Bioavailability of Non-Provitamin A Carotenoids

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Abstract: The role of carotenoids in human nutrition has gained increased interest, especially due to their associated health-beneficial effects for a number of chronic diseases, including certain types of cancer and cardiovascular disease. Whereas data is available on the intake and presence of carotenoids in foods, limited information exists on factors influencing their bioavailability, especially for the non-provitamin A carotenoids. However, carotenoid absorption strongly depends on a number of factors which are not entirely understood. These include mainly the release of carotenoids from the food matrix, their incorporation into mixed bile micelles, the transfer of carotenoids from micelles to the mucosa for passive or facilitated absorption (via SR-BI proteins), and the sequestration into chylomicrons. Thus, dietary compounds influencing carotenoid micelle incorporation, e.g. the amount fat present in an ingested meal or components competing for uptake, such as phytosterols and other carotenoids, can have a considerable impact on carotenoid bioavailability. However, the effect of many dietary factors, including dietary fiber, type of fat, or minerals on carotenoid absorption is not well understood. In addition, bioavailability also depends on the carotenoid structure; in general, polar carotenoids are preferably incorporated into mixed micelles and tend to be of higher bioavailability, as may be the case for free vs. esterified xanthophylls, and cis-isomers vs. their trans-form, due to apparent shorter chain-lengths. Whereas their importance as part of a healthy diet warrants an improved understanding of carotenoids, their dietary fate following ingestion, including their stability, efficiency of micelle-incorporation, and pathway of absorption are still marginally understood.

Keywords: Non-provitamin A carotenoids, bioavailability, absorption, micellarization, cis-isomers, diet.

INTRODUCTION

The role of carotenoids in human nutrition has gained increased interest due to their associated potential health benefits in relation to chronic disease prevention. Carotenoids are a class of isoprenoid compounds consisting of 40 C atoms (tetraterpenoids) which can be classified into oxygen containing carotenoids (oxocarotenoids or xanthophylls), and non-oxygen containing carotenoids (carotenes). Over 600 carotenoids have been specified to date, but only around 40-50 (Fig. **1**) play a role within the human diet [1, 2]. Carotenoids can be synthesized by all plants, most bacteria and fungi, but not by animals or humans, who therefore depend on their regular dietary intake. Many functions of carotenoids in plants and microorganisms are not entirely understood, but protection from photooxidation plays a predominant role [3]. All carotenoids exhibit free radical scavenging properties, either due to direct quenching reactions with singlet oxygen, or reaction with radicals such as the peroxyl ($ROO^{\cdot}$) or hydroxyl ($OH^{\cdot}$) radical [4], and can thus act as antioxidants. Carotenoids may be further classified into provitamin A carotenoids, which can be metabolized by humans into retinol, and non-provitamin A carotenoids, which exhibit no vitamin A activity in humans. Carotenoids with provitam A activity include alpha-, beta-, and gamma-carotene, and alpha- and beta-cryptoxanthin. Among the non-provitamin A carotenoids, especially lutein, zeaxanthin, and lycopene have been studied and associated with a variety of health beneficial effects. Lutein and zeaxanthin for example play an important role in the retina of the eye, in the prevention of macular disease [5, 6]. Furthermore, non-provitamin A carotenoid intake has been associated with reduced incidence of cardiovascular diseases (CVD), a variety of cancers such as prostate and breast cancer, and has been suggested to be beneficial for bone health [7-11]. However, many of the associations between carotenoid intake and their influence on disease prevention remain poorly understood and inconclusive. For example, while the observed relation between carotenoid intake and their potential health benefits are typically found for consuming carotenoids within foods, this is not necessarily true for the consumption within food supplements [12]. This inconclusiveness is partly due to a large number of confounding factors associated with intake of diets rich in carotenoids, such as dietary fiber and other phytonutrients, partly because of limitations of different techniques used to study the bioavailability and biodistribution of these compounds (Table **1**).

Upon ingestion, carotenoid bioavailability depends on a number of factors (Fig. **2**). To be bioavailable, carotenoids have to be released mechanically or enyzmatically from the food matrix and, due to their limited water solubility, to be incorporated into lipid droplets. They are then transferred into mixed micelles, together with lipids such as free fatty acids, monoglycerides and phospholipids (PL) such as phosphatidylcholine (PC) and bile salts, in the small intestine before being transferred to and absorbed by the enterocytes, either by passive absorption, or, according to more recent evidence, facilitated absorption, by a SR-BI transport protein [13-16]. They are then transported through the cells, and sequestered and released into the lymph within triacylglyerol-rich chylomicrons, carried to the bloodstream and

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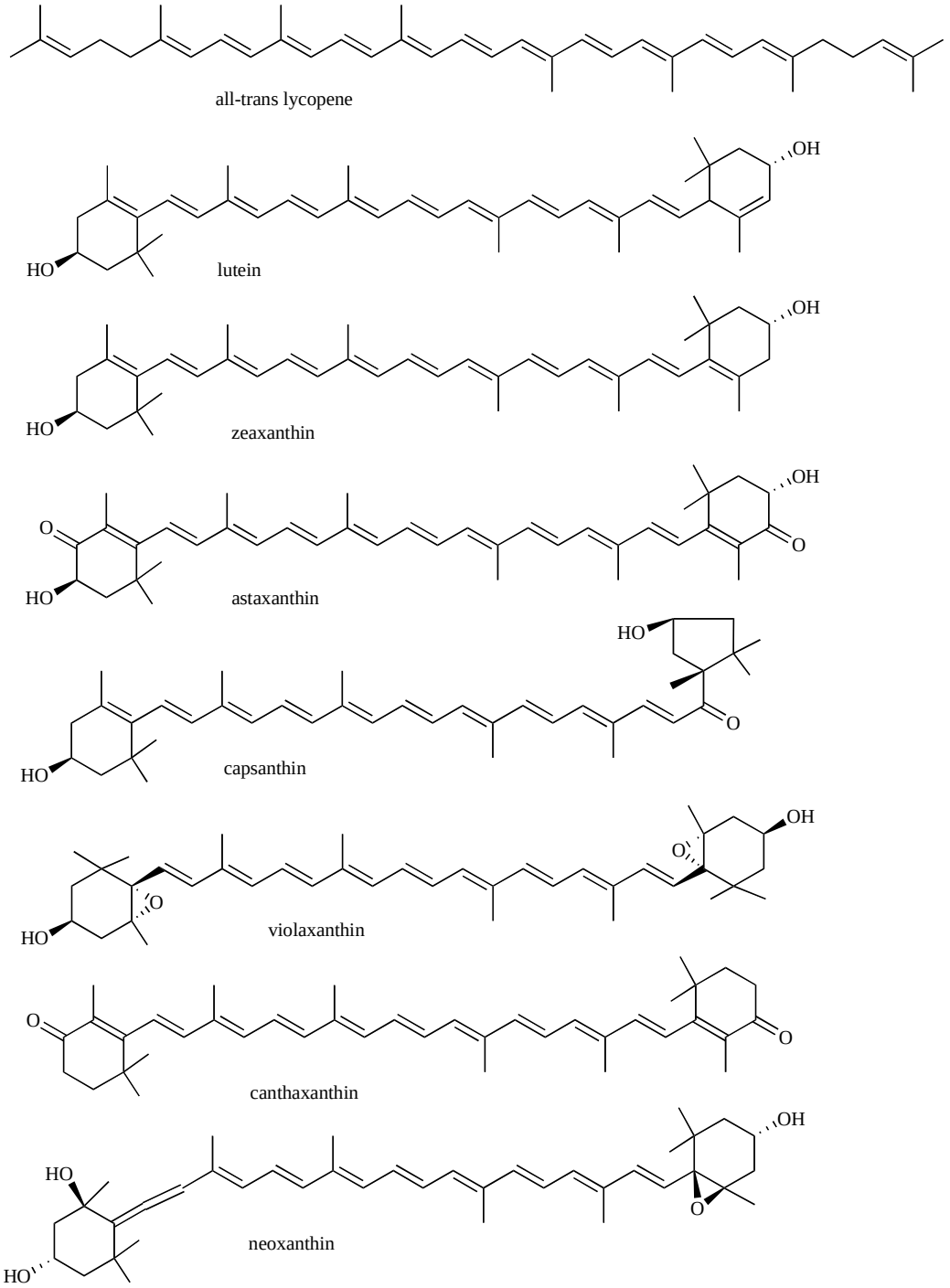

Fig. (1). Chemical structure of various non-provitamin A carotenoids typically consumed within the diet.

liver, where they are transferred to LDL and HDL particles. The further fate of carotenoids is only poorly understood. While carotenoid excretion via the urine is probably very low [17], the majority of carotenoids or their breakdown products are most likely excreted in form of endogenous losses within faeces. For the carotenoid fraction remaining in the body, very little information is available on their further metabolization, but degradation into smaller, more polar fragments via the formation of epoxides and carotenals seems likely [18].

