Beneficial or Detrimental Effects of Carotenoids Contained in Food: Cell Culture Models

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Abstract: Epidemiological studies have suggested a correlation between consumption of carotenoid-rich food and incidence of chronic diseases. In this review chemical structure, bioavailability and mechanisms of action of carotenoids most represented in human diet, mainly ß-carotene and lycopene, are reported, with focus on results obtained with cells in culture.

Key Words: Carotenoids, *in vitro* cell models, antioxidant, connexins, cancer.

INTRODUCTION

Carotenoids are natural coloured pigments, usually red, orange and yellow, isolated and characterized by chemists since the early 1900s [1].

They are widespread in nature, from microorganisms to plants and vertebrates, and have numerous important biological functions. Carotenoids are responsible for the fascinating colours of flowers, fruits, roots, birds plumage or crustacean shell that are essential for the attraction of pollinator insects or, in the case of animals, of sexual partners. In plants they exert many essential functions, as light harvesting during photosynthesis, protection from the photo-oxidation damage and synthesis of the plant hormone abscisic acid; in animals they act as anti-oxidants, precursors of vitamin A and, as evidentiated by recent research, in modulating molecules involved in cell proliferation [2].

All the described functions are related to the physical and chemical properties of carotenoids, determined by their molecular structure.

STRUCTURE

All carotenoids share essential chemical features: i) the basic polyisoprenoid structure, which contains 40 carbon atoms and a long conjugated chain of double bonds in the central part of the molecule, and ii) a good symmetry around the central double bond. The carotenoid structure is derived from the tail-to-tail linkage of two C_{20} geranylgeranyl diphosphate molecules, that are synthesized from the condensation of eight isoprene units (Fig. (**1**)).

Different carotenoid structures descend from the described basic frame by reactions of cyclization at one (as γ-carotene) or both (as β-carotene: ß-C) ends of the molecule, by reduction of certain double bonds or by addition of oxigen-containing functional groups. In general, the category can be divided into *carotenes*, hydrocarbon carotenoids with unsubstituted rings, and *xantophylls*, carotenoids with at least one oxygen atom in their structure [3].

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In (Fig. (**2**)) is shown the structure of some representative carotenoids.

It is the long conjugated double bond system that confers to carotenoids their peculiar protective properties, since they are able to accept excitation energy derived from other molecules in close proximity (as excited chlorophyll or free radicals produced by metabolic or pathological processes) and quench the activity of these reactive species by dissipating the excitation energy in a harmless form, mainly by delocalizing electrons over the entire chain.

Since carotenoids absorb in the blue region of the spectrum (from 400 to 600nm, responsible of their typical yellow to red coloration), they are able to harvest light at a wavelength not covered by chlorophylls, therefore in plants they function as accessory photosynthesis pigments as well as protectors from photo-oxidative damage.

The overall chemical structure and the size of the different carotenoids influence their position in the cell structures and their interaction with other molecules and therefore their function. Most carotenoids are highly lipophylic molecules, usually localized in specialized cell organelles, such as chloroplasts and chromoplasts, dispersed in the aqueous cytoplasm as microcrystalline aggregates (as in tomatoes, carrots, oranges), or as lipid-protein complexes, or embedded in the cellular membranes. The molecular structure of carotenoids determines their location within the cell membrane, for instance ß-C and lycopene (Lyc) are immersed in the inner part of the membrane parallel to the surface and present a certain degree of mobility, while lutein and zeaxanthin, with hydroxyl groups that interact with phospholipids, are held in a spanning position across the membrane [4-6]. Thus carotenoids are able to influence thickness, fluidity and function of membranes.

Particularly important is the interaction of carotenoids with different proteins that exert a protective role against the rapid oxidation to which the carotenoids are naturally susceptible and help the carotenoid molecule itself to maintain the correct position and orientation in the membranes and respect to other molecules, factors of great importance for

