CHAPTER 9

Metabolic Fate of Bioaccessible and Non-bioaccessible Carotenoids

TORSTEN BOHN

Luxembourg Institute of Health (LIH), Population Health Department, rue 1 A-B, Thomas Edison, L-1445 Strassen, Luxembourg
*E-mail: torsten.bohn@gmx.ch

9.1 Introduction

Carotenoids are among the most abundant lipophilic secondary plant compounds consumed in the diet. Carotenoids include mostly C-40 tetraterpenoids, though also C-30 tri-[1] and C-50 pentaterpenoids[2] have been reported, at least in various bacteria, though due to their minute intake, they are not of dietary relevance. However, their dietary variety is increased when also counting the many apo-carotenoids (*i.e.* carotenoid metabolites derived from oxidative cleavage of carotenoids, which may occur in bacteria, fungi and plants, but also in animals).[3] Major plant-derived apo-carotenoids include, for example, bixin, crocin, picrocrocin, abscisic acid, strigolactone and mycorradicin.[4]

Though several hundreds of carotenoids have been described in nature, it is estimated that only a few dozen or so predominate in our daily diet.[5,6] Their dietary intake as well as circulating blood levels have been related to

Food Chemistry, Function and Analysis No. 5
Non-extractable Polyphenols and Carotenoids: Importance in Human Nutrition and Health
Edited by Fulgencio Saura-Calixto and Jara Pérez-Jiménez

Published by the Royal Society of Chemistry, www.rsc.org

the reduced risk of several chronic diseases, including certain cardiometabolic conditions such as the metabolic syndrome[7] and certain types of cancer,[8,9] but also allergies[10] and asthma.[11] A carotenoid health index based on total plasma carotenoids has been proposed by Donaldson, with <1 μM total carotenoids constituting a very high health risk[12] regarding total mortality (lower threshold), and less so for metabolic syndrome and cancer (higher threshold).

Some compounds such as beta- and alpha-carotene as well as beta-cryptoxanthin possess a beta-ionone ring, and these compounds can be cleaved by the human body into vitamin A active compounds such as retinal. In addition, lutein and zeaxanthin, belonging to the oxygen-carrying xanthophylls (as opposed to the oxygen-free carotenes), have been related to improved vision aspects in subjects with age-related macular degeneration (AMD),[13] the major cause of blindness in the elderly. While in the past, most health benefits have been attributed to the radical-scavenging properties[14] and light-absorbing properties[15] of the native carotenoids, more recent reports have rather emphasized the potential of carotenoids and their metabolites to interact with nuclear transcription factors, such as with NF-κB and Nrf2,[16,17] influencing gene expression following their binding to nuclear receptors, which then regulate many inflammatory agents such as cytokines (IL-6, IL-1β) and antioxidant-related enzymes, including superoxide dismutase (SOD), catalyze (CAT) and heme-oxygenase (HO-1).

These latter mechanisms have therefore been highlighted as relating to reduced inflammation and strengthening the body's own antioxidant defense system. However, it has also been suggested that concentration-dependent effects could occur, with lower physiological concentrations fostering these health-beneficial effects, while higher concentrations may bear the risk of pro-inflammatory, pro-oxidant and thus adverse health effects.[18,19] Such negative effects were found in several supplementation trials in which beta-carotene was given concomitantly with vitamin E and A, as in the ATBC[20] and CARET trials,[21] respectively, and were found to increase, not decrease, the lung cancer rate, possibly due to pro-carciongenic intermediate formation following reaction with cytochrome oxidases. Thus, these results have not only questioned the dose-related effects, but also the nature of the bioactive constituents (*i.e.* native carotenoids *vs.* their metabolites and/or degradation products).

In fact, carotenoids have only low bioavailability – the amount that is absorbed and used for physiological functions and or storage (Table 9.1) – of approximately between 5% and 30%. The majority of carotenoid absorption is thought to take place in the small intestine. Absorption in the stomach, due to their lipophilic nature requiring micellization, is thought to be non-existent, while absorption from the colon remains an open question, but has never been shown.[18] Most bioavailability trials show absorption occurring between 2 and 8 hours following their intake, as measured by their appearance in the chylomicron-rich fraction of the plasma.[22–24] The low bioavailability of carotenoids is mostly due to the low solubility of these highly

Table 9.1 Concentration of carotenoids in human blood plasma/serum and various tissues.

