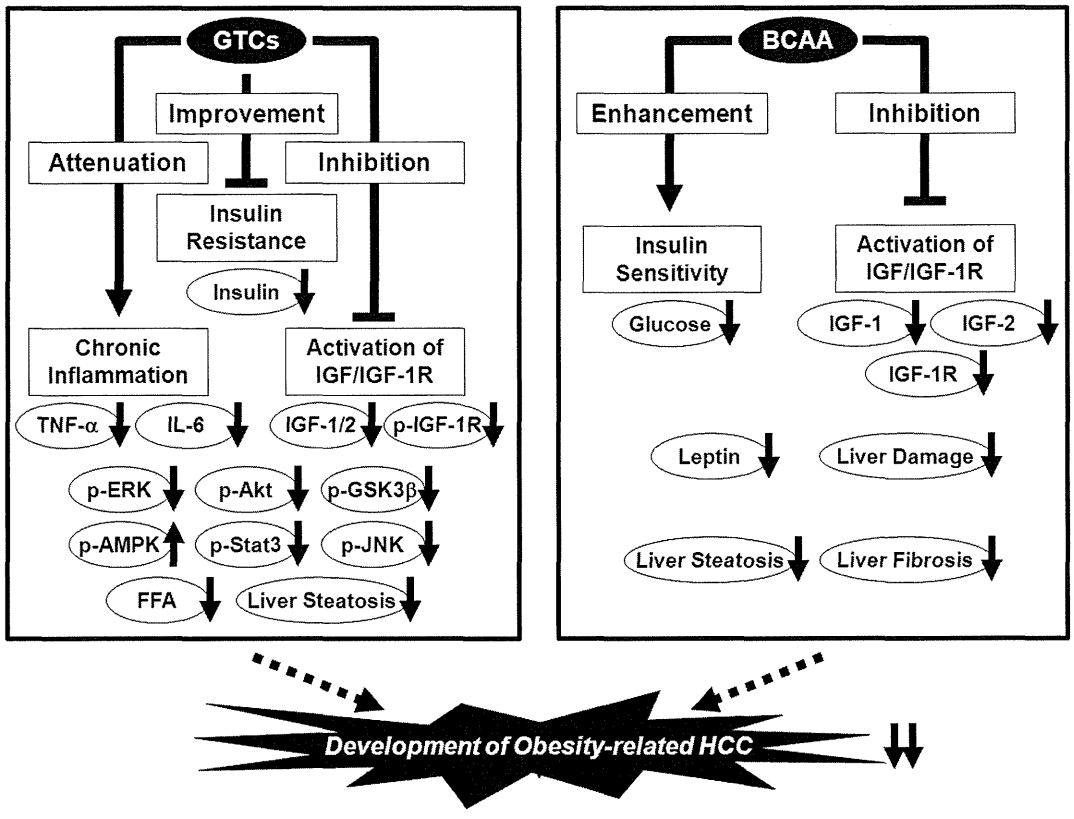
which is involved in CRC development because it mediates inflammatory signaling pathways and can therefore be an important target for chemoprevention (Figure 2) [100].

7. Prevention of Obesity-Related HCC via the Nutraceutical Approach—BCAA and GTCs Effectively Prevent Obesity-Related Liver Carcinogenesis

In addition to established risk factors such as hepatitis and alcohol consumption, obesity and its related metabolic abnormalities increase the risk of HCC [6–8,11]. NASH is also an important pathological condition when considering the prevention of obesity-related HCC because it progresses to cirrhosis and finally develops into HCC [53,54]. In order to elucidate the pathogenesis of obesity- and NASH-associated HCC and evaluate the mechanisms of how chemopreventive agents suppress these diseases, we developed a useful preclinical model using *db/db* mice and a liver carcinogen *N*-diethylnitrosamine (DEN) [101]. We found that *db/db* mice, which have severe steatosis, are more susceptible to DEN-induced liver tumorigenesis than the genetic control mice, and this oncogenic sensitivity is associated with the activation of the IGF/IGF-1R axis and induction of chronic inflammation in the liver [13,101–103].