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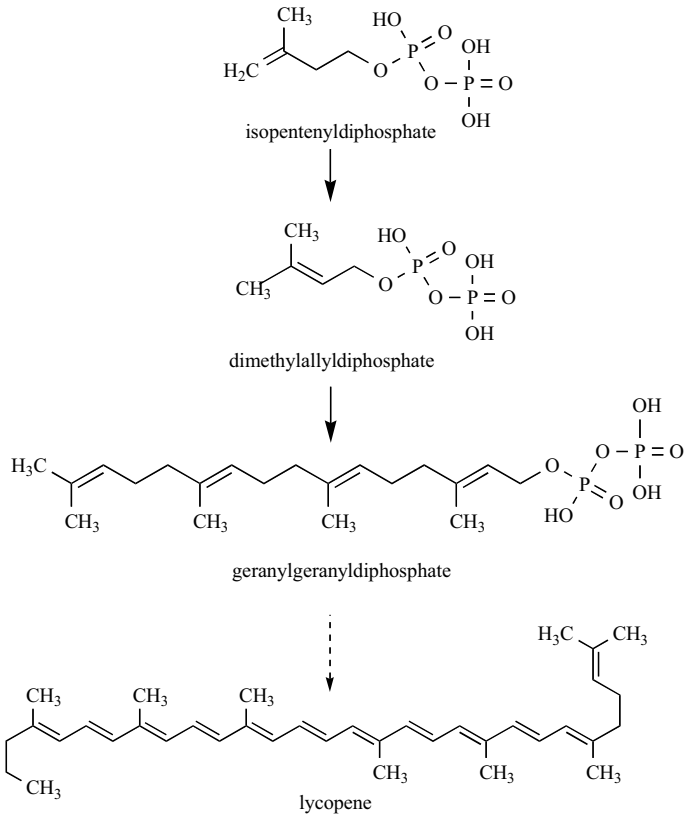

Fig. (1). Key steps in lycopene synthesis.

their function. Moreover, proteins can function as carriers in aqueous environments as cytoplasm and plasma [7].

FOOD CONTENT AND AVAILABILITY

More than 600 carotenoids have been identified in nature [8], indeed this number would highly increase considering all the possible geometric *cis/trans* conformations generated by isomerization around the double bonds of the polyene chain. However, only a reduced number of isomers are possible due to the steric hindrance and the energy state. In conclusion only a small portion of this crowded family, about 60 molecules, is present in vertebrate diet and has a biological activity, in particular the highly stable *trans* isomers are more common [9], even if they are generally more prone to aggregate respect to *cis* isomers and are less readily solubilized, absorbed and transported.

A great number of molecules generated by chemical modification of carotenoids have been identified in biological tissues: carotenoids may undergo isomerization, enzimatically cleavage to active molecules or oxidation by reaction with active oxygen [10-12]; these molecules, and their metabolites, have been shown to possess specific activity in crucial cellular processes. Work is in progress to identify and understand these specific functions.

The most abundant carotenoids in human body are ß-C, Lyc, α-carotene, lutein, cryptoxanthin and zeaxanthin, all assumed with the diet mainly from plant sources, in particular: leafy vegetables, apricots and carrots for ß-C, tomatoes for Lyc, broccoli, spinach and peas for lutein, sweet red peppers, oranges and papaya for cryptoxanthin [13].

ß-C is the most important vitamin A precursor in human body, while Lyc is involved in the regulation of some cell types proliferation, and both of them are essential in human body for their anti-oxidant activity; zeaxanthin and lutein protect the eye from blue light damage. Therefore, an unbalanced supply of these molecules, either in defect or in excess, can cause great damage to the organism.

To date, different epidemiological studies have established an inverse correlation between vegetable/fruit-rich diet and the incidence of several pathologies as cardiovascular [14], degenerative and ophthalmologic [15] diseases, metabolic disorders and some types of cancer [16, 17]. These protective effects of fruit and vegetables may be, at least in part, ascribed to their content in carotenoids. However, high doses of supplemented carotenoids could provoke unwanted effects, as the case of ß-C that has been shown to increase lung cancer incidence in smokers and asbestos workers [18].

The correlation between the amount and the variety of carotenoids assumed with the diet and their beneficial effects is related to their intestinal bioavailability that is dependent on several factors [19]. The main determinants in these process heve been summarized with the acronym **SLAMENGHI**: **S**pecies of carotenoid, **L**inkages at molecular level, **A**mount of carotenoid, **M**atrix, **E**ffectors, **N**utrient status, **G**enetics, **H**ost-related factors and **I**nteraction among these factors [20]. This scheme shows how complex is the way from carotenoids ingestion to their utilization in the organism, and also how difficult is the evaluation of the optimal uptake of such bioactive molecules in single inviduals with determined genetic features.