Carotenoid	Tissue	Concentration (nmol g^{-1} or nmol mL^{-1})[a]	Reference
Beta-carotene	Plasma, male/female	0.36 ± 0.01/0.54 ± 0.01	56
Lycopene	Plasma, male/female	0.74 ± 0.01/0.71 ± 0.01	56
Lutein	Plasma, male/female	0.38 ± 0.01/0.44 ± 0.01	56
Zeaxanthin	Plasma, male/female	0.09 ± 0.01/0.09 ± 0.01	56
Alpha-carotene	Plasma, male/female	0.12 ± 0.01/0.20 ± 0.01	56
Beta-cryptoxanthin	Plasma, male/female	0.23 ± 0.01/0.34 ± 0.01	56
Total carotenoids	Plasma, male/female	1.94 ± 0.02/2.35 ± 0.03	56
Zeta-carotene	Plasma, females	0.11 ± 0.09	57
Phytoene	Plasma, females	0.04 ± 0.02	57
Phytofluene	Plasma, females	0.17 ± 0.07	57
Lutein + zeaxanthin	Retina	65–225 pmol	58
Beta-carotene	Adipose tissue	0.32 ± 0.10	Reviewed in ref. 6
Lycopene	Adipose tissue	0.36 ± 0.23	Reviewed in ref. 6
Lutein/zeaxanthin	Adipose tissue	1.19 ± 0.56	Reviewed in ref. 6
Beta-carotene	Liver	5.9 ± 6.3	Reviewed in ref. 6
Lycopene	Liver	8.4 ± 11.5	Reviewed in ref. 6
Lutein/zeaxanthin	Liver	2.2 ± 1.6	Reviewed in ref. 6
Total carotenoids	Skin	0–0.7	59
Total carotenoids	Lung	1.95 ± 2.82	53
Beta-carotene	Lung	0.35 ± 0.44	53
Alpha-carotene	Lung	0.23 ± 0.27	53
Beta-cryptoxanthin	Lung	0.42 ± 0.75	53
Lycopene	Lung	0.57 ± 1.11	53
Lutein	Lung	0.48 ± 0.66	53
Total carotenoids	Kidney	3.05 ± 4.21	53
Beta-carotene	Kidney	0.55 ± 0.73	53
Alpha-carotene	Kidney	0.30 ± 0.40	53
Beta-cryptoxanthin	Kidney	0.45 ± 1.04	53
Lycopene	Kidney	0.62 ± 0.62	53
Lutein	Kidney	1.21 ± 2.83	53
Beta-carotene	Prostate	0.54 ± 0.09 (SEM)	60
Lycopene	Prostate	0.80 ± 0.08 (SEM)	60
Lutein	Various brain tissues[b]	0.02–0.08	61
Zeaxanthin	Various brain tissues	0.01–0.03	61
Beta-cryptoxanthin	Various brain tissues	<0.01	61
Beta-carotene	Various brain tissues	0.01–0.03	61
Total carotenoids	Adrenals	9.4 ± 7.8 (SEM)	62
Beta-cryptoxanthin	Adrenals	0.66 (0.01–2.90)	62
Lycopene	Adrenals	1.90 (0.19–5.60)	62
Alpha-carotene	Adrenals	1.22 (0.11–7.52)	62
Beta-carotene	Adrenals	5.60 (0.68–31.83)	62

[a]Unless otherwise stated, mean ± SD.
[b]Infants: prefrontal cortex, frontal cortex, hippocampus, auditory cortex and occipital cortex.

apolar constituents (log *p* values ~8–12[25]) in the gut and limited availability for absorption (*i.e.* poor bioaccessibility).[26] In addition, following cellular uptake into the enterocyte, carotenoids may be further metabolized by beta-carotene oxygenases 1/2 (BCO1/2) to produce several apo-carotenoids.[27–29] These may then be re-esterified[30,31] prior to their further sequestration by chylomicrons, as is known for retinol, and later experience tissue distribution *via* low-density lipoproteins/high-density lipoproteins in particular.

However, little information exists regarding potential metabolites, their concentrations in various tissues and also the fate of the large fraction of intestinally non-absorbed carotenoids, as these may be further fermented by the microbiota, similarly to, for example, polyphenols, though this remains unknown.[18] In addition, recent studies on naturally occurring apo-carotenoids such as on norbixin or bixin from annatto seeds or crocetin from safflower suggest that these compounds appear to be highly bioavailable[32,33] and may also be very bioactive, as has been shown for abscisic acid, a plant hormone derived from carotenoids, which was shown to improve glucose tolerance[34] and inflammation-related aspects in inflammatory bowel diseases (IBD).[35] For several of these apo-carotenoids, it is also not known whether they can be produced in the human body or if they are entirely derived from consuming plant-based foods.

This is of importance, as it appears that several of the carotenoid metabolites may be bioactive – in fact, more than the native compounds, a fact that may be ascribed to their better cytosolic solubility and potential to interact with transcription factors, as well as their higher electrophilicity, resulting in better binding to, for example, cysteine residues of NF-κB[36] and Nrf2.[37] In addition, other metabolites, such as those of lycopene, have been reported to interact with retinoic acid receptor (RAR)[38,39] and retinoid X receptor (RXR) nuclear receptors,[40] thus mimicking the immune-related functions of vitamin A active compounds. However, the further fate of these more polar carotenoid derivatives remains largely speculative.

This review tries to summarize our current knowledge regarding the metabolic fate of both bioaccessible and non-bioaccessible carotenoids and to point out the boundaries of our knowledge regarding the metabolism of these mostly colorful dietary constituents and their relation to health aspects.

