.0 mg β-carotene/100 g fresh weight [48,49].

Vitamin A deficiency is the single most important cause of childhood blindness in developing countries around the world, and subclinical levels can contribute significantly to increased child mortality [44]. Vitamin A can be consumed in the diet as both preformed retinoids from animal tissues and as pro-vitamin A carotenoids found in plant tissues. The major sources for vitamin A for most populations worldwide are the plant-based pro-vitamin A carotenoids. The same factors that may limit carotenoid bioavailability (see Section 5) also affect vitamin A status. The bioconversion of pro-vitamin A carotenoids is mediated by a predominantly cytosolic enzyme, β-carotene 15,15′-deoxygenase, present in the intestinal mucosa, the liver, and the corpus luteum [44]. The activity of this enzyme is most efficient for β-carotene; however, other carotenoids can be metabolized to yield retinal (α-carotene and cryptoxanthins). After conversion of the pro-vitamin A carotenoids, vitamin A is transported and stored in the liver mainly as retinyl esters. Metabolism of vitamin A occurs following esterification, conjugation, oxidation, and/or isomerization reactions, after which retinal forms participate in maintenance of epithelial cell differentiation, reproductive performance, and visual functions [44].

The chemical structure of β-carotene makes it an efficient *in vitro* neutralizer of singlet oxygen ($^1O_2$), and to a lesser extent, an effective agent at reducing lipid peroxidation. Early research that demonstrated both its antioxidant and antigenotoxic properties resulted in β-carotene being one of the most extensively studied cancer chemopreventative agents in research supported by the National Cancer Institute [50]. Unexpectedly, results from three separate clinical trials revealed that β-carotene supplements, either alone or in combination with vitamin E, administered for cancer prevention actually increased incidences of lung cancers in heavy smokers and asbestos workers [51,52]. Based on the results showing pro-oxidant behavior in the presence of tobacco smoke, it appears plausible that β-carotene could be causing stimulation of pre-existing latent tumors under these conditions, rather than initiating tumorigenesis [50]. However, research showed β-carotene supplementation in animal models significantly increased phase I carcinogen enzymes in the lung, including the cytochrome P450s of CYP1A1, CYP1A2, CYP3A, CYP2B1, and CYP2A [53]. It is hypothesized that high β-carotene supplementation may increase tissue oxidative stress, or could act synergistically with known carcinogenic chemicals (present in tobacco smoke) in CYP induction. In a current review on the subject, Paolini et al. [50] clearly demonstrate that detrimental effects of individual β-carotene supplements are possible when subject individuals are exposed to environmental mutagens and carcinogens. The authors still encourage a diet high in fruit and vegetables, but warn of the possible dangers in consuming high concentrations of one or more isolated supplements.

4.2 Vegetable Sources of Lutein and Zeaxanthin and Their Impacts on Human Health

The roles that lutein and zeaxanthin carotenoids play in photosynthesis, excess light energy dissipation, and general antioxidant functions cause them to be ubiquitous in leaf and fruit tissues of vegetable crops (Table 40.1). The highest concentrations of lutein can be found in dark green leafy vegetables such as kale, collards, and spinach. Values in kale and spinach can range from 5.8 to 12.9 mg lutein/100 g fresh weight [46,48]. Significant positive correlations exist between chlorophyll and carotenoids pigments in leafy vegetable crops [48,54,55]. Thus, the darker green the leafy

tissues, the greater the concentrations of lutein that will be expected to be present. Zeaxanthin accompanies lutein in both leaf and fruit tissues; however, it is usually only found in minor concentrations in most vegetables. The exceptions are sweet corn kernel (*Zea mays* var. *rugosa* L.) and tabasco pepper (*Capsicum frutescens*), where zeaxanthin can accumulate to levels of 0.5 and 2.0 mg/100 g fresh weight, respectively [56,57].