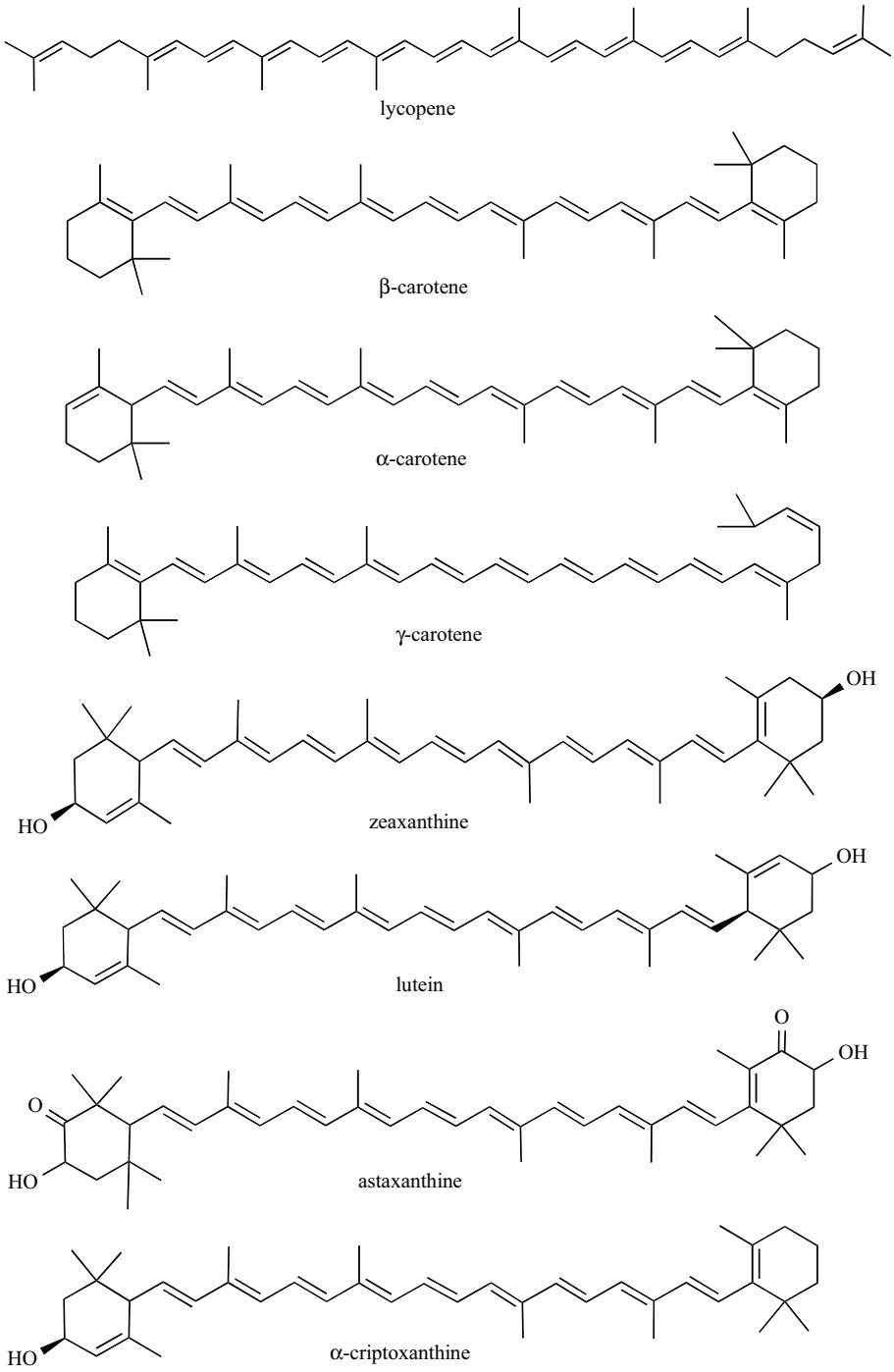

Fig. (2). Structure of some common carotenoids.

The release of carotenoids from the food matrix and their solubilization in micelles through the digestive process can be defined as *bioaccessibility*, and it is the first step toward the final *bioavailability* of these molecules and hence their action in the organism.

Bioaccessibility of carotenoids from food matrix is mainly determined by the structure of the single carotenoid, its localization in the cell as well as the nature of food matrix itself [21]. Food processing (mechanical disruption, heating, etc) also greatly influences the carotenoid availability, since the disruption of food matrix, in particular in the presence of fat, promotes the micellarization of hydrophobic carotenoids and improve their uptake from intestinal cells [22]. A clear example is given by Lyc release from tomatoes, that results much more efficient after cooking than from raw fruit [23, 24].

In vitro digestion experiments that mimic the physiological digestive process by treating food samples with pepsin, pancreatin and bile salts [25] or characterize micelles formation during the initial phase of digestion in the upper gastro-

intestinal tract [26] have been widely used to assess carotenoid bioaccessibility. Recently this technique has been improved by including reactions that resemble the colonic fermentation [27].

The following step in bioavailability studies is to understand how efficiently the carotenoids released from food are absorbed, metabolized and transported to the site of action.

Essential is the use of appropriate models. For instance, some of the most widely used animal models, such as rat or mouse, have been demonstrated to metabolize carotenoids very differently from humans. Ferrets or gerbils have been used recently, since they are more similar to human in carotenoids metabolism [28].