However, only little information is present about factors influencing bioavailability of carotenoids, perhaps because, with the exception of beta-carotene, studies investigating correlations between carotenoids and their role in disease prevention remains scant. Major factors influencing carotenoid absorption have often been described by the acronym "SLAMENGHI" including the: i) species of carotenoid; ii) molecular (chemical) linkage; iii) amount of carotenoids consumed; iv) food-matrix; v) effectors of absorption and bioconversion such as the presence of enhancers and inhibitors; vi) nutrient status of the host; vii)

Table 1. Techniques Used to Determine Aspects of Carotenoid Bioavailability

Method	Advantage	Disadvantages	References
***in vitro* Techniques**			
- *in vitro* digestion	cheap and easy to perform	only micellarization, not cellular uptake is studied	[162]
- *in vitro* digestion coupled with Caco -2 cells	relatively cheap to perform	Caco-2 cells not exactly comparable to complex small intestinal environment, large intestine not simulated, not all transporter/cleavage enzyme expressed, no mucus formed	[19]
Studies Involving Animals			
- animal model studies with animal organs	real biological system	only part of the metabolization pathway can be studied	[20]
- animal absorption studies, i.e. with ferrets	real biological system	carotenoid metabolism might differ depending on species	[21]
Human Studies			
- chemical fecal balance	non-invasive	oxidation of carotenoids in large intestine and feces, varying endogenous losses, retention by micro-organisms	[22]
- chemical fecal balance with ileostomists	non-invasive	does not mimic large intestine	[23]
- blood-plasma response	relatively easy to measure	usually relatively weak response to physiological doses, clearance rate could differ between subjects and carotenoids	[23]
- chylomicron response	good response to physiological doses over approx. 10 hours after test meal ingestion	long term bioavailability can be different due to dietary adaptation, measurement time often too short, fast clearance or transfer to other lipoproteins might impede interpretation of results, large plasma volumes (5 mL) needed	[24]
- LDL or HDL carotenoid response	studying further metabolisation	late appearance in lipoproteins (ca. 16-48 hours)	[148]
- radioisotopes	relatively easy to measure, excretion in feces and urine (fragments) can be studied, can be non-invasive	sophisticated equipment needed, ethical concerns	[25, 26]
- stable isotopes	absorbed label can be differentiated from other endogenous carotenoids	sophisticated equipment needed, labeling can be difficult and expensive	[27]
- compartment modeling	biodistribution can be studied	accurate model required, sophisticated software and sampling methods, isotopes needed	[28]

genetic factors; viii) host-related factors such as GI passage time; and ix) interactions between those factors. The objective of this review is to summarize the present knowledge about factors influencing carotenoid bioavailability, with a focus on dietary factors impacting the less studied non-provitamin A carotenoids.

CAROTENOID BIOSYNTHESIS

When investigating carotenoid bioavailability and metabolism, it is important to understand their origin and the functions these compounds exhibit in the plant kingdom. In higher plants, carotenoids are synthesized primarily in the plastids, especially the chromoplasts. In lower organisms such as bacteria, carotenoids are formed in the cytosol. The biosynthesis of carotenoids can be described in 5 stages: i. Formation of isopentyl diphosphate (IPP); ii. formation of phytoene from IPP; iii. desaturation of phytoene to lycopene; iv. cyclisation; and v. xanthophyll formation. All of these reaction cascades require specific enzyme systems and often activation of the molecules by active phosphate groups. The precursor molecule for all carotenoids is IPP, a C-5 molecule derived from either acetyl-CoA via mevalonic acid (MVA pathway, in cytosol) or pyruvate and D-glyceraldehyde-3 phosphate (MVA independent pathway, in plastids). Four of these C-5 isoprene units then form, via a chain elongation reaction, the C-20 molecule geranylgeranyl pyrophosphate (GGPP), of which 2 of these condense to form phytoene, a colorless symmetrical C-40 chain molecule with a total of 9 double bonds, of which 3 are conjugated. Its most prevalent form is the 15-cis geometric isomer. All other carotenoids are formed from this compound. Phytoene can be further desaturated to phytofluene, then to zeta-carotene, and further all-trans lycopene, the pathway being somewhat different between higher plants and bacteria or fungi. One or both

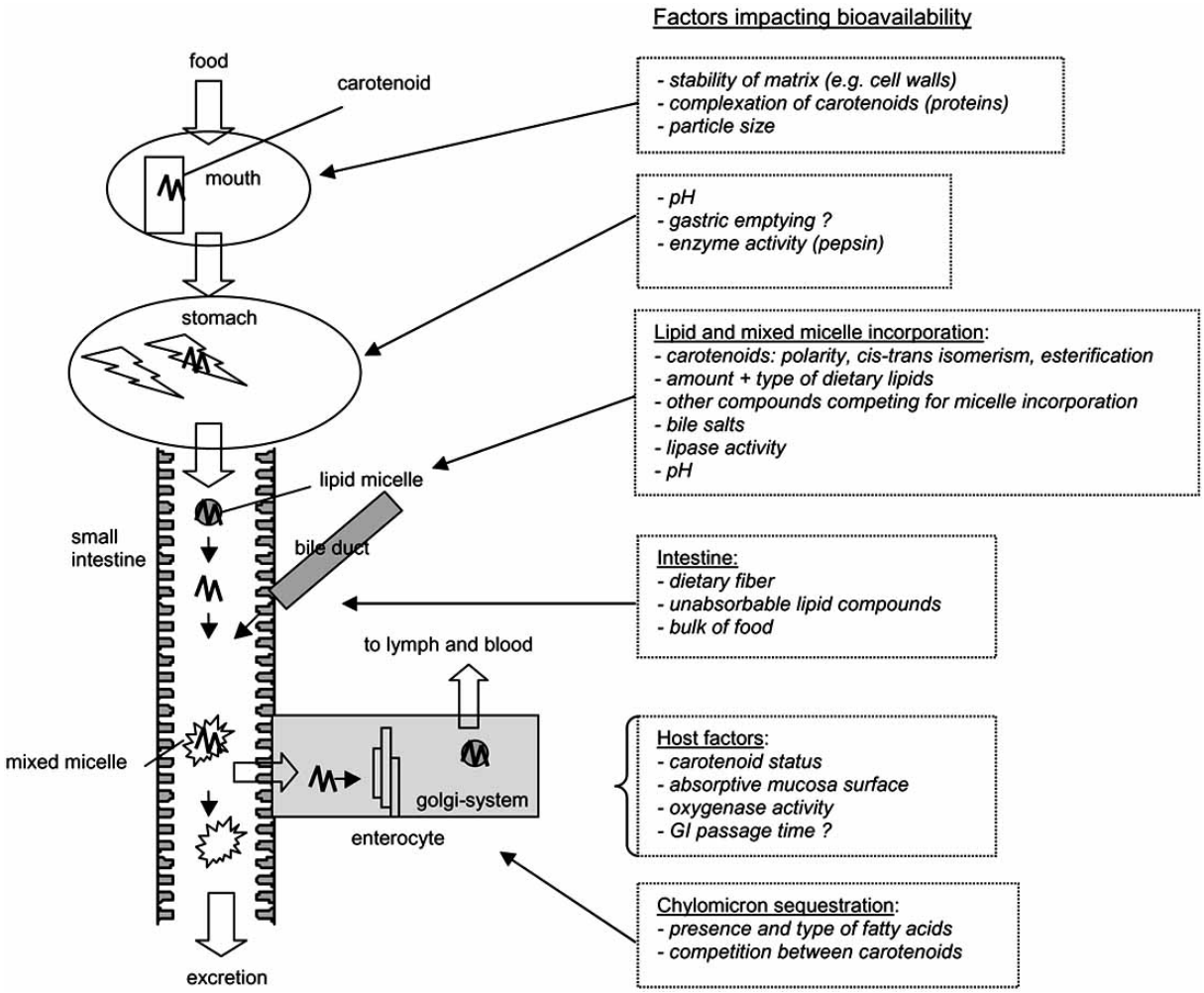

Fig. (2). Main factors and control points governing carotenoid absorption from the diet.

ends of the lycopene molecule can then undergo cyclisation to form alpha or beta ionone rings, differing only in the position of 1 double bond in the cyclohexane ring. The resulting compounds are alpha, beta, gamma, delta, or epsilon-carotene. These carotenes can then be hydroxylized to form a number of xanthophylls. Two pathways for example are the C-3 hydroxylation of alpha-carotene to lutein via alpha-cryptoxanthin and the C-3' hydroxylation of beta-carotene to zeaxanthin via beta-cryptoxanthin. Further epoxi-dation and de-epoxidation reactions may occur, resulting in a variety of products such as capsanthin, taraxanthin and violaxanthin. In addition, different isomers may be formed, such as cis-trans isomers, enantiomers, and diastereomers. The formation of cis-isomers is thought to follow a non-enzymatic reaction [29]. For more details on the biosynthesis the reader is referred to recent reviews [10, 30, 31].