Using this experimental model, we also investigated the possible inhibitory effects of BCAA and EGCG on obesity-related liver tumorigenesis. We found that BCAA supplementation significantly suppressed the development of hepatic preneoplastic lesions, known as foci of cellular alteration (FCA), in obese and diabetic *db/db* mice by inhibiting the expression of IGF-1, IGF-2, and IGF-1R in the liver [101]. The development of liver neoplasms, including hepatic adenoma and HCC, was also reduced by BCAA supplementation and this was associated with improvement of insulin resistance, reduction of serum levels of leptin, and attenuation of hepatic steatosis and fibrosis [101]. Yoshiji *et al.* [104] also reported that the chemopreventive effect exerted by BCAA supplementation against HCC in obese and diabetic rats was associated with the suppression of vascular endothelial growth factor expression and hepatic neovascularization. In addition, drinking water containing EGCG significantly inhibited the development of FCA and hepatic adenoma, and improved hepatic steatosis [103]. The serum levels of insulin, IGF-1, and IGF-2 and the phosphorylation of the IGF-1R, ERK, Akt, and GSK-3β proteins in the liver were reduced by EGCG consumption [103]. EGCG also decreased the levels of free fatty acids and TNF-α in the serum and the expression of TNF-α, IL-6, IL-1β, and IL-18 mRNAs in the liver, indicating that it prevents obesity-related liver tumorigenesis by inhibiting the IGF/IGF-1R axis, improving hyperinsulinemia, and attenuating chronic inflammation [103]. Thus, both BCAA and GTCs may be useful for the chemoprevention of liver carcinogenesis in obese individuals (Figure 3).

Figure 3. Mechanisms of action of GTCs and BCAA in the inhibition of obesity-related liver carcinogenesis.



8. Conclusions

In the present social and medical circumstances, the consequences of obesity and its related metabolic abnormalities, including cancer, are critical issues that need to be resolved. Among human cancers, CRC and HCC are the most representative malignancies affected by obesity. In this review, we indicate the possibility that the nutraceutical approach for targeting and restoring metabolic homeostasis may be a promising strategy to prevent the development of obesity-related CRC and HCC. Tea catechins, especially GTCs, are considered one of the most practical agents for the prevention of obesity-related carcinogenesis because the safety and efficacy of GTCs as chemopreventive agents have been demonstrated in recent interventional trials [69,71]. BCAA is also a feasible agent because its preparations are widely used in clinical practice for patients with chronic liver diseases, and a randomized controlled trial has shown that BCAA supplementation can prevent HCC in such patients who are obese [8,20]. Thus, active intervention using GTCs and BCAA might be an effective approach for the chemoprevention of obesity-related CRC and HCC.

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Conflict of Interest

The authors declare no conflict of interest.

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Review

Cancer Chemoprevention by Carotenoids

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Abstract: Carotenoids are natural fat-soluble pigments that provide bright coloration to plants and animals. Dietary intake of carotenoids is inversely associated with the risk of a variety of cancers in different tissues. Preclinical studies have shown that some carotenoids have potent antitumor effects both *in vitro* and *in vivo*, suggesting potential preventive and/or therapeutic roles for the compounds. Since chemoprevention is one of the most important strategies in the control of cancer development, molecular mechanism-based cancer chemoprevention using carotenoids seems to be an attractive approach. Various carotenoids, such as β-carotene, α-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, fucoxanthin, canthaxanthin and astaxanthin, have been proven to have anti-carcinogenic activity in several tissues, although high doses of β-carotene failed to exhibit chemopreventive activity in clinical trials. In this review, cancer prevention using carotenoids are reviewed and the possible mechanisms of action are described.

Keywords: carotenoids; xanthophylls; cancer chemoprevention; mechanisms

Abbreviations

ABCA1, ATP-binding cassette transporter 1; AFB_1, aflatoxin B_1; Akt, protein kinase B; AMD, age-related macular degeneration; AOM, azoxymethane; AP-1, activator 1; ARE, antioxidant response element; CAR, constitutive androstane receptor; Cdks, cyclin-dependent kinases; CHRP, β-cryptoxanthin- and hesperidin-rich powder; CMO-1, β-carotene 15,15'-monooxygenase; COM2, β-carotene 9',10'-monooxygenase; COX, cyclooxygenase; CUSM, citrus unshiu segment membrane; CVD, cardiovascular disease; CYP, cytochrome P450; DMH, 1,2-dimethylhydrazine; EGF, early growth response gene; ERK, extracellular signal-regulated kinase; GJIC, gap junctional intercellular communication; GSK3β, glycogen synthase kinase 3β; GSTs, glutathione *S*-transferases; HDL, high-density lipoproteins; HO-1, heme oxygenase-1; IGF, insulin growth factor; IGFBPs, IGF binding proteins; IL, interleukin; LDL, low-density lipoproteins; MJ, satsuma mandarin (*Citrus unshiu* Marc) juice; MMP, matrix metalloproteinases; NF-kB, nuclear factor kappaB; 4-NQO, 4-nitroquinoline 1-oxide; NQO1, NAD(P)H:quinone oxidoreductase; Nrf2, NF-E2-related factor 2; OH-BBN, *N*-butyl-*N*(4-hydroxybutyl)nitrosamine; PPARs, peroxisome proliferator-activated receptors; PSA, prostate-specific antigen; RAR, retinoic acid receptor; ROS, reactive oxygen species; RXR, retinoid X receptor; SXR/PXR, steroid and xenobiotic receptor/pregnane X receptor; TCF/LEF, transcription factors T cell factor/lymphoid enhancer factor; TNF, tumor necrosis factor; TRE, TPA response element; UV, ultraviolet; VDR, vitamin D3 receptor.