Many studies on carotenoids absorption in humans have been carried out by measuring their rise in plasma following acute and chronic administration and the intake-excretion balance following oral loading; this approach only provide indirect measures of carotenoids absorption but does not offer the possibility of quantifying and determining the characteristics of the differential steps [29, 30].

In parallel, many interesting cell culture models that are fundamental for a molecular approach and are complementary to *in vivo* human studies have been developed to study carotenoid metabolism: human intestinal cell lines (for instance Caco-2 and HT-29 cells) to study absorption/transport mechanisms, or immortalized Met Murine Hepatocytes - MMH - to study nutrients storage and delivery [31, 32].

In sum, carotenoid metabolism is a species-specific complex process that depends on several factors, and can only be drawn by combining the results obtained with different experimental procedures and models.

Finally, an important experimental aspect in these studies concerns the methods that allow the isolation and the analysis of different carotenoid species. This has been possible thank to the development of analytical techniques, mainly HPLC chromatography, in particular reversed-phase HPLC, that represents a powerful tool to characterize carotenoids in biological samples; recently these techniques have been further enhanced by the introduction of NMR and mass spectometry that can be coupled to HPLC separation to obtain a very sophisticated method of analysis [33].

ABSORPTION AND TRANSPORT

In the last decade new insights about the mechanisms underlying intestinal transport of carotenoids have been provided by the use of human intestinal cell lines. Several attempts to obtain differentiated intestinal cells from normal tissues have been not successful. However, established differentiated intestinal lines have been developed from human colon adenocarcinoma [34].

The majority of *in vitro* transport studies reported in the literature have been performed with the Caco-2 cells; despite their tumor origin, these cells show *in vitro* most of the characteristics of the small intestine enterocytes [35] and have been extensively used to study intestinal transport of nutrients, drugs and xenobiotics [36, 37, 38]. The differentiative process of Caco-2 cells is a spontaneous event dependent on the time that cells are maintained in culture; after 15-20 days at confluency cells give rise to a monolayer of polarized epithelial cells joined by a functional tight junction system and display several biochemical and functional characteristics typical of the mature absorptive enterocyte. For transport experiments cells are plated in special culture systems that represent the intestinal mucosa environment in which the lumen is separated from the bloodstream by the intestinal epithelial monolayer; in these systems cells are plated on porous membranes and trans-epithelial passage of molecules from the apical to the basolateral side of the monolayer can be easily measured in different experimental conditions, thus allowing to discriminate factors involved in transport mechanisms (Fig. (**3**)).

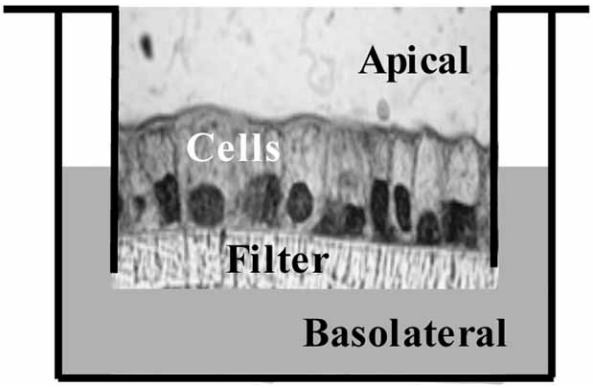

Fig. (3). Schematic representation of Caco-2 grown on filter.

Although these *in vitro* models represent a simplification of the intestinal environment, they provide a very useful tool to evaluate specific mechanisms of trans-epithelial transport; infact, even if many factors that contribute to the overall absorptive process like the presence of mucous layer, intestinal peristalsis and lamina propria are absent in the *in vitro* model, Caco-2 cells reproduce the selective vectorial transport of nutrients accomplished by the enterocytes .

Carotenoid transport has been studied mainly on two different Caco-2 clones: the parental ATCC Caco-2 clone, and the TC7 subclone [39]; when cultured on filters and supplemented with oleic acid and taurocholate for 16 hours these cells are able to assembly and secrete chylomicron (CM) in the basolateral medium [40]; comparison of the amount of CM secreted by the two cell lines has shown that, despite the fact that TC7 subclone is able to synthesize triglycerides to a larger extent, the parental Caco-2 line is more efficient in CM secretion. CM secreted by Caco-2 cells display a composition similar to the *in vivo* secreted CM, containing apolipoprotein B and being rich in newly synthesized triglycerides and phospholipids [41].