9.2 Carotenoid Concentrations: from Diet to Tissue Levels

The most frequently consumed carotenoids in the diet include beta-carotene, lycopene, lutein, alpha-carotene, beta-cryptoxanthin, phytofluene, phytoene, neoxanthin, violaxanthin and zeta-carotene, though the sequence of intake may change according to dietary habits. For example, in the USA and Italy, due to the intake of tomato products including ketchup, lycopene appears to constitute the most frequently consumed carotenoid, with an intake of up to approximately 10.5 and 7.5 mg day^{-1} in those countries, respectively.[41,42] Total

carotenoid dietary intake ranges typically between 10 and 20 mg day^{-1}.[43–46] In plant-based food items, concentrations vary widely, but can reach up to 20 mg/100 g in some cases, such as for spinach and tomatoes,[47] though other leafy vegetables such as cabbages,[48] as well as carrots,[49] sweet potatoes[50] and a few animal foods such as eggs and salmon[47] constitute important and frequently consumed dietary carotenoid sources.

As not all carotenoids and their food sources have comparable bioavailability, human tissue and plasma levels do not necessarily reflect total dietary intake (Table 9.1), though significant and reasonably strong correlations between various tissue carotenoid concentrations have been reported, such as for plasma with skin and buccal cells[51] as well as dermal levels,[52] and also between various organs such as kidney, liver and lung,[53] suggesting that plasma may serve as a reasonable indicator for comparing and perhaps assessing carotenoid status. At least for several tissues, only limited discrimination occurs between the concentration in the circulatory system and the tissues (Table 9.1), though certain tissues such as the liver and retina appear to store carotenoids at higher concentrations (see Chapter 8).

It is also interesting to note that, compared to other frequently consumed phytochemicals such as polyphenols, which are consumed in much higher amounts (up to 1 g day^{-1}), plasma concentrations are at least equally high, even when taking into account polyphenol metabolites such as glucuronides/sulfates.[54] In fact, carotenoids appear to constitute the most predominant phytochemicals in human plasma, owing to their reasonably long half-life, due to their more limited metabolism compared to, for example, polyphenols. In a human depletion study by Burri *et al.*[55] with 18–42 year-old females, half-lives of 27 days (lycopene), 35 days (beta-carotene) and 76 days (lutein) were found, likely reflecting losses from deeper body pools such as the liver and adipose tissue, perhaps suggesting increased metabolism of the carotenes compared to the xanthophylls. Similar half-lives were corroborated in other studies, as reviewed by Bohn *et al.*[6]

9.3 Carotenoid Bioaccessibility During Gastrointestinal Digestion

9.3.1 Overview of Factors Influencing Bioaccessibility

In order to come into contact with the small intestinal epithelium, where carotenoid absorption takes place, these very lipophilic molecules need to be solubilized in the aqueous environment (*i.e.* packaged into mixed micelles of approximately 5–10 nm in diameter).[25,63] These are formed during intestinal digestion and consist of a mixture of partially digested lipids (free fatty acids, mono- and di-glycerides), bile salts, phospholipids and other liposoluble micro-constituents from the diet, such as cholesterol or vitamin E.[64,65] Following their formation, mixed micelles can then diffuse through the mucus layer[66] to the unstirred water layer of the epithelium, where they interact with the cell membrane, following liberation of carotenoids and their cellular

uptake *via* either transporters or passive diffusion (see Chapter 4). The factors fostering adhesion to the epithelium and uptake include adhesion (governed by surface charges) and mobility in particular, with negatively charged particles resulting in generally higher transport rates.[67] This solubilization and becoming available for further absorption is also termed bioaccessibility.

Thus, micellization is a crucial step and a prerequisite for carotenoid bioavailability. Though only constituting the first important step of bioavailability after matrix release, several studies have reported a good correlation of carotenoid bioaccessibility with carotenoid bioavailability measures in human subjects,[68,69] also highlighting the pivotal role of carotenoid bioaccessibility (reviewed by Biehler and Bohn[70]).

Consequently, all factors that impinge on bioaccessibility are likely factors that can alter carotenoid bioavailability. These factors have often being summarized by the mnemonic term "SLAMENGHI," comprising carotenoid species, their molecular/chemical linkage, amount of carotenoids, dietary matrix, effectors of absorption/bioconversion, nutrient status of the host, genetic factors, other host factors such as diseases, and their interactions.[71] Regarding the metabolic fate of carotenoids, a first important consideration is therefore their bioaccessibility. While an extensive review of dietary and host-related factors is beyond the scope of this book chapter, and the reader is referred to other more comprehensive reviews,[5,6,26,72] the most important considerations that determine carotenoid bioaccessibility will be briefly highlighted in the following.