Lutein and zeaxanthin are two of seven major carotenoids found in human blood serum; however, they are the only carotenoids present in the retina and lens of the eye [58]. In the retina, lutein and zeaxanthin are selectively deposited and are chiefly responsible for the yellow pigmentation collectively referred to as macular pigment [59]. Lutein and zeaxanthin have been found in human retinal and lens tissues, and in other areas of the eye where concentrations of long-chain polyunsaturated fatty acids and the potential for tissue oxidation are the highest. The yellow pigments are postulated to participate in photoprotection, and diminished macular pigment may be related to retinal damage [60,61]. Possible modes of action for photoprotection of the retinal carotenoids include their ability to filter harmful short-wave UV (blue) light and their function as antioxidants. Factors that increase macular pigment include increased antioxidant ingestion, high fruit and vegetable consumption, high dietary carotenoid intake and the resultant elevated serum carotenoid concentration, normal body mass index, and history of no tobacco use. Many of these same factors are also associated with a decreased risk of developing age-related macular degeneration and suggest there may be a causal relationship [62,63]. However, a direct correlation between macular pigment levels and development of macular eye diseases has not been established [60,64], although strong associative relationships are reported. Strong epidemiological associations also demonstrate that increased intakes of lutein and zeaxanthin, but not β-carotene, α-carotene, lycopene, or β-crytoxanthin, are associated with decreased risks of developing cataracts [65].

Research by our group revealed differences in serum carotenoids and responses to macular pigment optical density evaluations in human subjects administered different doses of lutein from mono-molecular supplements and whole food sources (spinach). Serum carotenoid levels increased from baseline after ingestion of 10 mg or 30 mg lutein supplements, or spinach fortified with 8 or 12 mg lutein per 100 g fresh weight. Significant increases in macular pigment optical density (indicative of photoprotection) from baseline to the end of a 12-week intervention occurred in subjects administered 30 mg lutein supplements and spinach with 12 mg lutein per 100 g fresh weight [46]. Conclusions from this study support earlier research showing the ability to modify both serum carotenoid concentrations and macular pigment through increased consumption of carotenoid-rich plant foods. Unique to the study is the demonstration that serum carotenoid concentrations and macular pigment optical density were affected by the concentrations of spinach tissue lutein. Results may imply a dose-dependent nutritional impact of carotenoid enhancement in plant tissues, and emphasize the importance of phytochemical enhancement efforts in fruit and vegetable crops.

Xanthophyll carotenoids can also possess antimutagenic and anticarcinogenic properties. Mechanisms may include selective modulation of cellular apoptosis, inhibition of angiogenesis, increased gap junction intercellular communications, induction of cellular differentiation, and the prevention of oxidative damage [65]. Lutein serum status is inversely associated with cytochrome CYP1A2 activity, a hepatic enzyme responsible for activation of several human carcinogens [66]. Although human studies regarding the impacts of dietary lutein and zeaxanthin on the risk of breast, lung, colorectal, prostate, and other cancers have been

mostly inconclusive, there have been positive results from cell bioassays and animal models that may support a protective role of the xanthophylls [65]. In many of the studies reported in the review by Ribaya-Mercado and Blumberg [65], it is clear that greater cancer protection may be afforded by consuming a variety of vegetables (supplying both carotenes and xanthophylls) as compared to only consuming foods rich in one particular carotenoid.

4.3 Vegetable Sources of Lycopene and Its Impact on Human Health

Tomatoes and tomato-based products such as juices, pastes, and sauces are frequently consumed in the diets of populations around the world. The red color of the tomato is due to the concentration of its major carotene, lycopene, which accumulates as the fruit ripens. Minor concentrations of β-carotene, and the colorless precursors phytoene and phytofluene can be found in ripe tomato fruits; however, lycopene accounts for >90% of the total carotenoids [1]. Lycopene concentrations for whole tomatoes can reach levels of 5.0 mg/100 g fresh weight; however, fruit lycopene concentrations are highly dependent on ripeness stages [67]. Processed tomato products can range from 2.2 to 10.7 mg lycopene/100 g fresh weight [67]. Another major dietary contributor of lycopene is watermelon. An evaluation of lycopene concentrations among commercial seeded and seedless watermelons reported average values to be 6.1 mg lycopene/100 g fresh weight [68]. The predominate pigment among the watermelon cultivars was all-*trans*-lycopene, with minor amounts of *cis*-lycopene, β-carotene, and phytofluene present in the fruit.