A second important feature related to carotenoids metabolism that differs in the two intestinal lines, is the expression of ß-C 15,15'-dioxygenase, the cytosolic enzyme which cleaves ß-C into retinal, the direct precursor of retinol and retinoic acid. This enzyme is expressed in the human small intestine *in vivo* and in the TC7 subclone, but not in the parental Caco-2 cells [42].

The characteristics described above make the two intestinal lines suitable models for different purposes regarding intestinal transport of carotenoids; while the parental Caco-2

line represents a better model to study carotenoids trans-epithelial absorption because of its efficient CM production, the TC7 subclone is suitable for studying the intestinal metabolism of carotenoids due to the expression of the 15,15'-dioxygenase enzyme.

Several important aspects of intestinal carotenoid absorption have been characterized with the help of these *in vitro* models and the most relevant findings in this field will be summarized in the present review. However it has to be mentioned that a comprehensive description of the data reported is complicated by the employment of different experimental conditions by the various authors [43].

An important step that regulates intestinal absorption of lipophylic molecules is their assembly in lipidic micelles and several evidences indicate that the composition of the micelles may influence the extent of uptake of lipidic compounds [44].

This aspect of carotenoids transport has been evaluated on Caco-2 cells [45]; cells were incubated for 2 hours with several carotenoids solubilized in mixed micelles containing bile salts, fatty acid, monoacylglycerol, cholesterol and increasing amounts of phosphatidylcholine (PC) or Lyso-phosphatidylcholine (Lyso-PC); in these experimental conditions, uptake was increased by the presence of Lyso-PC in micelles, while was inhibited by the presence of PC, moreover, phospholipaseA2 hydrolysis of PC to lyso-PC significantly enhanced carotenoids uptake. These results confirmed the effects of micellar composition on carotenoids absorption and indicated that the presence of Lyso-PC probably facilitates carotenoids passage from the micelles to the intestinal cells. More recently it has been shown that it is the length of the acyl residues contained in PC or Lyso-PC molecules, that plays a crucial role on the extent of ß-C uptake [46]. Mixed micelles containing no phospholipids and PC or Lyso-PC with short-, medium- and long fatty acids chains were employed to study ß-C uptake. These experiments showed that PC containing medium-chain acyl moieties and Lyso-PC containing long chain acyl groups displayed similar ability of enhancing ß-C uptake, suggesting that those phospholipids with comparable amphiphilic properties, may facilitate carotene permeation, probably by altering cell membrane permeabilities.

Transport experiments performed on both the parental Caco-2 line and the TC7 subclone strongly suggest that intestinal carotenoids absorption occurs at least in part by specific transport systems.

Garrett and coworkers [47] have studied ß-C uptake in differentiated Caco-2 cells grown on plastic. When added to the cells in mixed micelles for 20 hours, ß-C showed a saturable kinetic of uptake. In these conditions 9.6% of the ß-C given to the cells was absorbed, transport reaching a plateau at 18 μM concentration; lutein transport in the same experimental condition was lower (6,3% of the initial concentration) compared to ß-C.

Similar characteristics of ß-C transport performed on Caco-2 cells grown on filters have been reported by other authors [41]; in this study ß-C and other carotenoids solubilized in Tween-40 micelles were added for 16 hours to Caco-2 cells, induced to synthesize CM. In these conditions 11% of ß-C was transported to the basolateral side and transport showed a saturable kinetic that reached a plateau at 10 μM; most of the ß-C transported was found to be associated to the CM fraction. These authors also demonstrated stereospecificity of ß-C transport showing that the *all-trans* isomer was transported at a greater extent as compared to 9- and 13-*cis*-ß-C. Differential absorption of carotenoids was reported with the following ranking: *trans* ß-C (11%) > α-carotene (10%) > lutein (7%) > Lyc (2,5%). Interestingly, despite these differences in transcellular net transport, intracellular uptake did not vary at the same extent (15-18%), indicating that incorporation into CM could be the crucial step that determines the degree of net absorption of these carotenoids.

Lutein trans-epithelial passage in TC7 cells showed several characteristics typical of a mediated transport mechanism: transport was highly polarized, temperature dependent, saturable and significantly decreased after trypsin treatment, these results strongly indicate the involvement of a carrier protein differentially expressed in the two membrane domains in lutein absorption [48].

Despite the results described above, that suggest the occurrence of transport carrier, a passive diffusion passage was proposed [40] for carotenoid intestinal absorption. The authors calculated a linear relationship between the extent of uptake of several carotenoids on differentiated Caco-2 cells and their octanol/water partition coefficient and suggested a passive diffusion transport dependent on carotenoids hydrophobicity. Since no kinetic analysis was performed in the study and only single concentration transport rate was calculated, the existence of saturable transporter cannot be excluded and the data reported may reflect different partition ability of the carotenoids from the micelles to the plasma membrane.