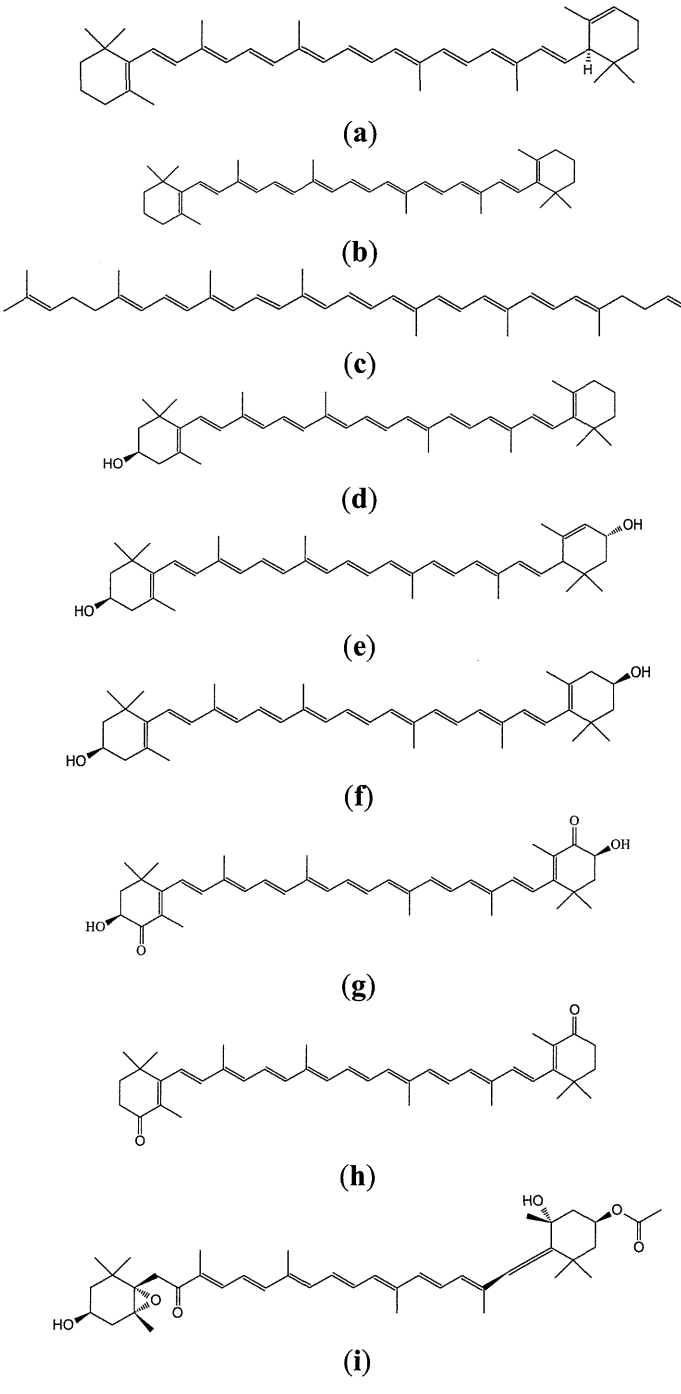
1. Introduction

To date, the cancer problem and the failure of conventional chemotherapy to achieve a reduction in the mortality rates for common epithelial malignancies such as carcinomas of the lung, colon, breast, prostate and pancreas, indicates a critical need for new approaches to control cancer development [1,2]. One of these approaches is chemoprevention, which is a pharmacological approach to intervention with the objective of arresting or reversing the process of multi-step carcinogenesis. The carcinogenic process may be driven by mutation(s), and followed by subsequent alterations in phenotypic, epigenetic and genetic events. Pharmacologic modulation of these regulatory pathways, involving the effective use of drugs, micronutrients and non-nutrients that block mutational damage of DNA, thus offers great potential for cancer prevention.