PRECURSORS AND DEGRADATION/METABOLIZATION PRODUCTS

Investigations on carotenoids have been carried out predominantly for the most abundant carotenoids, and still limited information is present on minor carotenoids and carotenoid precursors in foods or factors influencing their stability and absorption following ingestion. Carotenoid tetraterpenoids precursors expected to possess antioxidative properties include e.g. phytoene, phytofluene, zeta-carotene, and neurosporene [29]. The level of these precursors in plants and therefore average intake is usually assumed to be low [10, 32], however, they can accumulate in some varieties. For example, phytoene and phytofluene are abundant especially in tomato and watermelon and their products [33, 34]. However, there is only limited knowledge on the presence of these compounds in other foods, their stability, and bioavailability. Even though, for instance, some studies with rodents on phytoene and phytofluene have been conducted [35, 36] suggesting good bioavailability and uptake by various organs, and good fractional absorption of phytofluene was found in a human study [37], many factors associated with their absorption, such as the efficiency of micellarization and the uptake by mucosa cells have not yet been thoroughly investigated.

The same is true for carotenoid degradation products (Table **2**), formed in foods or upon ingestion [24, 38-40] (Fig. **3**). A first metabolization step is often cis-trans isomer formation [15] which has been reported in human tissues and fluids [33, 40]. The intake of cis-carotenoids is typically low, as most foods contain predominantly the all-trans form, unless they are rigorously processed. A number of

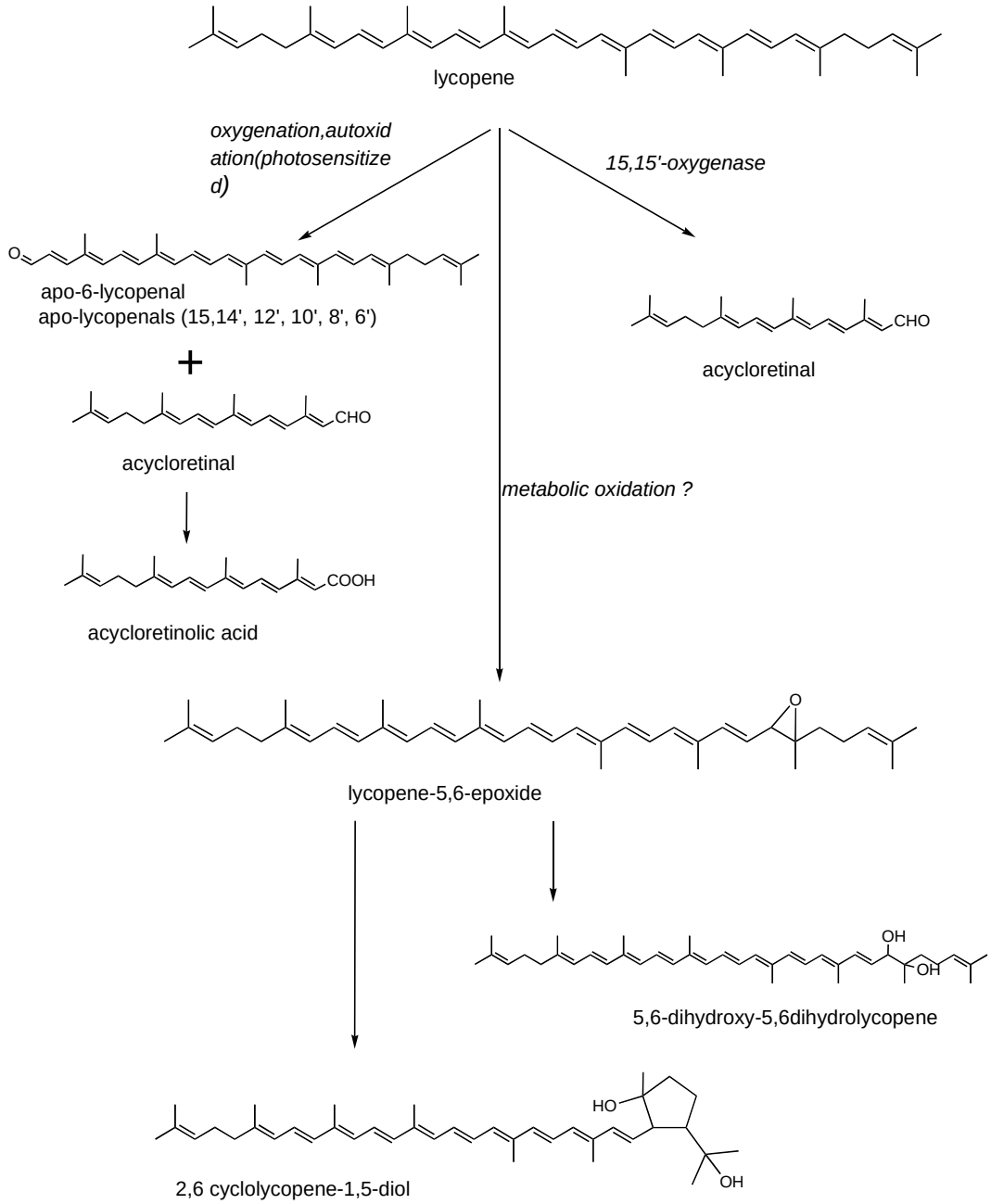

Fig. (3). Possible degradation products of lycopene following ingestion, detected in human body fluids according to [49, 53].

cis-carotenoids such as tetra-cis lycopene have been discussed to be more bioavailable than its parent all-trans carotenoid [41]. Other stereoisomers such as epimers may also be formed. Recently, the 3' epimer of lutein was detected in processed sorrel using cooking procedures such as steaming, its formation being facilitated under acidic pH [42]. This could be of interest, as differences in the absorption between stereoisomers of astaxanthin esters have been reported in humans, due to selective cleavage by the esterase activity [43]. Carotenoid degradation may then occur either enzymatically or non-enzymatically. Enzymatic breakdown products of carotenoids following digestion can include oxidative cleavage products, such as those originating from carotenoid oxygenases, resulting in the formation of apo-carotenals, even from non-provitamin A carotenoids [44-46], though the oxygenase effect on non-provitamin A carotenoids is relatively poorly understood. For pro-vitamin A carotenoids, both central enzymatic cleavage (by 15,15' monooxygenase), and asymmetric cleavage at the 9'10'bond resulting in the formation of beta-ionone and beta-apo-10' carotenal have been reported [47, 48].

Non-enzymatic reactions include autoxidation, e.g. by singlet oxygen (photosensitized oxygenation), co-oxidation with lipoxygenase, direct chemical reactions, or reaction with free radicals [58]. It has been suggested that the general typical carotenoid degradation pathway follows isomeri-

zation, oxidation, and fragmentation, with heat, light and low pH facilitating isomerization, and light, enzymes, presence of pro-oxidative metals and reaction with unsaturated lipids resulting in oxidation [18]. A number of products similar to those formed via the enzymatic pathway have been reported. For lycopene, epoxy-products [18], apo-lycopenals [49, 50], and acycloretinal and acycloretinolic acid [49] have been reported *in vitro*; 5,6-dihydroxy-5,6-dihydrolycopene [53], and 2,6-cyclolycopene-1,5-diol [33] have been found in humans. Fragments from carotenoid containing ionone rings may also be formed during degradation. *In vitro*, beta-ionone and 5,6-epoxy-beta ionone from beta-carotene were formed [reviewed by 18]. It can be hypothesized that similar products might be formed from other carotenoids and also during *in vivo* reactions. Thermal influence, as during food processing, has likewise been reported to result in similar products (Table **3**), however, a larger variety of compounds including cyclocitral, xylene, and cyclohexanone has been reported as compared to oxidation, at least for beta-carotene.

Lutein and zeaxanthin degradation pathways and products have been described by Khachik *et al.* [53]. Fatty acid esters of xanthophylls are generally assumed to be cleaved *in vivo* by carboxylic ester hydrolase originating from pancreas, prior to absorption. Lutein is then thought to undergo mainly acid catalyzed dehydration to form 3-hydroxy-3',4'-dihydro-β,γ-carotene and 3-hydroxy-2'3'-didehydro-β,ε-carotene. Other possible degradation pathways of both lutein and zeaxanthin follow oxidation to monoketocarotenoids and diketocarotenoids such as ε,ε-carotene-3,3'-dione. In the human retina, a number of additional products have been detected (Table **2**).

Information on degradation of other xanthophylls is scarce, but both oxidation products and cleavage products have been described, at least *in vitro* (Table **2**). Cantha-xanthin, has been assumed to be converted into their respective hydroxy-compounds, at least in chicken [59].

Thus, at present, there is very limited information on most carotenoids and their potential degradation products under physiological conditions, and at which stages during absorption and further transport metabolization/degradation occurs. As a consequence, absorption of these resulting compounds has not yet received much interest.