The involvement of a specific transport system is also suggested by competitive inhibition of transport between different carotenoids. In Caco-2 cells trans-epithelial transport of ß-C was competitively inhibited by Lyc and α-carotene but was not affected by the presence of an excess of lutein [41, 47]; conversely in TC7 cells [48] lutein transport was slightly but significantly decreased by the presence of ß-C and was not affected by Lyc. These data, despite some discrepancy in the behaviour of the two cell lines, suggest the existence of a mediated transport mechanism shared by the non-polar carotenoids.

Recently the SR-BI receptor (Scavanger Receptor class B type I) has been proposed as a possible candidate for intestinal lipid transport. This membrane protein is involved in cholesterol homeostasis and intestinal transport but its exact role has not been fully understood [49]; an homologous of the mammals SR-B1 receptor has been recently cloned in *Drosophila* and it has been demonstrated that this protein is responsible for carotenoids uptake [50].

SR-B1 is expressed in differentiated Caco-2 cells [51] and several evidences demonstrate its involvement in carotenoids transport in this cell line.

Transport experiments of lutein in TC7 cells and of ß-C in Caco-2 cells have demonstrated that increasing concentra-

tion of antibodies against the SR-BI receptor, significantly inhibited transport of both carotenoids. Increasing concentration of BLT1 (blocks lipid transport 1), a chemical inhibitor of the selective transfer of lipids by SR-BI, significantly decreased absorption of lutein in TC7 cells, while in Caco-2 cells ß-C transport was significantly inhibited by high cholesterol concentrations (25μM). In Caco-2 cells, ezetimibe, a specific inhibitor of cholesterol absorption, caused a 50% decrease of ß-C and α-carotene transport, moreover, in this study, it was also demonstrated that ezetimibe caused a concomitant downregulation of the expression of the two apical SR-BI, CD36 (cluster determinant 36) membrane proteins and of the basolateral ABC1 (ATP binding cassette transporter, subfamily A) transporter that are likely involved in cholesterol transport [48, 52]. Finally, a study demonstrating the involvement of SR-BI in vitamin E absorption in TC7 cells has shown that lutein is able to inhibit vitamin E absorption providing an indirect evidence of the role of SR-BI receptor in carotenoids intestinal passage [53].

In conclusion, the data reported above suggest the presence of a specific transport system for intestinal carotenoids absorption; a possible role of the SR-B1 receptor in carotenoids and lipids transport has been proposed but its exact function in this event has not been completely elucidated and the involvement of other not yet identified transporters cannot be excluded.

MECHANISMS OF ACTION

For many years carotenoids have been considered essential only as vitamin A precursors. In fact, they are the only source of vitamin A for a large part of the world population eating almost exclusively vegetable food. Several carotenoids are transformed by the intestinal cells into retinol through a series of enzymatic steps and with different efficiency [54]. However, not all carotenoids are vitamin A precursors, and since several years epidemiological, clinical and experimental evidences have established that carotenoids have another physiological important function as antioxidant molecules [55-58]. ß-C contained in fruit and vegetable has the highest vitamin A precursor activity; Lyc, the main carotenoid that confers the red colour to ripe tomatoes has no provitamin A activity, but its level in plasma and tissues has been inversely correlated with risk of prostate, lung, and other types of cancer [59, 60].

Results of early studies demonstrating the ability of dietary carotenoids to prevent infections were attributed to their action as vitamin A. Subsequent studies however, carried out to demonstrate the specific action of individual dietary carotenoids, have utilized carotenoids without provitamin activity such as lutein, canthaxanthin, Lyc and astaxanthin. These non provitamin A carotenoids were as active, and at times more active than ß-C in enhancing cell-mediated and humoral immune response in animals and humans [61].

Carotenoids are excellent scavengers of singlet oxygen and other reactive oxygen species [54]. Lyc can trap singlet oxygen and reduce mutagenesis in the Ames test [62]. Multilamellar liposomes were used to assay the antioxidant activity of the different carotenoids by measuring the rate of inhibition of thiobarbituric acid-reactive (TBAR) substances formation, in these experiments, Lyc has been demonstrated to be the most potent antioxidant, with the ranking: Lyc >α-tocopherol > ß-cryptoxanthin > zeaxanthin > ß-C > lutein. Mixtures of carotenoids were more effective than the individual compounds presumably for the different specific positioning in membranes [63]. The higher potency of Lyc can be explained with its highly conjugated structure that allows, more efficiently than other carotenoids, to quench reactive oxygen species, scavenge free radicals and terminate lipid peroxidation reactions.