9.3.2 Matrix-related Factors, Bioaccessibility and Carotenoid Fate

In order to become bioaccessible, carotenoids first have to be released from the food matrix. In the following, they then have to be packed into mixed micelles. Consequently, all factors that influence these two steps determine whether carotenoids will be absorbed or, alternatively, passed on to the colon. The major factors influencing carotenoid release from the food matrix and their transfer to mixed micelles are shown in Tables 9.2 and 9.3, respectively, and include the type of carotenoid, type of matrix, food processing aspects, preparatory aspects and the amount of dietary fat and fiber present in the carotenoid containing meal. Food processing in particular appears to be a double-edged sword, as there is a critical balance between heat application that may macerate the matrix and contribute to the release of carotenoids and the negative influence *via* facilitating air and oxygen access, which together with heat may result in carotenoid oxidative degradation.

However, when summarizing the most crucial factors, it can be stated that softer food matrices with macerized cells wall, those containing carotenoids in rather oily droplets compared to crystalline forms, low amounts of dietary fiber, sufficient presence of dietary lipids and small particle size are the main factors enhancing carotenoid absorption[5,73,74] and reducing the amounts of unabsorbed carotenoids that are passed on to the colon. Additional dietary

Table 9.2 Aspects of food processing influencing carotenoid release from the matrix and therefore bioaccessibility and bioavailability.

Type of processing	Food matrix	Carotenoid(s) investigated	Type of study	Effect on bioavailability marker	Explanation of effects	Reference
Heating	Water fruit juice, milk fruit juice, soymilk fruit juice	Total and individual carotenoids	*In vitro* digestion	Reduced bioaccessibility following heat treatment	Unclear	99
Heating, homogenization	Tomato juice	Lycopene	Human postprandial trial	Higher bioavailability from heat-induced *cis*-isomers	*Cis*-isomer formation, higher micellization	100
Heating, homogenization	Carrot juice	Beta-carotene	*In vitro* digestion	Up to 20-times higher bioaccessibility of carotenes from juice than from raw carrots	Cell wall maceration, cell rupture	101
Cooking + pureeing	Carrots: raw *vs*. cooked + pureed	Beta-carotene	Human study with ileostomists	>20% higher absorption from processed carrots	Cell wall maceration	102
High-pressure homogenization	Tomato-based systems	Beta-carotene, lycopene	*In vitro* digestion	Decreased bioaccessibility (50→30%) of beta-carotene with high-pressure homogenization when insoluble fiber was present	Forming more stable fiber network during high-pressure homogenization	103
High-pressure homogenization	Water fruit juice, milk fruit juice, soymilk fruit juice	Total and individual carotenoids	*In vitro* digestion	No consistent negative effects on juices in general	Slight changes due to fiber network rearrangements and cell maceration	99

(*continued*)

Table 9.2 (*continued*)

Type of processing	Food matrix	Carotenoid(s) investigated	Type of study	Effect on bioavailability marker	Explanation of effects	Reference
High-intensity pulsed electric fields	Water fruit juice, milk fruit juice, soymilk fruit juice	Total and individual carotenoids	*In vitro* digestion	No clear negative effects on juices in general	Slight changes due to fiber network rearrangements and cell maceration	99
Particle size	Carrots and tomatoes	Beta-carotene, lycopene	*In vitro* digestion	Beta-carotene and lycopene bioaccessibility increased with particles <125 μm	Destruction of cell wall as major barrier of carotenoid release	104
Grinding, particle reduction	Different leafy vegetables	Beta-carotene	*In vitro* digestion	Several-fold improved bioaccessibility of ground leafy vegetables	Enhanced surface and improved release kinetics	105

Table 9.3 Aspects of food matrix and co-consumed dietary constituents influencing carotenoid micellization and therefore bioaccessibility and bioavailability, showing exemplary studies.[a]

Type of dietary constituents	Food matrix	Carotenoid(s) investigated	Type of study	Effect on bioavailability marker	Explanation of effects	Reference
Presence as crystals *vs.* dissolved in lipids	Mango, papaya, tomato, carrot	Beta-carotene, lycopene, lutein, beta-cryptoxanthin	Bioaccessibility following *in vitro* digestion	Fractional liberation and bioaccessibility of beta-carotene several times higher from mango/papaya than carrot/tomato	Different physical states, with crystalline forms taking more time to dissolve	106
Dietary fiber	Supplements given with or without either pectin, guar, alginate, cellulose or wheat bran	Beta-carotene, lycopene, alpha-carotene, canthaxanthin, lutein	Human postprandial	Decreased plasma AUC (24 h), 33–43% for beta-carotene, 40–74% reduction for lycopene, no effect on other carotenoids	Binding of fiber to bile salts and phospholipids, effect on lipase, desquamination of mucosal cells	107
Dietary fiber	Alginates, carboxymethylcelluloses, and methylcelluloses, pectins added to purified carotenoids in oil	Lutein, beta-carotene	*In vitro* digestion and cellular uptake (Caco-2 model)	Up to 31% reduced micellization and up to 45% cellular uptake	Increased viscosity, bile salt binding	108
Dietary lipids	Avocado fruit/oil	Alpha-carotene, beta-carotene, lutein	Human postprandial	Increase: 7.2-, 15.3- and 5.1-fold of TRL-AUC, respectively *vs.* no added lipids	Enhanced micellization of carotenoids with lipids	24
Dietary lipids	Yellow peppers with medium-chain triglycerides, long-chain triglycerides or indigestible lipids	Beta-carotene	*In vitro* digestion	Higher bioaccessibility, especially with long-chain FA	Enhanced micellization of carotenoids with lipids	109