CAROTENOID ABSORPTION

In humans, carotenoids are absorbed in the small intestine, while absorption in the colon could not yet be demonstrated [60], even though lutein, lycopene and beta-carotene have been suggested to be available for absorption in the colon, based on *in vitro* studies [61]. Results from several studies have suggested that carotenoid absorption follows a dose dependent, saturable process, which would indicate at least the participation of an alternative absorption pathway in addition to passive diffusion, which was earlier believed to be the only route for absorption. In a recent human pharmacokinetic study, no significant differences between total lycopene absorption from a tomato paste oil mixture as determined by chylomicron response were observed for oral doses varying from 10 to 120 mg. Plasma levels though differed significantly, the highest increase in plasma levels however was found between 10-30 mg; it was concluded that lycopene absorption is saturated at relatively low intake, at about 6 mg [62, 63]. Similar results were obtained in an earlier rat-study in which total lycopene absorption was found to be independent from concentrations infused [64]. These studies also indicate that different methods can lead to different results when determining fractional absorption. It appears that there exists a limited rate of incorporating lycopene into chylomicrons, however, some lycopene can be stored in the enterocyte for later release, which usually happens with the intake of a subsequent meal containing fat. This is supported by results in canines with relatively high oral doses of lycopene, absorption was mostly limited by lymphatic transport, and, in addition, by intraluminal solubility at higher amounts [65]. A similar behavior can be assumed for other carotenoids. As for lycopene, intake of higher oral doses of zeaxanhtin (10 mg) versus lower doses (1mg) from a synthetic source was associated with 40% poorer fractional absorption [66] as determined by human plasma response. Despite their limited uptake, carotenoids can be absorbed rapidly, with a maximum appearance in plasma between 2 to 16 hours, depending on their polarity, the form of meals consumed, and esterification [43].

Recently, there is growing evidence that carotenoid absorption might, at least partly, follow a facilitated transport involving the scavenger receptor class B type 1 (SR-BI) belonging to the ABC class transport proteins. In a recent study *in vitro*, the effect of ezetimibe, an inhibitor for cholesterol absorption, and antibodies of CD36 and scavenger receptor class B, type 1, were studied in Caco-2 cells [15]. Ezetimibe and antibodies to SR-BI, but not antibodies to CD36 acted as inhibitors of alpha and beta-carotene, beta-cryptoxanthin, lycopene, and lutein absorption, in an additive way. However, the results suggested differences between the degree of inhibition, being stronger for the more apolar hydrocarbons, suggesting that there may be more than 1 transporter or pathway involved in the uptake of carotenoids. Further evidence is that some carotenoid isomers (cis vs. trans) seem to be incorporated preferentially into enterocytes [19]. A similar effect of the participation of SR-B1, and also CD36 based on *in vitro* cell and in mice studies has been shown for beta-carotene absorption [14]. Likewise, using Caco-2 cell lines, lutein absorption was significantly inhibited by blocking SR-BI receptors either chemically or by antibodies [67]. The presence of specific transporters might further explain why certain carotenoids compete for absorption while others do not. It can be speculated that different transporters exist for carotenoid uptake, and that the extent these transporters participate in carotenoid absorption differs between the carotenoids.

DIETARY FACTORS AND THEIR IMPACT ON CAROTENOID ABSORPTION

Food-Matrix

Within plants, carotenoids occur typically within chromoplasts or chloroplasts, where they have been suggested to be bound to proteins [68, 69], which would impede absorption following ingestion [70]. Carotenoids may exist both in a crystalloid form or dissolved in oil. In green leafy vegetables for example, alpha and beta- carotene exist primarily in chloroplasts in crystals of up to 1000 μm in

Table 2. Metabolites and Degradation Products of Non-Provitamin A Carotenoids Detected *In Vitro* and *In Vivo*

Name of Compound	Parent Carotenoid	Detected in	Suggested Mechanism	References
apo-lycopenals (6', 8'10'12'14',16'); acycloretinal; acycloretinoic acid; 6,10,14-trimethyl-3,5,7,9,13pentadecapentaein-2-one;	lycopene	*in vitro* (via ozone oxidation)	autoxidation	[49]
2-methyl-2-hepten-6-one; apo-6'-lycopenenal;	lycopene	*in vitro* (via photo-oxidation)	oxidation in presence of sensitizer via lycopene-5',6'-epoxide	[50]
lycopene-1,2-epoxide; 1,5-epoxyiridanyl-lycopene;	lycopene	*in vitro* oxidation	oxidation with m-chloroperbenzoic acid	[51]
lycopene-5,6-epoxide; 5,6-dimethoxy-5,6-dihydrolycopene; dimethoxy-prolycopene;	lycopene	tomato products	oxidation	[52]
2,6-cyclolycopene-1,5-diol; 2,6-cyclolycopene-1,5-diol;	lycopene	serum	metabolic oxidation via lycopene-5,6,-epoxide and 2,6-cyclolycopene-1,5-epoxides	[2]
5,6-dihydroxy-5,6-dihydrolycopene ;	lycopene	serum, tomato products	oxidative metabolites	[53]
bixin like products	lycopene	plants	oxidative metabolites	[54]
(all-E)-3'-epilutein; 3-hydroxy-3', 4'-didehydro-β,-γ-carotene; 3-hydroxy-2', 3'-didehydro-β,-β-carotene;	lutein	serum	oxidative metabolites	[2, 53]
3-hydroxy-ε,ε-caroten-3-one; 3-hydroxy-β,ε-caroten-3'-one; (Z)-3-hydroxy-β,ε-carotene-3'-one; ε,ε-caroten-3,3'-dione;	lutein or zeaxanthin	serum, retina	oxidative metabolites	[53, 55]
3-hydroxy-3', 4'-didehydro-β, γ-carotene; 3-hydroxy-2', 3'-didehydro-β,ε-carotene;	lutein	serum	dehydration products under acid conditions	[2]
(9'Z)-canthaxanthin-20-al; 9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin; (9'Z)-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin; (13'Z)-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin;	canthaxanthin	*in vitro* (via peroxide influence)	degradation product following reaction with nickel	[56]
4-oxo-β -ionone; (7E, 9E)-4-oxo-β-apo-11-carotenal; (7E, 9Z)-4-oxo-β-apo-11-carotenal; 4-oxo-β-apo-13-carotenone; 4-oxo-β-apo-14'-carotenal; 4-oxo-β-apo-12'-carotenal; 4-oxo-β-apo-10'-carotenal;	canthaxanthin	*in vitro*	cleavage product following oxidation with nickel-peroxide	[56]
4-oxo-retinoic acid	canthaxanthin	*in vitro*	oxidation	[57]

length [71], and lycopene in tomatoes exists in a similar crystalline form. In orange and yellow fruits such as pumpkin and sweet potato, beta-carotene is typically present dissolved in oil droplets. Carotenoids from oil and water soluble dispersions are usually well absorbed, over 50% [58], as the carotenoids are present in free form. In addition, it is well established that the processing of vegetables typically goes along with improved bioavailability of carotenoids. Absorption of lycopene was significantly higher from cooked and processed compared to raw tomatoes, most likely because of disruption of the cell matrix [72-75]. Likewise, processed and heated tomato paste showed higher lycopene absorption compared to yellow carrots in humans [76] and red carrots in gerbils, as measured based on their liver tissue concentration [77]. Interestingly another, less well studied lycopene source, watermelon juice, showed to be a good source of lycopene compared to processed tomato juice [78], perhaps indicating that the fruit matrix, its weaker cellular structure or less abundant protein-carotenoid complexes, or presence in oil droplets rather than in crystals allows for a better bioavailability as compared to raw tomatoes. Other approaches to serve lycopene in a more available form included entrapping lycopene with whey proteins [37] or encapsulation in gelatin capsules [79]. While no differences were seen in lycopene bioavailability as compared to a control (tomato paste), in the first study, bioavailability of the gelatin capsules has not yet been tested.

A number of studies have investigated the bioavailability of carotenoids, especially lycopene supplements. These include either synthetic lycopene or supplements enriched in natural lycopene, both of which seem to possess comparable lycopene bioavailability. Synthetic lycopene from tablets [80] served with minestrone showed to be as bioavailable as from tomato soup obtained from paste, and even superior to tomato juice in healthy humans based on plasma concentration measurements during 8 d feeding trials, which was attributed to increased amounts of present Z-isomers in the supplement and reduced matrix effects. In another study [81], human lycopene plasma response was significantly higher from tomato oleorosin capsules and juice than from raw tomatoes, with no remarkable difference between the first two sources. Similarly, elevations of plasma serum lycopene from water dispersible tomato beadlets were not different compared to tomato juice and oleoresin [82]. Two types of tomato beadlets, both almost free of tomato matrix, containing synthetic lycopene and natural tomato lycopene resulted in similar lycopene absorption in human subjects [83]. However, synthetic lycopene dissolved in corn oil showed to be 3 times more bioavailable than lycopene from unheated tomato puree when consumed in diets with equal amounts of fat [84], based on human plasma concentrations. It thus appears that lycopene from supplements, especially when containing a simple matrix and consumed with some fat, is absorbed equally as heat processed tomato products.

Similar as for lycopene, higher lutein absorption from supplements compared to spinach [85, 86] has been reported. Also, lutein absorption in humans showed to be higher from oil containing supplements than from complex food matrices such as spinach and broccoli, the latter having the lowest response [87], or yellow carrots [88], based on measured serum concentrations (all test meals containing about equal amounts of fat). In the first study however, broccoli was served as a whole, while spinach was served minced, which might at least partly account for lower bioavailability. Minced vs. whole leaf spinach showed to impact bioavailability of lutein in one human study [89], but failed to demonstrate an effect in a second [85]. No significant differences between lutein absorption from broccoli versus green peas in humans were found by van het Hof *et al.* [89]. It thus remains speculative if other factors, such as different types of fibers, and the location of carotenoids in vegetables, i.e. the association with chloroplasts or chromoplasts could result in differences in absorption.