There is a clear link between dietary intake or dietary habits and cancer development in man [3–5]. Dietary risk factors have ranked higher than smoking and much higher than pollution or occupational hazards in their association with death due to cancer [6]. However, a number of compounds naturally occurring in foods, particularly antioxidative compounds in plants, have shown promise as potential chemopreventive agents [2,6–8]. These phytonutrients include the yellow, orange and red carotenoid pigments that have recently been investigated. Epidemiologically, vegetable and fruit consumption has constantly been associated with a reduced incidence of a variety of cancers [7–9], and dietary carotenoid intake from these sources has similarly been correlated with a reduced cancer risk [10–12]. However, several recent large-scale intervention trials failed to find any chemopreventive effects due to long-term supplementation with β-carotene, the most abundant dietary carotenoid [13–15]. In contrast, several naturally occurring carotenoids other than β-carotene have exhibited chemopreventive

and/or anti-cancer activities [16–19]. Foodstuffs contain various carotenoids. Vegetables contain carotenoids such as α-carotene (Figure 1a), β-carotene (Figure 1b), lycopene (Figure 1c), β-cryptoxanthin (Figure 1d), lutein (Figure 1e), zeaxanthin (Figure 1f), capsanthin and crocetin. Citrus fruits contain β-cryptoxanthin and marine carotenoids include astaxanthin (Figure 1g), β-carotene, zeaxanthin, canthaxanthin (Figure 1h), fucoxanthin (Figure 1i) and lycopene.

Figure 1. Chemical structures of (**a**) α-carotene; (**b**) β-carotene; (**c**) lycopene; (**d**) β-cryptoxanthin; (**e**) lutein; (**f**) zeaxantin; (**g**) astaxanthin; (**h**) canthaxanthin and (**i**) fucoxanthin.



In this brief review, cancer prevention by means of carotenoids (Table 1), are summarized and the possible mechanisms of action are also described.

Table 1. Sources, function, and effects of different carotenoids.

Carotenoids	Dietary Sources	Function	Effects
α-Carotene	Yellow-orange vegetables (carrots, sweet totatoes, pumpkin) and Dark-green vegetables (broccoli, green beans, spinach)	Provitamin A activity; Anti-oxidant	Immune- enhancement; Stimulate cell to cell communication; Decreases risk of some cancers
β-Carotene	Green leafy vegetables and orange and yellow fruits and vegetables (carrots, apricots, spinach, sweet potetoes, pumpkin, pepper, kale, cantaloupe)	Provitamin A activity; Antioxidant	Immune-enhancement; Decreases risk of some cancers and some cardiovascular events; high-dose supplementation may increase the risk of lung cancer among smokers
Lycopene	Tomatoes, water melon, apricot, peaches	Anti-oxidant	Decreases risk of some cancers and some cardiovascular events, diabetes, and osteoporosis
β-Cyptoxanthin	Orange fruits (mandarin orange and papaya, *etc.*), corn, peas, and egg yolks	Provitamin A activity; Anti-oxidant	Anti-inflammatory effects; Inhibits risks of some cancer and cardiovascular events; Immune enhancement
Lutein/Zeaxanthin	Dark green leafy vegetables (spinach, kale), red peppers, maize, tomatoes, corn, and egg yolks	Anti-photosensitizing agent and photosynthetic pigment; Acts as antioxidants and blue light filters	Decrease age-related macular degeneration, cataract, and risk of cardiovascular disease and certain cancers
Astaxanthin	Green algae, salmon, trout, crustacea	Antioxidant; Coloration	Prevent certain cancers, cataract, diabetes, and inflammatory neurodegenerative and cardiovascular diseases
Canthaxanthin	Salmon, crustacea	Antioxidant; Coloration	Immune enhancement; Decreases risk of some cancers
Focoxanthin	Brown algae, heterokonts	Antioxidant	Anti-cancer, anti-allergic, anti-obese, anti-inflammatory, and anti-osteoporotic activities

2. Definition of Carotenoids

Carotenoids, which belong to the chemical group known as isoprenoid polyenes, are lipid-soluble, yellow-orange-red pigments found in all higher plants and some animals. The carotenoids can be categorized as follows: (a) vitamin A precursors that do not pigment such as β-carotene; (b) pigments with partial vitamin A activity such as cryptoxanthin, β-apo-8'-carotenoic acid ethyl ester; (c) non-vitamin A precursors that do not pigment or pigment poorly such as violaxanthin and neoxanthin; and (d) non-vitamin A precursors that pigment such as lutein, zeaxanthin and

canthaxanthin. Due to the numerous conjugated double bonds and cyclic end groups, carotenoids present a variety of stereoisomers with different chemical and physical properties. The most important forms commonly found among carotenoids are geometric (*E*-/*Z*-). A double bond links the two residual parts of the molecule either in an *E*-configuration with both parts on opposite sites of the plane, or a *Z*-configuration with both parts on the same side of the plane. Geometrical isomers of this type are interconvertible in solution. This stereoisomerism exerts a marked influence on the physical properties. Isomers differ not only in their melting points, solubility and stability, but also in respect to absorption affinity, color and color intensity. Animals cannot synthesize carotenoids, so their presence in the body is due to dietary intake of foods such as pink salmon flesh. The plumage of many birds owes its color to carotenoids. Plant, algae, fungal and synthetic (nature-identical) carotenoids are permitted as colorants in food products, but not animal carotenoids.