The activity of carotenoids in health maintenance and disease suppression comes from their unique chemical structures. Lycopene is a highly unsaturated straight-chain hydrocarbon which lacks any terminal β- or ε-ionic ring structures, and thus lycopene lacks pro-vitamin A activity. Similar to the other carotenoids, lycopene is subject to oxidative, thermal, and photo-degradation, causing structural isomerizations. These structural changes have significant impact on the degree of intestinal absorption, described in further detail later in this chapter. In general, the absorption of dietary lycopene in humans is between 10 and 30% [39]. It has been demonstrated that lycopene absorption from tomato-based products is greater than from fresh tomatoes, which may be attributed to the greater release of plant membrane-bound lycopene and/or higher incidences of *cis*-lycopene isomers during food processing activities. There are many different forms of lycopene present in vegetable crop tissues (Table 40.1). The presence of *cis*- and *trans*-isomers, different oxidized forms, and polar metabolites of lycopene have been isolated and identified from blood serum and tissues in animal models [69]. To date, there is very little information as to the metabolism and biological functions of lycopene *in vivo* in human systems [43]. Lycopene is believed to convey *in vivo* antioxidant properties, function in gene regulation, modulate hormonal and immune metabolism, participate in gap junction communication, and influence carcinogen metabolism, as well as affecting enzymes in the pathways associated with phase II drug metabolism [43].

The impact of lycopene on cancer prevention has clearly been established. The main body of research has focused on the influence of increased lycopene intake on the suppression of prostate cancer in adult men. Giovannucci et al. [70] first established the link between consumption of tomatoes and tomato-based products and reduced prostate cancer risks. The authors followed up that work with a comparative study of data gathered from more than 70 different epidemiological studies which established the role of dietary lycopene in the reduction of not only prostate cancer, but mammary,

cervical, ovarian, and liver cancer as well [71]. Antioxidants, such as β-carotene and lycopene, can reduce oxidative stress associated with the onset of osteoporosis. It has been demonstrated that dietary lycopene intake and serum lycopene levels may be associated with a reduced incidence of osteoporosis in postmenopausal women [72]. The study showed that lycopene intake reduced protein oxidation and specific bone resorption markers in subject participants. The antioxidant functions of lycopene can also reduce incidences of cardiovascular disease. Epidemiological studies have demonstrated that serum lycopene can reduce the oxidation of low-density lipoproteins (LDL) in subjects who consume tomato-based products or lycopene supplements [73]. The largest case study to date, conducted in Europe, showed increased adipose tissue lycopene status to decrease the risk of cardiovascular disease in a dose-dependent relationship [74]. More information on the association between dietary lycopene and other carotenoids in the reduction of cardiovascular disease prevalence can be found in a review of the subject [75]/ce:cross-ref>.

5. FACTORS THAT IMPACT CAROTENOID BIOAVAILABILITY

The bioavailability of carotenoids from plant foods is highly variable and is influenced by the crop species, the composition of carotenoid structures present in the food matrix, release of carotenoids from the food matrix, amount consumed and absorption in the intestinal tract, transportation within the lipoprotein serum fractions, the potential for *in vivo* biochemical conversions, tissue-specific depositions, as well as nutritional status of the ingesting host [12,14,76]. Carotenes are entirely lipophilic molecules located in the hydrophobic cores of plant membranes. Similarly, xanthophylls are largely hydrophobic molecules with their polar groups at opposite ends of a non-polar carbon skeleton [77]. Because of their lipophilic nature, biotic or abiotic activities that expose carotenoid molecules to potential oxidation, degradation, or isomerization will ultimately have an influence on carotenoid biochemistry and bioavailability.

Carotenoid localization in plant membranes may also influence their release from the food matrix. Results from a 12-week dietary intervention of two different spinach types, differing only in measured carotenoid concentrations, showed that lutein was more bioavailable from the spinach matrix than β-carotene, based on serum profiles in the subject participants [46]. One possible explanation for differences in bioavailability between lutein and β-carotene from the spinach matrix may come from differences in molecular orientation in plant membranes between carotenes and xanthophylls. Hydrophobic interactions and lack of polar end groups localize carotenes, such as β-carotene, within the hydrophobic core of biomembranes at several different orientations. The positions of xanthophyll carotenoids (lutein and zeaxanthin) are oriented to span the entire membrane, allowing for the positioning of polar groups outside of the hydrophobic core, or in the polar head-group region of the membrane. Differences in molecule positioning may be expected to affect release of carotene and xanthophyll carotenoids from the matrix of plant tissues, and thus affect bioavailability.