(*continued*)

Table 9.3 (*continued*)

Type of dietary constituents	Food matrix	Carotenoid(s) investigated	Type of study	Effect on bioavailability marker	Explanation of effects	Reference
Free and esterified plant sterols (2.2 g)	Beta-carotene isotopic marker in milk	Beta-carotene	Human trial, plasma appearance	50% reduced absorption with plant sterols	Competition for micellization	80
Proteins	Artificial micelle systems digested with milk cream or canola oil	Beta-carotene	*In vitro* digestion	Higher bioaccessibility of carotenoids from milk cream than canola oil	Degradation products of proteins aiding in emulsification	84
Calcium	Tomato paste	Lycopene	Human postprandial	83% reduced plasma-AUC of lycopene with 500 mg dose	Binding of calcium with bile salts and FA	76
DM: calcium, magnesium	Spinach, carrot juice, tomato juice, apricot juice, individual carotenoids	Beta-carotene, lycopene, lutein, neoxanthin	*In vitro* digestion and cellular uptake (Caco-2 model)	Reduction by up to 100% with equivalent full RDA intake	Binding of DM with bile salts and FA, causing precipitation of bile salts and poorly soluble soaps	75,110,111

[a]AUC: area under concentration *vs*. time curve; DM: divalent minerals; TRL: triacyl glycerol-rich lipoprotein fraction; RDA: recommended dietary allowance; FA: fatty acids.

factors that may play a role but require more studies are dietary minerals, compounds competing for micellization, such as phytosterols and other lipophilic vitamins, and proteins. Dietary minerals may reduce carotenoid solubilization in the gut due to binding of released fatty acids and bile salts,[75] though the two human studies conducted thus far remain somewhat contradictory.[76,77] Other lipophilic constituents such as cholesterol and phytosterols may also compete for micellization and therefore reduce carotenoid bioaccessibility. This was shown for high concentrations of cholesterol *in vitro*,[78,79] phytosterols limiting beta-carotene[80] but not beta-cryptoxanthin availability in humans,[81] and also according to a recent meta-analysis where plant sterol and plant stanol intake lowered carotene and, less so, xanthophyll concentrations in human blood plasma,[82] and vitamin E lowered canthaxanthin absorption in rats.[83] Proteins may produce peptides with emulsifying properties during digestion that could aid in the transition of carotenoids from lipid droplets to mixed micelles,[84,85] and their emulsifying properties have been employed for encapsulating carotenoids to enhance both shelf-life stability and also bioavailability aspects.[86] However, no human trials investigating the effects of proteins on carotenoid absorption have been reported.

Also, due to a typically lower aqueous solubility and the higher tendency to aggregate (especially lycopene) and to be present in the matrix in crystalline form, carotenes tend to be of lower bioaccessibility compared to xanthophylls (reviewed by Desmarchelier and Borel[5] and Bohn[87]), and this appears to cause their generally lower fractional absorption and bioavailability expressed as percentage of ingested dose,[24,88,89] resulting in a larger proportion of carotenes *vs*. xanthophylls reaching the colon. For xanthophylls, lower bioaccessibility and bioavailability of the densely packed H-aggregates compared to more loosely packed J-aggregates (present in the absence of hydrogen bonds between molecules such as at lower pH or when esterified) have been proposed.[90] In addition, *cis*-isomers of carotenoids appear to be of higher bioaccessibility, most likely due to their more bent structures and shorter apparent molecular lengths, which improve micellization[91,92] and often bioavailability.[24,93–95] For this reason, the rather apolar phytoene and phytofluene, predominantly present in their 15-*cis* form and as variable *cis*-isomers,[96] respectively, were found to be highly bioaccessible[91] and also bioavailable (almost 60%), as shown in a postprandial study with isotopically labeled phytoene,[97] though the nature of the isomer(s) given was not revealed. A high bioavailability was also shown for a *cis*-product of lycopene, tetra-*cis* lycopene or prolycopene, which showed an almost eight to ten-times higher area under the curve (AUC) than all-*trans* lycopene from tangerines compared to red tomato sauces[93,98]

9.3.3 Degradation in the Gastrointestinal Tract

Most carotenoids are present in the all-*trans* form in fruits and vegetables and their products, though through heat, light and oxygen exposure, *trans–cis* isomerization as well as oxidation to certain apo-carotenoids (mostly apo-carotenals) can occur, as reviewed previously.[27,112] In a recent study by