In another study, an over 2 times higher serum response of lutein from eggs compared to spinach or lutein supplements (both esterified and in free from) served in a test meal standardized for the amount fat was found in humans [90], while the lutein serum response between spinach, lutein and lutein ester supplements did not differ. The high lutein availability from egg yolk was explained by different composition in fatty acids high in saturated fatty acids within the egg, competing less strong for carotenoid transport inside the enterocytes for the fatty acid binding protein (FABP), an intracellular transporter. However, higher intake of fiber and different state of solubilization in the food matrix, might also explain these results, while matrix binding effects, such as to the light harvesting complex did not seem to negatively effect the release, as bioavailability was not different between the supplements and spinach. It is also interesting to note that the lutein chylomicron response between all test meals did not differ, which might be explained by a faster transfer of lutein between different lipoproteins [91, 92], or due to limited/ saturated chylomicron secretion from the enterocytes. However, an earlier study supplementing egg yolk already suggested high bioavailability (based on plasma) of lutein and zeaxanthin from this source in humans [93].

Apart from a direct matrix effect, the resulting pH following consumption of a test meal has also been suggested to influence carotenoid bioavailability, impacting the transfer from lipid droplets to mixed micelles, being lower for a decreased pH, around 2-4 [94] when compared to a pH between 5 and 7. In addition, it was reasoned that pH might impact lipase activity which in turn could negatively influence carotenoid bioavailability, and at low pH (2), micelle precipitation was observed. On the other hand it has been suggested that a lower pH decreases the negative surface charge of the micelles and the enterocyte [95], facilitating diffusion, suggesting that there could be a pH optimum for carotenoid absorption.

Lipids

It is known that a certain amount of fat is needed for both micellarization and chylomicron secretion and thus for carotenoid absorption. Dietary lipids also stimulate bile secretion, increase the quantity of carotenoids carried in micelles and micelle count [96]. The optimum amount of fat needed for absorption is still uncertain and might vary depending on the individual carotenoid, whether it exists free or esterified, and type of fat. Based on *in vitro* studies, 100 g triglycerides were able to solubilize 112-141 mg of carotenes or 22-88 mg of xanthophylls, respectively [91]. A similar low amount of fat to ensure optimal absorption has also been suggested for beta-carotene absorption, around 3-5 g fat/meal [97, 98]. In support of this assumption, a relatively low-fat diet (15% of energy intake) in 13 human subjects over 14 days versus a diet containing 38% of energy from fat did not affect serum lycopene levels during a lycopene rich diet [99]. In further accordance, while amounts of 3 or 36 g fat served within a spread containing 8 mg of carotenoids in an otherwise fat and carotenoid restricted diet over 7 d periods however did not impact alpha and beta-carotene plasma response, it did impact lutein response from lutein esters [98], suggesting that lower amounts of fat are needed to ensure maximum absorption of the carotenes as compared to xanthophyll esters, due to their higher hydrophobicity. In addition, limited dietary fat intake could go along with reduced excretion of esterase and lipase needed for ester cleavage. Likewise, in a study by Unlu *et al.* [24], a higher amount of fat (24 vs. 12 g) was needed for optimal lutein absorption determined via chylomicron response from mixed green salad as opposed to alpha and beta-carotene. On the other hand, in a Caco-2 cell study, low presence of fat, at 2.1-3.5% versus 10% reduced micellarisation of the more apolar alpha and beta-carotene, but not that of lutein from spinach [100], however, chylomicron response was not studied and results might not reflect human bioavailability. In another study, consumption of salad containing lycopene, beta-carotene and lutein with 6 g of oil versus 28 g of oil

reduced absorption of all carotenoids significantly, indicating 6 g of fat in the reduced-fat dressing was not sufficient to ensure optimal carotenoid absorption [97]. In summary, it appears likely that the amount fat needed for optimal carotenoid absorption depends indeed on the type of carotenoid, such as on the state of esterification.

Another important aspect of fat is during food processing to aid in the release of carotenoids, and to foster the formation of the potentially higher bioavailable cis-isomers. In a study by Unlu *et al.* [40], cis-lycopene formation was induced by up to 45% in a sauce rich in cis-lycopene isomers by adjusting the temperature to 127 ^{o}C (40 min) versus a controlled variety of about 5% by applying a mild processing temperature of 75 ^{o}C. While not all studies suggested an impact of heat on the formation of cis-lycopene isomers [101-103], it has been suggested that the addition of fat causes an increase in lycopene cis-isomer formation during tomato heat processing. Agarwal *et al.* [104] observed an increase from 6 to 29% cis-isomers when heating tomato juice (1 h) with 10% added corn oil. Similarly, van het Hof *et al.* [105] found significantly elevated levels of cis-isomers (about 55%) when tomato paste was heated to 100^{o}C in the presence of 10% corn oil, whereas it was lower with 5% oil addition and very low in the absence of oil. Thus, both fat and heat seemed to be required for lycopene release from the matrix and the formation of lycopene cis-isomers.

There also seem to exist differences of the type of dietary fat and their impact on carotenoid absorption, especially for lycopene, whereas few data exists on other carotenoids. In a number of human studies, long chain triglycerides (LCT) seemed superior to medium triglycerides (MCT) in enhancing lycopene absorption [106-108]. These lipids increase chylomicron secretion, whereas short chain and medium chain fatty acids are absorbed via the portal pathway. Different carotenoid absorption in dependence of various types of LCT have also been suggested, albeit these observations rest on chylomicron response alone and not on whole serum concentrations. Beef tallow rich in oleic, palmic, and stearic acid compared to sunflower oil rich in polyunsaturated fatty acid (linoleic acid) resulted in higher beta-carotene chylomicron responses in humans following ingestion of 47 μmol beta-carotene together with 60 g of either fat [109]. The reason for this has been speculated to rest in the decreased lipemic response of beef tallow due to its high content of stearic acid rich triglycerides. Polyunsaturated fatty acids have been reported to have a higher affinity to the FAB in the enterocytes, which might compete with carotenoids for intracellular transport [110]. In accordance with this, an earlier study in rats [111] found higher absorption of beta-carotene following infusion of oleic acid (18:1) as compared to linoleic acid (18:2), and astaxanthin recovery in the lymph of rats was higher when fed together with olive oil compared to corn oil [112]. No differences on carotenoid plasma appearance on the other hand have been reported for lycopene absorption when given with olive oil versus sunflower oil [113]. It is possible that the choice of methodology, i.e. plasma versus chylomicron response does impact the results. It is thus further confirmation needed that animal fats and fats rich in saturated fatty acids go along with increased bioavailability of carotenoids.

On the other hand, there is a fair amount of evidence that nonabsorbable lipid compounds might reduce carotenoid absorption. It appears that carotenoids form micelles with e.g. sucrose polyesters can be entrapped by the polyesters, making the carotenoids unavailable for absorption. Consumption of the non-digestible fat replacement olestra (a sucrose polyester) in amounts of 0-17 g/d during a 1 year study significantly decreased serum concentrations of lycopene, alpha- and beta-carotene, lutein, zeaxanthin, and beta-cryptoxanthin, in a dose dependent way. Lipophilic carotenes were more depleted compared to the more polar xanthophylls [114], presumably due to higher solubility of xanthophylls and association of carotenes with the lipid phase. Likewise, a negative effect of giving 0, 8, 20 or 32 g olestra/d on blood plasma carotenoids (lycopene, alpha-carotene, lutein and zeaxanthin) was found in an earlier 8 wk human intervention study with normal fat consumption (35% of energy), with partly dose-dependent and significant effects after 2 wks of olestra consumption, i.e. with plasma carotenoid concentrations of approx. 50% their original values [115]. In an earlier double blind, placebo controlled intervention study [116], even 3g/d consumption of a sucrose polyester similar to olestra resulted in significantly reduced beta-carotene and lycopene concentrations in plasma, 20 and 38%, respectively. In another study, less drastic albeit significant effects (a ca. 25% reduction for total carotenoids) following consumption of 18 g/d olestra were found on blood carotenoid concentrations during 16 wks [117]. On the other hand, in free living subjects during an observational study, consuming lower and perhaps more realistic amounts of olestra, no correlation was found between serum carotenoids and consumption of olestra [118]. It is possible that the observational diets were more prone to confounding factors, i.e. different food choices between subjects consuming olestra versus those who did not, whereas this is less likely in the randomized intervention studies.