Carotenoids owe their name to carrots (*Daucus carota*), and xanthophylls (originally phylloxanthins) are derived from the Greek words for yellow (*xanthos*) and leaf (*phyllon*). Together with anthocyanins, carotenoids are the most complex class of natural food colorants with over 750 different structures identified.

3. Absorption, Metabolism, and Bioavailability of Carotenes and Xanthophylls

Carotenoids, being mostly fat soluble, follow the same intestinal absorption path as dietary fat. Carotenoids are released from food matrices and solubilized in the gut. This is carried out in the presence of fat and conjugated bile acids. For carotenoid absorption, as little as 3~5 g of fat in a meal is sufficient [20,21]. Absorption is affected by the same factors that influence fat absorption. Thus, the absence of bile or any generalized malfunction of the lipid absorption system, such as diseases of the small intestine and pancreas, will interfere with the absorption of carotenoids. Chylomicrons are responsible for the transport of carotenoids from the intestinal mucosa to the bloodstream via the lymphatics for delivery to tissues. Carotenoids are transported in the plasma exclusively by lipoproteins. Oxygen functionalized carotenoids are more polar than carotenes. Thus, α-carotene, β-carotene and lycopene tend to predominate in low-density lipoproteins (LDL) in the circulation, whereas high-density lipoproteins (HDL) are major transporters of xanthophylls such as cryptoxanthins, lutein and zeaxanthin [22,23]. The delivery of carotenoids to extrahepatic tissues is accomplished through the interaction of lipoprotein particles with receptors and the degradation by lipoprotein lipase.

Although no less than forty carotenoids are usually ingested in the diet, only six carotenoids and their metabolites have been found in human tissues, suggesting selectivity in the intestinal absorption of carotenoids [24,25]. In contrast, thirty-four carotenoids and eight metabolites are detected in breast milk and serum of lactating mothers [26]. Recently, facilitated diffusion in addition to simple diffusion has been reported to mediate the intestinal absorption of carotenoids in mammals. The selective absorption of carotenoids may be due to uptake to the intestinal epithelia by means of facilitated diffusion and an unknown mechanism of excretion into the intestinal lumen. It is well known that β-carotene can be metabolized to vitamin A after intestinal absorption of carotenoids, but little is known about the metabolic transformation of non-provitamin A xanthophylls. The enzymatic oxidation of the secondary hydroxyl group leading to keto-carotenoids would occur as a common pathway of xanthophyll metabolism in mammals [24].

4. Distribution and Nature of Certain Carotenoids

Numerous studies have reported that carotenoids have the potential to prevent cancers, diabetes, and inflammatory and cardiovascular disease (CVD). Some of these carotenoids are listed below.

4.1. Hydrocarbone Carotenoids

Under EU legislation, plant carotenoids may be derived from edible plants, carrots, vegetable oils, grass, alfalfa and nettle. However, according to U.S. legislation carotenes may only be derived from carrots. A good source of plant carotenoids is the mesocarp of oil palm (*Elaeis guineensis*) fruits, which contains an oil rich in carotenes. After separation of the carotenes from the palm fruit oil, which is used for making detergents, the carotenes are suspended in vegetable oil at a concentration of 30%. The predominant carotenes are α- and β-carotene in the ratio 2:3. Other carotenes, including phytoene, phytofluene, ζ-carotene, γ-carotene and lycopene, which are all precursors in the biosynthesis of α- and β-carotene, are present in smaller amounts. Due to heat treatment of the oil palm fruit used in obtaining the oil, a complex mixture of geometric isomers is formed, with only 60% of α- and β-carotene as the *trans*-forms. Synthetic β-carotene is predominantly *trans*-β-carotene. The presence of β-carotene and *cis*-isomers of α- and β-carotene in palm fruit carotenes means that synthetic β-carotene is more orange than palm fruit carotenes, which is more yellow. Carotene from *B. trispora* is also mainly *trans*-β-carotene, with approximately 3% of other carotenoids. Carotene from *D. salina* also primarily consists of β-carotene with 5–6% of other carotenoids (α-carotene, lutein, zeaxanthin and β-cryptoxanthin); according to legislation, the content of *trans*isomers coming from this source should be in the range 50–71%. This means that its color shade would be between that of oil palm carotenes and synthetic β-carotene. Besides being used as colorants, carotenes are also used for nutritional purposes, such as provitamin A agents or as dietary supplements.