Kopec *et al.*,[113] it was investigated whether there were digestive losses of beta-carotene, lutein and lycopene dissolved in sunflower oil, as studied by a gastrointestinal (GI) *in vitro* digestion system from a simulated meal containing mostly sucrose and additional phospholipids. While *trans–cis* isomerization was found to be insignificant, losses of lutein, lycopene and beta-carotene during GI digestion with digestive enzymes (pepsin, pancreatin) were 40%, 20% and 40%, respectively. Metmyoglobin further increased losses of lycopene and beta-carotene to approximately 30% and 80%, respectively, possibly due to oxidative degradation as triggered by iron. It is possible that the formed microcrystals of lycopene protected it from further oxidation and thus losses were lower compared to beta-carotene. The absence of digestion enzymes even enhanced degradation, perhaps as proteins digested with enzymes conferred some protection by forming a protective layer. Similar losses of 20% and 30% for lycopene and beta-carotene, respectively, during simulated digestion in a dynamic model were reported earlier by Blanquet-Diot *et al.* based on tomato-rich meals.[114] In another study, losses of beta-carotene from digested spinach of up to 70% after the small intestinal phase were reported,[115] based on digested spinach (Table 9.3), while lutein losses were lower (25%), perhaps due to higher micellization and thus protection form degradation. Losses of carotenoid standards dissolved in acetone/oil were also considerably higher compared to carotenoids in juice, demonstrating that the matrix could have protective effects, preventing carotenoid oxidation. Other studies reported virtually no detectable losses of lutein during simulated digestion.[114,116] It is likely that differences in test meals (matrix *vs*. solvents), time and pH of digestion or even mechanical factors such as shaking speed did contribute to the observed differences. The absence of significant isomerization was in line with previous studies with lutein,[117] beta-carotene[118] and lycopene[92,119] *in vitro*, and also *in vivo* for beta-carotene.[69] In the study by Kopec *et al.*,[113] no further oxidation products were detected, which was different from an earlier report by the same authors employing artificial emulsifiers[120] and the presence of iron, where several epoxides and apo-carotenals were detected (Table 9.4). Thus, though losses of carotenoids appear to be quite variable, mostly depending on the original matrix and co-digested food constituents, typically 50% or more are expected to reach the site of absorption. Indeed, it can be postulated that a matrix that releases carotenoids rapidly during digestion ensures rapid transition of the lipid droplets to mixed micelles and is able to bind iron (otherwise triggering degradation), which would be associated with only minimal carotenoid degradation.

Exceptions regarding digestive stability exist for carotenoids that are unstable under acidic conditions, as these are more prone to structural changes during the gastric phase, where the pH could drop to as low as 2 (absence of meal). This has been shown for the epoxycarotenoids violaxanthin and neoxanthin, which undergo, at least partly, epoxide–furanoid transition during digestion.[75] Nevertheless, their bioaccessibility appeared to be high, at around 49% and 30% in the latter study, respectively. In another

Table 9.4 Degradation products of carotenoids formed during simulated digestion.

Degradation product	Parent compounds	Factors fostering degradation	Place of degradation	Type of study	Reference
Neochrome	Neoxanthin	Low pH	Likely gastric phase	*In vitro* digestion	75 and 127
Auroxanthin Luteoxanthin	Violaxanthin	Low pH	Likely gastric phase	*In vitro* digestion	75 and 128
(*E*)-beta-apo-13-carotenone (*E*)-beta-apo-15-carotenal(retinal) (*E*)-beta-apo-14′-carotenal (*E*)-beta-apo-12′-carotenal (*E*)-beta-apo-10′-carotenal (*E*)-beta-apo-8′-carotenal 5,6-Seco-beta-carotene-5,6-dione (*E*)-beta-carotene-5,8,5′,8′-diepoxide (*E*)- and (*Z*)-beta-carotene-5,6,5′,6′-diepoxide (*E*)- and (*Z*)-beta-carotene-5,6-epoxide (*E*)-beta-carotene-5,6,5′,8′-diepoxide (*E*)- and (*Z*)-beta-carotene-5,8-epoxide (*Z*)-β-carotene	Beta-carotene	Oxidizing agent (iron)	Gastric phase	Simplified gastric digestion model, pH 4	120 and 129

study, recovery of violaxanthin from raw spinach was below 10% in an *in vitro* digestion system. However, the pH of both models was adjusted to 2, which does not reflect a postprandial pH in the stomach, when the pH could rise to as high as pH 5.[121] However, following epoxide–furanoid transition, violaxanthin would react to form auroxanthin and luteoxanthin, and neoxanthin to from neochrome; epimers of these compounds can likewise be formed. These compounds have been shown to be taken up by Caco-2 cells as a model of the intestinal epithelium, following *in vitro* digestion with a gastric pH of 3.[111,122] However, concentrations in the human bloodstream[123] were shown to be very low, possibly supporting the fragility of the original epoxycarotenoids. In another human study with three subjects, none of the original compounds or breakdown products of violaxanthin and neoxanthin could be found in the plasma following 50 mg bolus doses.[124] It is also possible that these compounds react to form further unknown metabolites or are shuffled out of the epithelial cell back into the gut lumen.