Carotenoid accumulation in vegetable crops appears to be shaped by a plant species' physiological, genetic, and biochemical attributes, as well as environmental growth factors such as light, temperature, and fertility [48,78–81]. Significant differences among vegetable crop species for carotenoid accumulations have been reported [8,82,83]. Significant genetic variation within species has been found for carrot [47], corn (*Zea mays* L.) [56], kale [48,54,78], lettuce (*Lactuca* species) [55], potato (*Solanum tuberosum* subsp. *tuberosum* L.) [84], pepper (*Capsicum* species) [85,86], and edible green

soybean (*Glycine max* L.) [87]. Genetic variation both within and among vegetable crop species is of importance since concentrations of carotenoid present in the food matrix can influence serum carotenoids levels following intestinal absorption [46].

The increased coloration in vegetable and fruit tissues associated with maturity is often indicative of increases in carotenoid concentrations [57,85,86]. Carotenoid concentrations increase in leaf tissues with maturity [49,88] and decrease during senescence [1,89]. Manipulation of cultural growing conditions and time of harvest would therefore be influential on carotenoid concentrations of fruit and vegetable crops. Environmental growing conditions will also have a large influence on the accumulation of carotenoid in plant foods. Carotenoid accumulations have been shown to increase and decrease in response to environmental manipulations, with results differing among plant species. Changes in the growing air temperature [90], irradiance level [91], irradiance photoperiod [92], and nutritional fertility [93–95] all affect plant carotenoid accumulations.

The first step in carotenoid bioavailability is release from the food matrix. Absorption of carotenoids from raw, uncooked vegetables can be extremely low, in some cases less than 1–2%. Food processing activities, such as heating or pureeing, will act to increase intestinal absorption. Thermal processing, mincing, or liquefying results in changes to carotenoid chemistry, most likely through isomerization or oxidation reactions [35,42,76,96,97]. However, frozen or low-temperature storage generally preserves carotenoid concentrations by reducing potential enzymatic oxidation [96,97]. Processing activities usually increase bioavailability through increased release of bound carotenoids from the food matrix; however, thermal breakdowns of the exposed carotenoid compounds may adversely affect bioavailability in some food crops.

Absorption of carotenoids in humans is passive and follows digestive pathways similar to lipids. Once released from plant tissues, protein- or membrane-bound carotenoids must be dissolved in a hydrophobic domain (oils, fats, or bulk lipid emulsions). Carotenoid absorption requires the presence of dietary fat in the small intestine, which stimulates the release of emulsifying bile acids by the gallbladder [12]. Further studies provide evidence of increased absorption when carotenoids are ingested with dietary lipids [98,99]. Due to their hydrophobic nature, carotenoids in the mostly aqueous environment in plant foods must be transferred to bulk lipids or intestinal micelles in the digesta [14]. After release from the food matrix, carotenoids are assimilated and oriented into lipid micelles before uptake by intestinal mucosal cells. Once in the enterocyte, carotenoids are incorporated into chylomicrons, which are eventually delivered to the bloodstream, and ultimately to the liver. The distribution of carotenoids among the various transporting lipoprotein classes is influenced by their physical structures. The hydrocarbon carotenes are transported mainly in low-density lipoproteins, while the more polar xanthophylls are evenly distributed between the low-density and high-density lipoprotein serum factions [44]. Carotenoid compounds can remain in the liver, or get transferred to low-density or high-density lipoproteins before eventual tissue-specific deposition [12].