As opposed to dietary lipids, emulsifiers, such as phosphatidylcholine (PC) might, depending on their polarity, either increase or decrease carotenoid absorption. PC originate either from the diet or from bile. PC have shown to increase lymphatic absorption of lycopene in rats [119], as measured via cannulated lymph. However, the degree of increase depended on the type of PC, with PC from soy resulting in a higher increase in absorption compared to PC from eggs. In a Caco-2 cell uptake study, long-chain PC negatively impacted beta-carotene absorption while short-chain PC increased uptake in Caco-2 cells [120]. The influence of another emulsifier within 3 different lipid based formulations on astaxanthin absorption was studied in humans, as measured by blood plasma samples [121]. All, i) long chain triglycerides from palm oil with polysorbate 80, ii) glycerol mono-and dioleate with polysorbate 80, and iii) glycerol mono and dioleate polysorbate 80 with sorbitan monooeleate enhanced bioavailability compared to iv) the control fat-free formulation. The formulation containing high amounts of hydrophilic surfactant polysorbate 80, increased absorption the highest. Unfortunately, the effect of the polysorbate emulsifiers was not studied independently of the fatty acid composition in this investigation to allow conclusions of the effect of the type of fat on carotenoid absorption. It is assumed that emulsifiers can enhance the

formation and stability of micelles, similar to bile salts, but could also prevent carotenoid transfer to from the mixed micelles to the enterocytes due to its strong interaction, thereby reducing cell-uptake. In this case, the more polar carotenoids might be bound stronger with a resulting lower uptake. Reduced absorption of lutein in mice was found with added PC in the diet, while addition of the more polar lysophosphatidylcholine (hydrolyzed PC), increased absorption of lutein in mice [122]. Similarly, reduced uptake of especially lutein but also beta- carotene following the addition of PC have been found in Caco-2 cell studies, while lysophosphatidylcholine increased their uptake [123].

Dietary Fiber

Dietary fiber has been suggested to enact a negative effect on carotenoid absorption, by entrapping carotenoids and increasing excretion of bile acids and fecal output, inhibiting lipase activity and increasing loss of dietary lipids, or increasing enterocyte turnover [58]. A significant negative correlation of lignin and resistant protein on the release of lutein and beta-carotene was found *in vitro*, however, correlations were based on only 3 different lignin and resistant protein concentrations, respectively [124]. A negative effect of pectins on lycopene absorption has been speculated due to the formation of a complex [125]. Pectin, guar, alginate, cellulose and wheat bran (approx. 10 g) added to a single meal all decreased serum response of beta-carotene, lutein, lycopene (over 100 mg in total given orally) in humans [126], with no significant differences between the types of fibers consumed. Similarly, in a study by Rock and Swendseid, 12 g of citrus pectin reduced serum response of 25 mg synthetic beta-carotene consumed within a single meal [127]. Furthermore, a negative correlation between dietary fiber intake in form of pectin, guar and cellulose and antioxidant capacity following a carotenoid (lutein, lycopene, p-carotene, canthaxanthin)/tocopherol mixture also indirectly indicated reduced carotenoid bioavailability from single meals [128]. Long- term studies however usually failed to observe a negative effect of consuming dietary fiber on carotenoid absorption. Intake of non-starch polysaccharides (NSP) was not found to be correlated with reduced serum levels of carotenoids (beta-carotene, lutein, lycopene) in a prospective cohort study [129], as subjects consuming more NSP also consumed more carotenoids. Likewise, in an intervention study by Castenmiller *et al.* [85], addition of dietary fiber from sugar beet rich in pectins to enzymatically liquefied spinach had no negative effect on lutein (6.6 mg) or beta-carotene (9.8 mg) absorption during a 3 wk dietary intervention study, as determined by serum concentrations. However, differences in dietary fiber intake were low (31 g versus 28 g/d). It remains to be elucidated whether this lack of effect could be due to adaptive mechanisms by the body, or due to other confounding factors such as increased intake of cartotenoids with higher fiber intake. In summary, while it seems that short-term intake of high amounts of fiber can have negative effects on carotenoid absorption, this has not been clearly shown for free living subjects during prolonged periods.

Minerals, Vitamins, Polyphenols, Phytosterols

A negative, dose dependent effect of supplementing 0.8-3.2 g/d plant sterol esters for 3.5 wks on lycopene and the sum of alpha plus beta-carotene was found by Hendriks *et al.* [130], which was explained by competitive mechanisms, either at the stage of micellarization, or incorporation into the enterocytes, and by reduction of LDL particles in blood plasma. The fact that a negative effect of plant sterols on beta-carotene uptake could be shown for Caco-2 cells [131] supports the theory of competitive mechanisms at the stage of micellarisation. Similar negative effects of supplementing 0.8-6.6 g/d plant sterols/stanols or their esters for 1-4 wks were shown in a number of additional human intervention studies [132-135]. Richelle *et al.* [136] included isotopically labeled carotenoids in human supplementation studies with 2.2 g/d for 1 wk, either with free or sterol esters, minimizing interferences with endogenous carotenoids. Their study further suggested that the more apolar sterol esters had a stronger effect on lowering cartenoid absorption compared to free sterols, related to their stronger partitioning into the oil phase versus the micellar phase of the intestine, however, this would need to await confirmation by further studies. Lower concentrations of 1 g of stanyl esters however did not reduce plasma and chylomicron response following consumption of beta-carotene fortified foods [137], which was explained by high interindividual variations of beta-carotene responses. Likewise, a feeding trial of 4 wks with 0.8-3.2 g/d of plant stanol estes did not lower beta-carotene concentrations in plasma [138], perhaps due to non-standardized diets.

In a study by Reboul *et al.* [73], addition of naringenin, a relatively lipophilic polyphenol, significantly inhibited lutein absorption in humans and Caco-2 cells, while addition of vitamin E and C, and a number of other polyphenols had no effects, increasing the odds that results could have been obtained by chance. Inhibition of absorption was suggested due to competitive mechanisms involving absorption via the SR-B1 transporter or interaction between naringenin and membrane lipids, affecting invagination of lipid raft domains containing lutein receptors. In another study by Hageman *et al.* [139], high vitamin E concentrations decreased plasma response of canthaxanthin in the rat. Ascorbic acid has been suggested to enhance lutein absorption in young adults [140], absorption was faster compared to intake of the supplement alone; however, the area under the curves (AUC) did not differ significantly, and no explanation was offered. Thus, there is weak evidence that vitamins or phytochemicals in concentrations regularly consumed within a diet have a considerable impact on carotenoid absorption, however, competitive effects with other lipid soluble compounds, especially when consumed in large amounts, seem likely.

A potential factor for carotenoid bioavailability which has not yet received much attention is the interaction of carotenoids with minerals and trace elements. An earlier report stated that beta-carotene increased absorption of iron in humans [141] and increased iron bioaccessibility of Caco-2 cells [142]. However, the mechanism of such observation could not be explained. It has been speculated that some sort of complex between the 2 compounds did form, altering iron solubility [143]. Unfortunately, beta-carotene absorption was not investigated in these studies. It might be hypothesized that a high concentration of minerals present in the diet could alter micelle stability. Also, a high concentration of sodium has been suggested to influence uptake of micellarized

carotenoids [144]. However, this has not yet been thoroughly investigated.

Other Carotenoids Present

A number of studies have suggested interactions between different carotenoids impacting their bioavailability, either during micellarization, competition for gastrointestinal uptake into the enterocytes, for chylomicron incorporation, or for tissue uptake/release from lipoproteins [77]. Most studies have suggested that this interaction is most likely due to competition at the micellarization step. For example, the fact that beta-carotene absorption and retinyl palmitate was decreased to a similar extent following co-supplementation of lutein indicated interferences during the absorption step [145]. The fact however that this could not be demonstrated in all studies [146], and that transport proteins have recently been suggested to partake in carotenoid uptake [67], might also suggest that carotenoids compete for absorption at the stage of entering the enterocytes.

In some human studies, carotenes reduced the absorption of other carotenes and xanthophylls. In a recent study by Reboul *et al.* [73], addition of a mixture of lycopene and beta-carotene impaired lutein uptake by Caco-2 cells. Decreased serum levels [147] and LDL's [148] associated with lycopene were found following short term beta-carotene supplementation, even though doses were not physiologically but high bolus doses (over 100 mg/d). A similar decrease of serum lycopene was found with 300 mg/d during 3 wks of beta-carotene supplementation [149]. Using more dietary realistic concentrations, Kostic *et al.* [150] giving 0.5 μmol/kg body weight beta-carotene as a single dose together with equal amounts of primarily free lutein found decreased serum responses of the latter, 53-61% compared to giving lutein alone, whereas beta-carotene uptake was not significantly affected. A single dose of beta-carotene (25 mg) also decreased absorption of 25 mg free, synthetic canthaxanthin in humans [151, 152] but not vice versa [152], as measured by plasma, chylomicron and VLDL subfractions. In dietary intervention studies, Castenmiller *et al.* [85] and van het Hof *et al.* [86] reported decreased lycopene serum values during lutein supplementation, with 11 mg/d from spinach during 3 wks and 9 mg/d from safflower oil (mostly free lutein) during 4 wks, respectively. Slightly but significantly reduced serum lutein concentrations were also found in humans following a 6 wk beta-carotene supplementation of 30 mg/d [153]. Long-term studies might however result in different outcomes. In a supplementation study over 6.7 year, 20 mg/d beta-carotene decreased lutein serum concentrations [154], however, another study over 4 year with 50 mg/d beta-carotene did not find such results [155], as did a 4 year study with 25 mg/d beta-carotene in patients with colonic adeno-carcinomas [156]. In another long-term study, supplementing 20 mg beta-carotene/d for 2 years with patients suffering from colecteral adenocarcinoma even increased lycopene and alpha-carotene values [157]. This might indicate either adaptive mechanisms by the body, or antioxidant sparing effects by co-supplementation of carotenoids, or both.