On the basis of the evidence brought by many papers that individuals with high level of serum carotenoids have a lower risk of cancer, especially lung cancer [64, 65], human studies were initiated using chronic pharmacological doses of ß-C. Unexpectedly, human intervention trials failed to reduce and even increased the incidence of lung cancer in smokers [66]. Therefore a great deal of interest has been given to the understanding of the mechanism(s) of action by which ß-C and other carotenoids may modulate physiological functions and influence cell growth. A possible mechanism that could explain the dual role, beneficial and harmful, of carotenoids, could be their ability to modulate the intracellular redox state. These compounds may function as antioxidant, inhibiting free radical production [67, 68] or as pro-oxidant, propagating free radical-induced reactions, depending on their intrinsic properties as well as on the redox potential of the biological environment in which they act [55, 68, 69]. In both cases, carotenoids can contribute, but in opposite direction, to regulate different signalling pathways involved in cell proliferation and apoptosis [70].

Reactive oxygen species (ROS) have been described as second messengers for several growth factors and cytokines, and signal transduction by ROS involves alteration in cellular redox status and oxidative modifications of proteins [71, 72].

In order to verify if ß-C may modify cell growth through changes in intracellular redox potential, many studies have been performed utilizing as a model cells in culture [73]. It was shown that ß-C was able to modify ROS production in human colon adenocarcinoma cells [74, 75] as well as in human leukemia cells [76] and that the effect was accompanied by changes in cell growth. At low concentrations the carotenoids inhibited ROS production and were ineffective in modifying cell growth, while at high concentrations increased the formation of free radicals and inhibited cell growth by inducing apoptosis. In these cells the pro-oxidant effect of ß-C occurred at concentrations from 2.5 to 20 μM, amount that can be reached *in vivo* in the serum of individuals supplemented with 50 to 100 mg /day of ß-C.

The overproduction of ROS by ß-C was observed in cytoplasm and in mitochondria, as revealed by the use of specific fluorescent probes [75]. The potential mechanisms implicated in the production of ROS can be: ß-C 1- may act as an endogenous generator of ROS through the induction of various isoforms of P450 [78]; 2- can modify iron levels by increasing the production of endogenous free radical species through Fenton reactions [80]; 3- can undergo auto-oxidation, which has been reported to induce formation of singlet oxygen in cultured HL-60 and HP-100 cells; 4- can induce the formation of potential oxidative carotenoid products; and finally 5- can induce modifications of the content of other

endogenous compounds with a better antioxidant profile [71].

According to the last hypothesis, HL-60 leukemia cells treated with ß-C (10–20μM), exhibited a decrease in the intracellular concentration of reduced glutathione (GSH) and a concomitant increase of oxidized glutathione (GSSG). These changes were highly coincident with the ability of the carotene to induce apoptosis and to arrest cell cycle progression [77].

Several reports suggest that carotenoids can prevent DNA oxidative damage [81]. However, increasing evidence shows that carotenoid molecules at relatively high concentration may increase DNA damage. An enhancement of H_2O_2-induced oxidative damage by ß-C has been reported in HepG2 cells [82], whereas a failure of ß-C and of other carotenoids, such as Lyc, to protect human cells against free-radical-induced DNA damage has been recently observed in HT29 cells [83].

It can be concluded from *in vitro* experiments that ß-C acts in living cells as a redox molecule, modulating redox-sensitive molecular pathways involved in cell cycle progression and apoptosis. However, the results have to be confirmed *in vivo* in animals and finally in humans. Due to their high hydrophobicity, carotenoids are insoluble in aqueous systems and therefore poorly available to cells in culture. In most *in vitro* studies carotenoids are given to cells as water dispersible beadlets, detergent solutions, or dissolved in various solvents as alcohols, dimethyl sulphoxide (DMSO) and tetrahydrofuran (THF). These methods allow the evaluation of the potential effects of the pigments, but could mislead the interpretation of the physiological significance of the observed phenomenon. Moreover it has been demonstrated in *in vitro* studies that the oxygen tension of the environment may strongly modify the redox properties of the carotenoid molecule [71]: ß-C improves its antioxidant efficiency at low oxygen partial pressure, while it turns into a prooxidant molecule at high oxygen pressure. Given the low oxygen tension of human tissues, presumably the antioxidant character of the molecule may prevail on the pro-oxidant one. According to this hypothesis, the growth inhibitory effect of ß-C in SCC-25 tumor cells was decreased by an oxygen-poor environment [84], in which the pro-oxidant character of the molecule is minimized [85, 86]. However, cell growth-promoting effects of ß-C are mainly reported in tissues, such as lung, in which the oxygen tension is particularly high and therefore able to promote effective prooxidant effects of the carotenoid [66, 67]. Increasing evidence suggests that ROS are important in the regulation of intracellular signalling, by acting as intermediate molecules in mitogenic signalling promoted by growth factors and oncogenic molecules and by operating in the apoptotic cascade initiated by tumor suppressor proteins.