β-Carotene is the major source of vitamin A as a provitamin A carotenoid. Two metabolic pathways exist for its conversion to vitamin A, and they are known as the central cleavage pathway and the excentric cleavage pathway. For provitamin A carotenoids, central cleavage is the main pathway leading to the formation of vitamin A [27,28]. β-Carotene, α-carotene, and β-cryptoxanthin are cleaved symmetrically at their central double bond by β-carotene 15,15'-monooxygenase (CMO1), formerly called β-carotene 15,15'-dioxygenase. An alternative excentric cleavage pathway was also reported [29,30] and confirmed by molecular identification of an excentric cleavage enzyme, β-carotene 9',10'-monooxygenase (CMO2) in mice, humans, and zebrafish [31]. CMO2 has the ability to catalyze the asymmetric cleavage of β-carotene to produce β-apo-10'-carotenal and β-ionone [31]. Apo-β-carotenals can be precursors of vitamin A *in vitro* and *in vivo*, by further cleavage enzyme, CMO1 [32]. They can also be oxidized to their corresponding apo-β-carotenoic acids, which undergo a process similar to β-oxidation of fatty acids, to produce retinoic acid [33]. The coexistence of these two cleavage pathways reveals a greater complexity of β-carotene metabolism in organisms and raises a potential link between effects from β-carotene and/or its metabolites and anti-carcinogenesis. Common non-synonymous single-nucleotide polymorphisms (SNPs) exist in the human CMO1 gene and alter β-carotene metabolism [34,35].

4.2. Lycopene

Being a precursor in the biosynthesis of β-carotene, lycopene can be expected to be found in plants containing β-carotene, albeit usually at very low and sometimes undetectable concentrations. The best-known sources of lycopene are tomatoes, watermelon, guava and pink grapefruit. Lycopene may also be produced synthetically and by *B. trispora*. Lycopene is permitted as a food colorant in the EU and was also approved for use as a food supplement in the USA in July 2005. The only permitted source is tomatoes (*Lycopersicon esculentum*, Lycopersicon, meaning wolf peach). Besides lycopene, tomato oleoresin also contains appreciable amounts of β-carotene, phytoene and phytofluene. In solution, lycopene appears orange and not bright red as in the tomato. Lycopene is very prone to oxidative degradation, much more so than β-carotene.

Carotenoids absorb light, transfer energy to chlorophyll in the process of photosynthesis and protect against photo-oxidative damage [36,37]. In man, carotenoids function primarily as dietary sources of provitamin A. However, lycopene lacks the β-ionone ring structure required to form vitamin A and has no provitamin A activity. Therefore, lycopene has no known physiological function in man. However, some potential molecular targets in cells have been identified for lycopene. They include molecules that are involved in antioxidant activity, the antioxidant response element (ARE), apoptosis induction, cell cycle arrest, growth factors and signaling pathways, and invasion and metastasis [38–42].

4.3. Lutein and Zeaxanthin

Lutein and zeaxanthin are the two major components of the macular pigments of the retina. The macula lutea "yellow spot" in the retina is responsible for central vision and visual activity. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eye, and have dual functions in both tissues to act as powerful antioxidants and to filter high-energy blue light [43]. Lutein is found in high amounts in human serum [26]. In the diet it occurs in highest concentrations in dark green leafy vegetables (spinach, kale, collard greens and others), corn and egg yolks [44]. Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges and tangerines.

Lutein is also a very common carotenoid and one of the major xanthophylls present in green leafy vegetables. Lutein and zeaxanthin are known to selectively accumulate in the macula of the human retina. They are thought to function as antioxidants [45,46] and as blue light filters [47] to protect the eyes from oxidative stresses such as cigarette smoke and sunlight, which can lead to age-related macular degeneration (AMD) and cataracts. The name lutein is derived from the Latin word for yellow (compare xanthophyll, vide supra). The most interesting source is Aztec marigold (*Tagetes erecta*) in which lutein is primarily found esterified with saturated fatty acids (lauric, myristic, palmitic and stearic acid). Lutein made from Aztec marigold also contains some zeaxanthin (typically less than 10%). Containing only 10 conjugated double bonds, lutein is more yellowish-green than oil palm carotenes.

Zeaxanthin, the principal pigment of yellow corn, *Zeaxanthin mays* L. (from which its name is derived) is the compound that consists of 40 carbon atoms. It also occurs in egg yolks and some of the orange and yellow vegetables and fruits, such as alfalfa and marigold flowers [48]. Zeaxanthin exhibits no vitamin A activity. Zeaxanthin and its close relative lutein play a critical role in the prevention of AMD, the leading cause of blindness [49]. Zeaxanthin is isomeric with lutein; the two carotenols only

differ from each other in terms of the shift of a single double bond, so that in zeaxanthin all double bonds are conjugated. Zeaxanthin is used as a feed additive and colorant in the food industry for birds, swine and fish [50]. The pigment imparts a yellow coloration to the skin of birds and their egg yolk, whereas in pigs and fish it is used for skin pigmentation [51].