Somewhat contradictory to the above studies, it was earlier speculated that inhibition of micellarisation of the more polar xanthophylls is more difficult to achieve compared to the more apolar carotenes and that both carotenes and xanthophylls can inhibit inclusion of carotenes into micelles, but not that of xanthophylls [94]. If this should be the case, then micellarization is not the predominant step for determining bioavailability, as indicated by the number of studies showing a negative effect of carotenes on xanthophylls plasma occurrence mentioned above. However, in a study by van den Berg and van Vliet [145], 15 mg lutein from marigold extract rich in lutein diesters [158], but not lycopene (15 mg) reduced beta-carotene (15 mg) TRL response. Interestingly, Tyssandier *et al.* [159] found measurable interactions between carotenoid doses (about 30 mg lycopene, 24 mg lutein and 16 mg beta-carotene) when investigating carotenoid chylomicron incorporation postprandially, but not when measuring more mid-term plasma responses during 3 wk intervention trials (half the amount given in the postprandial trial). Both lutein supplements and lutein from spinach decreased lycopene chylomicron response from tomato puree and supplements significantly and vice versa, while beta-carotene response was only (negatively) affected by the lutein containing meals. In the same study, surprisingly, neither lutein nor lycopene plasma concentrations were negatively affected by the presence of the other carotenoid in the diet after 3 weeks of consumption of either tomato puree, tomato puree with spinach, or tomato puree with lutein supplements. On the contrary, plasma lycopene concentrations were significantly elevated almost 2-fold following consumption of tomato puree with spinach or lutein pills. This is in line with a recent 3 wk study of tomato puree added to spinach, indicating no decrease in lutein plasma concentration [160], again indicating either adaptive mechanisms, or antioxidant sparing effects. The study by Tyssandier *et al.* may also indicate that investigating the TRL fraction alone might not be a sufficient indicator for detecting carotenoid bioavailability, as some carotenoids might be sequestered preferentially into chylomicrons, delaying the response of other carotenoids present in the enterocytes, and highlights that distinguishing between short-term and more long-term effects might be prudent. In addition, the degree of esterification of the xanthophylls could impact results, as well as absolute amounts and the ratio of the carotenoids given. In summary, there is no evidence that carotenes or xanthophylls inhibit each other's status as expressed as serum or plasma levels based on a normal diet, even though acute responses may result in a short-term negative effect on absorption, as demonstrated especially for carotenes on xanthophyll absorption.

SPECIES OF CAROTENOID

In the majority of studies, the more polar xanthophylls tended to be of higher bioavailability compared to the carotenes [61, 73, 150]. Differences determining carotenoid bioavailability can be expected to result from varying release from the matrix, distribution into mixed micelles, discrimination during uptake by specific transporters, and chylomicron secretion and transport. The lower bioavailability of carotenes is usually attributed to their less efficient micellarization [161], based on their more apolar character. Indeed, lutein and zeaxanthin from spinach were micellarized more efficiently compared to the more apolar carotenes [162]. Lower lycopene uptake compared to beta-

carotene and lutein from various foods was reported based on Caco-2 studies [163], with uptakes of 2.5% for lycopene and 7% for lutein, their values being close to solubility (% micellarization) values [15], indicating that indeed the solubility in the mixed micelles could be a crucial limitating factor for carotenoid absorption, even though food matrix effects cannot completely be ruled out. In rats, canthaxanthin from olive oil was absorbed to a larger extent compared to lycopene [164], based on cannulated lypmph measurements. In humans, a higher plasma response of zeaxanthin and lutein compared to beta-carotene from betatene, an algae [165] and from various vegetables [86], was shown. While lutein content of betatene was low compared to beta-carotene, and dose effects could have explained results, this is not true for the various vegetable rich diet. Likewise, lycopene has been reported to possess lower bioavailability in humans compared to beta-carotene and lutein [166], based on chylomicron incorporation following ingestion of mashed vegetables.

Contradictory results have also been reported. Following ingestion of similar amounts of lutein (31 mg from marigold) versus lycopene and beta-carotene from single doses (capsules) resulted in lower TRL-AUC for lutein. It was suggested that this observation might have been due to faster lutein clearance from the TRL fraction and transport into other compartments, such as blood plasma or other tissues [167], as the mole polar xanthophylls have been reported to rest rather at the surface of the chylomicrons [91]. However, this could also reflect lower bioavailability from the natural lutein ester rich extract. A low serum response of lutein from a fruit and vegetable rich diet versus cryptoxanthin and alpha-carotene from the same diet was found by Yeum *et al*. [168] in human subjects during 15d periods, discussing the high beta-carotene content of the diet as a negative factor for lutein uptake. In another study, higher uptake of pure beta-carotene compared to pure lutein from DMSO solutions in Caco-2 cells was found [169], more stable emulsions were discussed to play a role [91], which might counterbalance, to some extent, poorer micellarisation.

Evidence that a lower apparent chain length through a lower number of double bonds could increase bioavailability originates from studies with lycopene precursors. Phytofluene was more bioavailable than lycopene, and zeta-carotene in rats fed a diet containing 10% tomato powder [170], based on liver and serum concentrations. Higher phytofluene plasma response compared to lycopene was also observed earlier in humans [37] based on plasma measurements following consumption of a supplement and tomato paste. However, both results might have been due to lower fractional absorption from the higher dose of lycopene.

Whether there exist differences in the bioavailability between xanthophylls remains controversial and the answer is impeded by the esterification. Bone *et al.* [171] found that zeaxanthin supplements were less well absorbed by humans than lutein supplements, but this was explained by the fact that zeaxanthin was given in a crystalline and not in esterified form dissolved in oil as the lutein supplement. Contrarily, Liu *et al.* [169] found higher absorption of zeaxanthin compared to lutein (unknown sources) from DMSO solutions in Caco-2 cells. Native (mostly esterified) capsanthin, found in pepper, was well absorbed by humans from paprika juice, as detected by plasma occurrence [172], while only poor chylomicron uptake of capsanthin (35 mg) and capsorubin (2 mg) from paprika was found in humans following ingestion of a single dose from oleoresin [173], which was attributed to the absence of lipases able to cleave the ester, due to matrix effects, or metabolism. Similarly, no absorption of the native xanthophyll-epoxides, violaxanthin (zeaxanthin 5,6, 5'6' diepoxide) and taraxanthin (lutein 5,6-monoepoxide), which can be relatively abundant in leafy vegetables and legumes, could be observed in a human study with 3 subjects [174], being in contrast to absorbable beta-carotene epoxides [175]. Furthermore, very low plasma concentrations of the epoxides neoxanthin and fucoxanthin from spinach and wakame were detected after a 1wk intervention in 5 healthy adults [176]. It is possible that conversion of these epoxides into their furanoid form takes place under acid pH, as reported for the conversion of neoxanthin into neochrome [177]. Limited micellarization does not appear to be a problem, as neoxanthin and fucoxanthin micellarisation were found to be comparable to that of lutein [176]. Also, good absorption of purified, unesterified neoxanthin and fucoxanthin was measured in mice [177]. It has even been suggested that the human lipase cannot well cleave esters of a number of carotenoids [178], however, the effectiveness of lipase in cleaving different carotenoid esters remains to be elucidated.

Chemical derivatives of carotenoids have also been investigated as a dietary source, and may be a good source of carotenoids, but studies remain scant. A synthetic and water dispersible astaxanthin, the disodium disuccinate derivative, was, probably due to its increased polarity, highly bioavailable in mice, as measured by plasma appearance, however, no control group was included [179]. In conclusion, it appears that polar carotenoids tend to be of higher bioavailability than apolar species. A number of xanthophyll esters and epoxides on the other hand seem to be of low bioavailability. It appears possible that some of the discrepancies might result from varying degrees of esterification within the food matrix.