Taken together, losses from test meals have been reported to average around 20–30% for most carotenoids. The absence of *cis*-isomer formation during digestion suggests that only minor isomerization occurs or that isomers quickly further degrade, which appears less likely as *cis*-isomers appear to be generally as stable during digestion as the all-*trans* form.[114] Several metabolites have been reported during GI digestion, as summarized in Table 9.4. Whether these can be further absorbed is unclear. Studies that have investigated more polar carotenoid metabolites from plants indicate that shorter, more polar apo-carotenoids can be absorbed. For example, in a human postprandial study with crocetin from saffron from crocus flowers,[32] plasma AUC following doses of 7.5, 15 and 22.5 mg were 670, 1130 and 1840 ng hour^{-1} mL^{-1} (100, 200 and 250 ng mL^{-1} peak concentrations), respectively, apparently not saturable at these doses, and bioavailability levels were comparable to native carotenoids (*e.g.* 250 ng hour^{-1} mL^{-1} following the consumption of 19 mg lycopene from tomato soup[76]), though plasma appearance was slower for lycopene. Also, doses of 16 mg of bixin and 0.5 mg of norbixin were well bioavailable, reaching 12 and 58 ng mL^{-1} plasma peak concentrations.[33] In a very recent study, various apo-carotenals were show to be taken up by Caco-2 cells, also emphasizing that these appear to be absorbable.[125] Likewise, in a mouse study, apo-10′-lycopenoic acid, a tentative *in vivo* metabolite of lycopene, was shown to be bioavailable in mice and stored in the liver, while acting on SIRT1 and decreasing hepatic fat accumulation.[126]

9.4 Host-related Factors Governing Carotenoid Digestion, Cellular Uptake and Absorption

9.4.1 Carotenoids during GI Digestion

Host-related factors predominantly include the available intestinal surface area for absorption, which can be diminished *via* various diseases such as IBD[132] or surgically removed short bowel syndrome following, for example, cancer surgery and factors that influence the amount of digestion enzymes

and bile salts needed for carotenoid absorption, such as pancreatitis.[133] Also, older age may contribute to decreased intestinal surface[134] and result in lower circulating carotenoid concentrations. Recently, these factors have been reviewed and found to contribute to inter-individual variation of plasma carotenoid concentrations.[6]

Again, during intestinal digestion, lipase and bile salts appear to constitute the most crucial agents fostering carotenoid solubilization, and several genetic aspects (*i.e.* single-nucleotide polymorphisms [SNP]) have shown to be associated with altered carotenoid bioavailability as measured by plasma or plasma-triacylglycerol-rich lipoprotein (TRL) fractions. These included *PNLIP* (encoding pancreatic lipase) and *CLPS* (encoding colipase), which were shown to be related to lycopene[23] and cholesterol absorption,[135] respectively. Several *in vitro* studies have shown that without either bile or pancreatin, carotenoid bioaccessibility was severely compromised. While some studies emphasized that the absence of pancreatin had a more drastic effect,[78] others emphasized that the absence of bile more drastically reduced carotenoid bioaccessibility,[122,136] possibly differing due to specific interactions with the matrix or digestion conditions.

Not much is known regarding the importance of gastric lipase, which presumably digests about 25% of ingested lipids,[121] thus contributing to carotenoid transfer from lipid droplets to mixed micelles, due to the fact that this enzyme has not been commercially available. Also in the gastric phase, low pH (due to an empty stomach) again may foster the degradation of epoxy-carotenoids (violaxanthin, neoxanthin) to their furanoid forms. Pepsin is not known to have any significant influence on carotenoid bioaccessibility, as most carotenoid-containing food items are rather low in total protein content, though in one study, higher pepsin concentrations enhanced lycopene bioaccessibility from a tomato puree *in vitro*,[137] perhaps due to the formation of emulsifying peptides.

Esters from xanthophylls, such as those present in several fruits (mango and papaya, rich in, for example, beta-cryptoxanthin esters) and also some leafy vegetables (mangold, rich in lutein esters) are even more apolar than the non-esterified carotenoids and likewise rely on micellization for their absorption. It remains unclear to what extent cleavage by carboxyl-ester lipase (also termed cholesterol esterase) or also triacylglycerol lipase occurs, which are both secreted by the pancreas,[138] with the latter also present in intestinal cells[139] and possibly active at the brush border, as reviewed by Reboul.[30] *In vitro*, cholesterol esterase does not seem to constitute an effective enzyme for cleaving xanthophyll esters,[140] while *in vivo* cleavage appears complete, perhaps suggesting the involvement of other enzymes. However, as native esters of carotenoids in the plasma appear only at very low concentrations,[141] it is assumed that esters are cleaved prior to absorption.[142] On the other hand, esters have been found to be taken up by Caco-2 cell models.[140] It is thus possible that they are further cleaved intracellularly or are not further absorbed and/or re-secreted in the GI tract, though more research is needed here. It is also worth noting that there are indications that xanthophylls, upon absorption, may be re-esterified, perhaps when present at high concentrations, as shown earlier with lutein in humans.[141]

9.4.2 Carotenoid Cellular Uptake

It is likely that at lower, rather physiological concentrations, uptake *via* transporters predominates, while higher (supplemental) concentrations may favor passive diffusion. In fact, a saturable absorption curve (for doses from 10 to 120 mg) has been determined for lycopene, for example,[143,144] supporting the notion that at these concentrations the transporter uptake predominates. Meanwhile, several transporters have been identified or suggested to participate in carotenoid cellular uptake from the small intestine, including scavenger receptor class B type 1 (SRB1), cluster of differentiation 36 (CD36), Niemann-Pick C1-like 1 (NPC1L1) and ATP binding cassette subfamily A member 1 (ABCA1) for further uptake to the basolateral site.