The effects of carotenes on cell growth and transformation are attributed by some authors to modification in cell-cell communication. All human tumors examined are deficient in gap junctional communication (GJC) and its restoration by forced expression of connexins (Cxs) reduces indices of neoplasia. Gap junctions (GJ) are composed of a pore-like structure (connexon) made of several Cxs. The structure allows molecules of about 1000 daltons to pass between adjacent cells. The expression of Cx43, the most widely expressed Cx family member, is upregulated by retinoids and carotenoids with cancer-preventive properties and this correlates with the suppression of carcinogen-induced transformation in C3H/10T1/2 cells [87]. However, according to some authors, only provitamin A carotenoids show this activity [88]. Following the observation that retinoids could modify cell-cell communication [89], Bertram and his associates reported that carotenoids could also enhance GJC in C3H/ 10T1/2 cells [90]. However, these authors did not find a relationship between the ability of carotenoids to increase GJC and antioxidant action [91]. Many of the effects of carotenoids on GJC have been summarized in a review [92]. After the publication of the review, many more results have been published using other cell lines and other carotenoids, like Lyc, that are not vitamin A precursors. In human oral tumor cells [93] it was found that the inhibition of cell proliferation exerted by Lyc and ß-C was associated with upregulation of Cx43 mRNA and protein expression, concomitant with enhanced GJC. Other authors, [94] have investigated the molecular mechanism by which cancer preventive retinoids and carotenoids upregulate the expression of Cx43 in normal and preneoplastic cells; they found that the retinoic acid receptor antagonist Ro 41-5253 was able to suppress retinoid induction of Cx43 luciferase reporter construct in F9 cells, but did not suppress reporter activity induced by the carotenoids astaxanthin or Lyc, indicating that retinoids have separate mechanisms of gene activation than non-provitamin A carotenoids. With site directed mutagenesis experiments, the authors were able to localize the responsive region of the gene.

In most cases examined, Cxs are modified post-translationally by phosphorylation. This modification has been implicated in the regulation of GJC at several stages of the Cx "lifecycle" such as trafficking, assembly/disassembly, degradation, as well as, gating of gap junctional channels. In fact many Cxs not only contain protein kinase "consensus phosphorylation sequences", but they also have been demonstrated to be phosphorylated by kinases *in vitro* and in tissues. Many Cxs (Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50 and Cx56) have been shown to be phosphoproteins by a shift in their electrophoretic mobility or by direct incorporation of ^{32}P [95]. However, growth factors may also modulate GJC through mechanisms indipendent on phosphorylation of Cx43. Treatment of a kidney epithelial cell line with Epithelial Growth Factor (EGF) for few hours was reported to upregulate GJC maybe through the synthesis and transport of Cx43 [96], and EGF was found to increase the expression of Cx43 in porcine granulosa cells [97] in the absence of changes in Cx43 phosphorylation and may be involved in the regulation of early folliculogenesis .

One of the most intriguing actions of carotenoids [98], is their ability to induce xenobiotic metabolizing enzymes in rodents; this topic had been reviewed [99] and it has been suggested that modulation of these enzymes might be relevant to humans [100].

CONCLUDING REMARKS

This short review is focused on the use of cell culture models to study the effects of carotenoids contained in food

and shows how useful these models are in biomedical investigation. However when *in vitro* models are employed, it has to be considered that they cannot represent the complexity of physiological systems *in vivo*. It is therefore crucial to ask the proper questions to the right cells. As shown by the data described, *in vitro* models are a very advantageous tool to characterize the effects of compounds and their mechanisms of function, but in order to validate the results obtained, these have to be compared and confirmed with the observations coming from epidemiological and *in vivo* studies.

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ABBREVIATIONS

ß-C	=	ß-carotene
Lyc	=	Lycopene
Cx	=	Connexin
DMSO	=	Dimethyl sulphoxide
THF	=	Tetrahydrofuran
GSH	=	Reduced glutathione
GSSG	=	Oxidized glutathione
TBAR	=	Thiobarbituric acid-reactive
GJC	=	Gap Junctional Communication
GJ	=	Gap Junctions
EGF	=	Epithelial Growth Factor
CM	=	Chylomicron
PC	=	Phosphatidylcholine
Lyso-PC	=	Lyso-phosphatidylcholine
SR-BI	=	Scavanger Receptor class B type I

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