ISOMER FORM OF CAROTENOID

In contrast to all-trans-beta-carotene, which has been reported to be the dominant beta-carotene isomer form in plasma [58], cis-lycopene concentrations in human plasma are comparatively higher than in foods [180], with relative concentrations typically ranging around 50% or higher in plasma as compared to <30% in foods [181]. The reasons are not entirely understood, but could be explained by preferred incorporation of the cis-form into the micelles (due to shorter apparent molecule length due to the bended structure), preferential absorption via the enterocytes, or isomerization from all-trans to cis forms, or both. Indeed, both isomerization of all-trans to cis-isomers and preferential absorption of lycopene cis-isomers *in vivo* have been suggested in a recent study investigating the effect of heat processing to increase the cis-isomer lycopene content in tomato products; baseline corrected TRL-AUC response of both cis and trans lycopene isomers was significantly higher (by about 20%) after consumption of a cis-lycopene isomer rich tomato

sauce compared to a very similar, all-trans rich sauce [40], with an increased shift to higher cis:trans isomer ratios in the TRL fraction, especially following the cis-rich sauce. A number of lycopene isomers in plasma have been reported, 5-cis, 9-cis, 13-cis, and 15-cis lycopene having the highest concentrations [182]. While 5-cis lycopene was detected in comparable high concentrations in plasma following tomato feeding studies [180, 182], the concentration of this isomer was relatively low following consumption of a tangerine sauce rich in tetra-cis lycopene, hypothesizing that 5-cis lycopene is predominantly formed from all-trans lycopene [41]. Where in the body a potential conversion of the all-trans to cis form of lycopene takes place has been subject of discussions. Conversion has been shown to take place under a sufficient acid pH such as in the stomach, but still depends on the matrix, as a stable matrix has been shown to stabilize trans-lycopene and to prevent isomerization under acidic pH [95]. This matrix stabilization effect could explain why not all studies could clearly indicate a trans-cis isomerization effect in the stomach [166]. Boileau *et al.* [183] found significantly higher percentage of cis-lycopene isomers in mesenteric lymph (77%) compared to dose, stomach and intestinal contents (6-18%) in ferrets. It has thus been suggested that there is a continuous isomerisation of all-trans lycopene to cis-lycopene isomers in the body, with higher proportions of cis-lycopene following the order tissue> serum>chylomicrons [181]. This is also supported by a previous human study by the observation that upon cessation of dietary (predominantly all-trans) lycopene intake, the percentage of cis-isomers of total lycopene increases in plasma [184]. It has further been suggested that isomerization continues toward an equilibrium between both lycopene forms in the plasma TRL fraction. An earlier *in vitro* study investigating cis-trans isomer distribution in organic solutions suggested a ratio of 50% all-trans to 50% cis-lycopene to be the most stable [101], values which were close to the ones observed in human studies [40].

Besides the degree to which carotenoids are incorporated into the micelles, micelle stability and the location of carotenoids within micelles has been suggested to be of equal importance [91], unfortunately, information of factors influencing carotenoid stability in mixed micelles is scarce. It has been suggested that cis-isomers are less likely to crystallize compared to their trans-forms, thus having a higher solubility and tendency to be incorporated into mixed micelles [185]. In accordance with this, Ferruzzi *et al*. found higher micellarization of cis-beta-carotene compared to its all-trans form, while the further uptake into Caco-2 cells did not seem to discriminate between the two forms [186]. Similar results for beta-carotene micellarization were reported by Tyssandier *et al.* [166]. On the contrary, the all-trans-form of beta-carotene was found to be easier absorbable from the micelles into Caco-2 cells [15], suggesting specific transporters for facilitated uptake to explain why this is the predominant iso-form in humans.

Similar as for lycopene, isomerization of lutein from all-trans to cis-forms was suggested during micellarization, but not after uptake into Caco-2 cells [162]. In humans, astaxanthin Z- ismomers accumulated preferentially in blood plasma compared to the all-E form [43, 187]. Accumulation of the Z-form has also been reported for canthaxanthin in the plasma of ferrets [188].

ESTER FORM VERSUS FREE FORM, CHEMICAL DERIVATIVES

There is scientific controversy on the bioavailability of xanthophyll esters. Xanthophylls in nature are present both in the esterified (mono and diester) and free form [189-191]. It appears that these esters cannot be absorbed and transported intact in the human bloodstream, or at least not to a significant extent with normal dietary intake [192, 193] and thus have to be cleaved by human bile salt stimulated lipase, acting at the lipid-water interface of micellular or emulsified substrates, or pancreatic cholesterol esterase [13, 178, 194]. I has been suggested that this cleavage takes place in the gut, albeit it has also been reported that xanthophylls esters can be taken up intact by Caco-2 cells [194, 195]. However, because of higher apolarity, incorporation into mixed micelles and Caco-2 cell uptake was found to be lower compared to their free forms. Due to its higher polarity, *in vitro* studies have therefore suggested that free lutein is better accommodated in O/W microemulsions [196], compared to its ester form. In accordance with this hypothesis, micellarization and apical uptake by Caco-2 cells was found to be lower for zeaxanthin esters from wolfberry compared to its free form [197]. Likewise, beta-cryptoxanthin esters were less bioaccessible in a Caco-2 cell study compared to its free form [194]. However, it is not sure whether lipase activities in the *in vitro* studies are comparable to *in vivo* conditions. On the other hand, it was speculated that the more apolar esters are situated in the core of the liposomes during digestion, and are thus better dissoluted and or protected, especially in a fat/oil rich matrix. Interestingly, lutein esters tended to be in fact more bioavailable than free lutein in a study with healthy human subjects, based on serum concentrations [192], but results were not significant, and the supplements were of different formulations, a crystalline suspension in oil (lutein) form vs. powder (lutein ester). For zeaxanthin, a slightly but significantly higher plasma response in humans of the dipalminate ester from wolfberry was observed as compared to its free form [198]. In contrast, a recent human study found no differences between the bioavailability of lutein supplements from its ester vs. free form in oil as measured by the TRL response and serum concentrations [90]. Likewise, comparable plasma responses of esterified and non-esterified beta-cryptoxanhtin from a yoghurt drink in humans was found [199]. Thus, a potential lower micellarization of the xanthophyll esters might be counterbalanced by better protection/dissolution/stability within the micelles, so that other matrix factors could have a more predominant impact on their bioavailability.

HOST-RELATED FACTORS, GENETIC FACTORS, NUTRIENT STATUS

It has been speculated that atrophic gastritis, occurring in ca. 20% of elderly adults, affects cartotenoid bioavailability due to changes (increase) in pH [200]. In deed, a decreased chylomicron response of lycopene, but not lutein and beta-carotene was found in subjects 60-75 years of age compared to 20-35 years of age [201]. In addition, Bowen *et al*. [192] found a negative correlation of age and lutein serum

appearance in a supplementation study with lutein esters, hypothesizing that the efficiency of the esterase enzymes could decline with age; however, more long term studies in this area are needed. Besides age, the impact of gender has also been discussed. Women are known to have higher levels of carotenoids in the plasma, albeit this is more likely to be related to higher consumption of carotenoids in relation to a lower plasma volume. However, also estrogen has been speculated to increase carotenoid absorption due to effects on the lipid metabolism such as chylomicron turnover [202].

A slower GI passage was associated with higher lutein absorption (15 mg) in ileostomy subjects from whole leaf spinach whereas beta-carotene (10 mg) absorption was not effected [23]. The authors concluded that GI-transit time is more crucial for lutein compared to the less polar beta-carotene, albeit methodological limitations of the chylo-micron measurements or varying doses might also explain results. Future studies will have to confirm these results. As with decreased passage time, parasite infections and effective intestinal surface available for absorption have also shown to effect carotenoid absorption. After deworming, beta- carotene bioavailability as measured as plasma retinol was significantly higher from dewormed children [203]. Similarly, increased absorption measured via liver uptake of alpha and beta-carotene was found in rats following the administration of antibiotics due to decreased intestinal flora and decreased metabolization of carotenoids through gut bacteria, or due to increased GI transit time or bile salt concentration when compared to the intact flora [204].

The presence of enzymes, such as lipase, has also reported to influence carotenoid bioavailability. Triacyl-glycerol lipolysis has been suggested to play a role, as after solubilization in the fat matrix, carotenoids have to be transferred from the fat to the aqueous phase [166]. Whereas the incorporation of carotenes into mixed micelles seemed to depend on the concentration of pancreas lipase and increased with higher lipase concentration, the incorporation of xanthophylls was not influenced [13, 91, 94], which might be related to the fact that the more polar xanthophylls tend to be present at the surface of the micelles, whereas the carotenes rest inside the core of the micelles. Likewise, in order to be released from the mixed micelles, activity of pancreatic phospholipase A2 has been suggested to be of importance to cleave phospholipids and to allow for transfer of carotenoids from the mixed micelles to the enterocytes [205]. Again, it could be speculated that the activity is more important for the carotenes resting in the core of the mixed micelles.

Correlates of the sum of lutein and zeaxanthin concen-tration in serum and host related factors were investigated by Gruber *et al*. [206]. The investigators found significant correlations between lower serum lutein and zeaxanthin and smoking, heavy drinking, being white, female, not being physically active, higher fat-free mass, a higher percentage of fat mass, a higher waist-hip ratio, lower serum cholesterol, a higher white blood cell count, and high levels of C-reactive proteins. However, a high number of variables have been included, increasing the risk of wrong positive findings, even though some of these results have been reported earlier by other investigators. Furthermore, despite incorporating a high number of variables, the final correlation between the model and serum lutein+zeaxanthin did not increase over 0.18, highlighting that many predictors of serum carotenoids are unknown. Limited information is also present on the concentration of carotenoid status and carotenoid absorption. While vitamin A status has shown to impact absorption of beta-carotene [58], no such dependency between status and absorption has been reported for the other carotenoids.

CONCLUSIONS

Numerous factors can impact carotenoid absorption, including the amount and possibly type of dietary lipids consumed, the polarity and isomer form of the carotenoid, the stability of the matrix to which the carotenoid was bound, and additional dietary factors such dietary fiber. Albeit there is information present on some of these aspects influencing carotenoid absorption in humans, information on other factors, including precursors and metabolites of carotenoids, the impact of their state of esterification, and interaction with other dietary components such as phytosterols or minerals is still scarce. The importance of carotenoids for human health and the limited knowledge on their interaction with a number of food components merits further investigations regarding their bioavailability.

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