4.4. β-Cryptoxanthin

β-Cryptoxanthin is found in human blood together with α-carotene, β-carotene, lycopene, lutein and zeaxanthin. Unlike other abundant carotenoids, β-cryptoxanthin is not found in most fruits or vegetables but only in specific ones, namely hot pepper, persimmon and Satsuma mandarin (*Citrus unshiu* Marc.) [52]. Satsuma mandarin, also known as table orange or Satsuma in Western countries, is one of the most popular citrus fruits in Japan. It is sweet, tasty and rich in vitamin C. It is notable that Satsuma mandarin is one of the most common β-cryptoxanthin rich fruits in the world. The edible part of the Satsuma mandarin contains about 1.8 mg/100 g of β-cryptoxanthin, while the β-cryptoxanthin content is 0.2 mg/100 g in Valencia orange and almost nothing in grapefruits. As β-cryptoxanthin is rarely found in most fruits or vegetables, the serum β-cryptoxanthin concentration in the Japanese population is almost parallel to their consumption of the Satsuma mandarin, and is higher than in western populations [53]. Although the nutritional functions and metabolism of abundant carotenoids, for example β-carotene and lycopene, have been well studied [54,55], those of β-cryptoxanthin have not been examined in detail. Recent reports strongly suggest a significant negative correlation between serum β-cryptoxanthin concentrations and disease morbidity such as liver disorders [56,57], cancer [58,59] and mutagenesis [60], and post-menopausal osteoporosis [61–63]. β-Cryptoxanthin intake is beneficial for human health. The anti-obesity effects of β-cryptoxanthin have recently been reported [64,65]. The major xanthophyll, β-cryptoxanthin, was also reported to decrease the gene expression of interleukin (IL)-1α in mouse macrophage RAW264 cells [66], to promote osteoblastic differentiation of mouse MC3T3 cells [67] and to prevent a decrease of calcium content in the bone of ovariectomized rats [63].

4.5. Astaxanthin

Astaxanthin contains two keto groups on each ring structure as compared with other carotenoids, resulting in enhanced antioxidant properties. This compound occurs naturally in a wide variety of living organisms including microalgae (*Haematococcus pluvialis*, *Chlorella zofingiensis* and *Chlorococcum* sp.), fungi (*Phaffia rhodozyma*, red yeast), complex plants, seafood and some birds such as flamingos and quail; it has a reddish color and gives salmon, shrimp and lobster their distinctive coloration [68]. The microalga *Haematococcus pluvialis* has the highest capacity to accumulate astaxanthin at up to 4–5% of cell dry weight. Astaxanthin has been attributed with the extraordinary potential of protecting the organism against a wide range of diseases. It also has considerable potential and promising applications in the prevention and treatment of various diseases such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, CVD, gastrointestinal and liver diseases, and neurodegenerative diseases [69]. Astaxanthin cannot be manufactured in animals or converted to vitamin A, and therefore must be consumed in the diet. Astaxanthin and canthaxanthin have antioxidant activity, are free radical scavengers, potent quenchers of reactive oxygen species

(ROS) and nitrogen oxygen species, and chain-breaking antioxidants. They are superior antioxidants and scavengers of free radicals as compared with the carotenoids such as β-carotene [70]. Astaxanthin is even called superantioxidant.

4.6. Canthaxanthin

Canthaxanthin was first isolated from the edible mushroom, *Cantharellus cinnabarinus*. In addition, canthaxanthin is said to be produced at the end of the growth phase in several green algae, and also in blue-green algae, as secondary carotenoids instead of, or in addition to, primary carotenoids. It has also been found in bacteria, crustacea and various species of fish including carp (*Cyprinus carpio*), golden mullet (*Mugil auratus*), annular seabream (*Diplodus annularis*) and trush wrasse (*Crenilabrus tinca*). Canthaxanthin is not encountered in wild Atlantic salmon, but represents a minor carotenoid in wild Pacific salmon. It has also been reported in wild trout (*Salmo trutta*). Canthaxanthin is used widely as a drug or as a food and cosmetic colorant (skin tanning), but it may have some undesirable effects on human health. These are mainly caused by the formation of crystals in the *macula lutea* membranes of the retina. This condition is called canthaxanthin retinopathy [71]. It has been shown that this type of dysfunction of the eye is strongly connected with damage to the blood vessels around the locations of crystal deposition.