Carotenoid bioavailability is most often assessed in blood serum after ingestion in dietary trials or cohort studies. The relatively simple analysis quantifies carotenoid changes in serum at various time intervals following ingestion of whole foods or supplements. Some caveats to interpreting serum carotenoid bioavailability include: 1) serum responses to single oral doses of carotenoids are highly variable; 2) carotenoids measured in serum signify an equilibrium between intestinal absorption, breakdowns, tissue uptake, and tissue

release; and 3) there are high concentrations of endogenous carotenoids (α-carotene, β-carotene, lycopene, lutein) already present in serum [45]. Other studies have also utilized *in vitro* Caco-2 human intestinal cell lines to assess carotenoid bioavailability [100–102]. In these studies, pure carotenoid compounds and whole food samples are brought through an *in vitro* digestion model and reacted with Caco-2 human intestinal cells. Absorption potential is measured using standard high performance liquid chromatography (HPLC) carotenoid analysis. Current results demonstrate that *in vitro* Caco-2 cells can accurately predict carotenoid bioavailability from supplements and whole foods [100–102]. Increases in serum carotenoid concentrations typically result after ingestion of carotenoids from whole food or mono-molecular supplements [33,42,45,76,98,99,102]. *In vitro* and *in vivo* studies show that carotenoid bioavailability is influenced by source (whole food vs. supplement), degree of processing, interactions with other carotenoid compounds, the degree of isomerization before, during and after absorption, transit time in the intestine, and the nutritional status of the human subjects [14].

6. ENHANCEMENT EFFORTS TO INCREASE VEGETABLE CROP CAROTENOIDS

Carotenoid enhancement efforts in plant foods have advanced through both traditional breeding and molecular approaches. Some reviews have chronicled molecular advances in carotenoid pathway manipulation to improve biosynthesis and partitioning [13,15,103]. Successful approaches have centered on modification of the biosynthetic pathway to change the flux and end products, increasing preexisting carotenoids, and engineering carotenogenic behavior in tissues completely devoid of carotenoid activity [13]. Identification and cloning of genes and cDNAs in the carotenoid pathway have facilitated genetic manipulations. Most studies have demonstrated that phytoene synthase holds greatest control over fluxes in the carotenoid pathway; however, success has also been achieved overexpressing phytoene desaturase enzymes [15]. A 2–3-fold increase in tomato fruit-specific carotenoid accumulation was achieved by utilizing a bacterial phytoene synthase (*crt*B) and a tissue-specific promoter [104]. Genetic strategies have also increased β-carotene production in canola (*B. napus* L.) using seed-specific promotion [105], xanthophyll carotenoids in potato using tuber-specific promotion and suppression of epoxidation reactions [106], and most notably, xanthophyll and carotene production in rice (*Oryza sativa* L.) endosperm ('Golden Rice') using exogenous cyclase and desaturase enzymes [107]. There are obvious advantages to genetic engineering techniques, but they must be accompanied by a clear understanding of how these manipulations will ultimately affect plant physiology. Advances in carotenoid biosynthesis have clearly been facilitated through molecular approaches, but the question still remains if the general public will accept genetic modification of whole, unprocessed foods.

Programs designed to increase carotenoid bioavailability in food crops must begin with a firm understanding of carotenoid chemistry and biosynthesis. Before selecting candidate crops for enhancement efforts, care must be taken to outline specific research impacts, whether combating widespread vitamin A deficiency or fighting aging eye diseases. Understanding how carotenoids in the food matrix respond to food processing activities and any subsequent changes to carotenoid chemistry will be of primary importance. Lastly, there is a necessity for collaborative activities to assess bioavailability in model systems or human subjects to gauge progress in enhancement efforts. Epidemiological data firmly establish the link between increased carotenoid intakes and chronic disease suppression;

however, properly designed dietary intervention studies with varying levels of vegetable carotenoid treatments are needed to clearly demonstrate the *in vivo* health attributes of higher consumption of vegetable crops high in carotenoids.

7. SUMMARY

Dietary guidelines recommend consuming 7–9 servings of fruits and vegetables daily, which has been positively associated with reduced chronic disease risk. Specifically, carotenoid compounds in fruits and vegetables provide improved health maintenance. Research demonstrates the antioxidant activity of β-carotene, lutein, zeaxanthin, and lycopene in promoting disease suppression, and their activity is affected by the amount consumed, conditions of the food matrix, intestinal absorption, and biometabolism. Genetic and environmental effects strongly influence carotenoid content in fruits and vegetables. Therefore, current carotenoid enhancement efforts and assessment of carotenoid consumption on human health need to consider many complicated and interrelated factors.

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