Canthaxanthin is one of the carotenoids without provitamin A activity, but may have anti-carcinogenic, immune-enhancing, antioxidative activities. The mechanisms by which canthaxanthin may exert anti-tumor activity are associated with its antioxidant properties through radical trapping or chain-breaking processes [72,73], or its enhancement of gap-junction cell to cell communication through upregulation of the gap-junction protein, connexin [74].

4.7. Fucoxanthin

The allenic carotenoid fucoxanthin is one of the most abundant carotenoids, and contributes to nature more than 10% of the estimated total production of carotenoids in nature, especially in the marine environment [75]. Fucoxanthin is a naturally occurring brown- or orange-colored pigment that belongs to the class of non-provitamin A carotenoids. Fucoxanthin acts as an antioxidant under anoxic conditions. The typical antioxidants are usually proton donors (ascorbic acid, α-tocopherol and glutathione). Fucoxanthin, on the other hand, donates an electron as a part of its free-radical quenching function. A combination of these distinct properties is very rarely found among naturally occurring compounds [76,77]. During normal metabolism the body produces heat. Fucoxanthin increases the amount of energy released as heat in fat tissue, a process known as thermogenesis. In a published study it has been reported that fucoxanthin affects multiple enzymes involved in fat metabolism causing an increase in the production of energy from fat [78].

Fucoxanthin is present in *Chromophyta* (*Heterokontophyta* or *Ochrophyta*), including brown seaweeds (*Phaeophyceae*) and diatoms (*Bacillariophyta*) [79]. Based on its unique molecular structure, fucoxanthin has remarkable biological properties similar to neoxanthin, dinoxanthin and peridinin, which make it different to other carotenoids. Fucoxanthin does not exhibit toxicity and mutagenicity under experimental conditions [79–81]. Fucoxanthin may have the ability to increase circulating cholesterol levels in rodents as a common feature [79].

5. Clinical Trials with Long-Term β-Carotene Supplementation

Epidemiologic studies have shown an inverse relationship between the presence of various cancers and dietary or blood carotenoid levels [82]. However, three [13–15] out of four intervention trials [13–15,83] using high-doses of β-carotene supplements did not show protective effects against cancer or CVD. Rather, the high-dose intervention trials showed an increase in cancer and angina pectoris [13–15,83]. Therefore, carotenoids may promote health when taken at dietary levels, but may have adverse effects when taken high doses by subjects who smoke or who have been exposed to asbestos.

The epidemiologic observations of the possible protective effects of high dietary (not supplemental) β-carotene intakes against cancer, along with what is known about carotenoid biochemical functions, has led to further study of the effect of β-carotene on cancer risk. Long-term large randomized intervention trials were designed to test the efficacy of high doses of β-carotene (20–30 mg/day) in the prevention of cancer (Table 2). As stated above, the results from two trials provided possible evidence of harm from β-carotene supplements in relation to cancer among high-risk individuals such as smokers and asbestos workers [15], but no effect (either beneficial or detrimental) in a generally well-nourished population [84]. Moreover, the Linxian (Chinese) Cancer Prevention Study [83] found that supplementation with β-carotene, vitamin E and selenium led to a significant reduction in total mortality (9%), especially from cancer (13%) and stomach cancer in particular (21%) (Table 2). The positive results of the Chinese study probably reflect the correction of a vitamin A deficiency in the study population. A number of mechanisms have been proposed to account for the association between β-carotene supplementation and lung cancer in smokers and asbestos workers, including an imbalance of other carotenoids or antioxidants, a pro-oxidant activity of β-carotene at the high oxygen tensions found in the lungs, induction of P450 enzymes and the production of damaging β-carotene oxidation products by components of cigarette smoke [85]. The Women's Health Study [86] indicated no statistically significant differences in incidence of cancer, CVD, or total mortality, although the treatment duration is short (a median treatment duration of 2.1 years and a median total follow-up of 4.1 years).

Table 2. β-Carotene supplementation trials.

Studies	Study Designs				Ref.
	Population	Intervention	Duration	Cancer outcome	No.
ATBC	29,133 Finish male smokers (50–69 years of age)	β-carotene, 20 mg/day; vitamin E, 50 mg/day	5–8 years	18% increase in lung cancer; 8% increase in mortality	13
CARET	18,314 men and women and asbestoss workers (45–74 years of age)	β-carotene, 30 mg/day; vitamin A, 25,000 IU	<4 years	28% increase in lung cancer; 17% increase in deaths	15
PHS	22,071 male physicians (40–84 years of age)	β-carotene, 50 mg on alternate days	12 years	No effect of supplementation in incidence of cancer	14