Several gene association studies were conducted by Borel *et al.* and other researchers, and several SNP of transport enzymes were found to be associated with newly absorbed carotenoids, as determined by their concentration in the plasma-TRL fraction: *SCARB1* (encoding for SRB1; transport of lutein/zeaxanthin, beta-cryptoxanthin, lycopene, beta-carotene, alpha-carotene likely), *CD36* (lycopene, alpha-carotene, lutein/zeaxanthin, beta-cryptoxanthin transport likely), *ABCA1* (beta-carotene, lycopene, lutein/zeaxanthin transport likely) and *NPC1L1* (lycopene, lutein/zeaxanthin transport likely), as reviewed recently.[6] Despite the fact that the selectivity of some of these transporters remains to be further elucidated, it is not quite clear whether at this step of absorption a huge discrimination between the various carotenoids exists. Not considering micellization, compared to all-*trans*-beta-carotene, beta-carotene *cis*-forms were shown to be poorly taken up, at least in Caco-2 cell experiments,[145] similarly to lutein and lycopene. The higher cellular uptake of beta-carotene compared to lycopene in such cell models was found earlier,[146] also observed by O'Sullivan *et al.*[147] when comparing beta-carotene and lutein Caco-2 cellular uptake, though secretion was not effected systematically. However, differences in cellular uptake/absorption efficacy may be lower between other carotenoids, such as between lycopene and astaxanthin cellular uptake into HT-29 cells.[148] In another study by O'Sullivan *et al.*, astaxanthin and also lutein showed high cellular uptake into Caco-2 cells.[149] Differences in cell lines, passage number, time of incubation, dosing and preparation of artificial micelles may contribute to such obtained differences.

As several of these transporters also accept other substrates, these may, at least at higher concentrations, compete for one another. For example, SRB1, CD36 and ABCA1 are all also cholesterol transporters, while NPC1L1 has also shown some selectivity for vitamins E, K and D, CD36 for vitamins D and K and SRB1 for vitamins E, K and D, as reviewed by Reboul and Borel[150] and Yamanashi *et al.*[151] However, no human postprandial studies have investigated the interactions of high doses of vitamins with carotenoid absorption, though it has been observed in an intervention that high doses of plant sterols and sterol esters reduced beta-carotene absorption in humans by approximately 50%.[80] As these sterols (2.2 g day^{-1}) are normally poorly absorbable, however, it is also more likely that their negative influence originates from competition for micelle incorporation (Table 9.2).

In summary, carotenoid cellular uptake is at least in part occurring *via* transporters, and thus higher doses are expected and have been shown to result in lower fractional absorption, also questioning the intake of high doses of individual carotenoids at the same time, such as in high-dose supplements.

9.4.3 Further Carotenoid Metabolism in the Enterocytes and Other Tissues

Once taken up by the enterocyte, carotenoids may be further cleaved, either centrally by BCO1 or eccentrically by BCO2. While BCO1 is present in the cytosol, BCO2 is present in the mitochondria.[152] While beta-carotene appears to be a particular target for BCO1, resulting in the production of provitamin A active retinal, BCO2 has shown to be able to produce apo-carotenals from beta-carotene, at least apo-10′-beta-apo-carotenal, as shown in HepG2 liver cells[153] and in mice studies.[154] BCO2 has further been suggested to cleave lycopene into apo-10′-lycopenal,[38,155] but also apo-8′- and apo-12′-lycopenal[156] were found in the livers of rats. While BCO1 thus appears to favor full-length provitamin A carotenoids (beta-carotene, alpha-carotene and beta-cryptoxanthin, though not lycopene, lutein and 9-*cis*-beta-carotene[157]), BCO2 favors the cleavage of xanthophylls.[158] However, cleavage is far from being complete and species dependent, and has been estimated for xanthophylls and BCO2 to be 10–40-times weaker than in mice.[159] It is also noteworthy that longer apo-carotenoids are themselves substrates for BCO1/2, as shown for beta-apo-4′-carotenal, beta-apo-8′-carotenal, beta-apo-10′-carotenal and beta-apo-12′-carotenal in chickens and rats, as reviewed by Kim *et al.*[3]

The further fate of these metabolites is not entirely clear. While retinal can react to form retinol and is then likely to be re-esterified by LRAT prior to further absorption, this is not the case for uncleaved carotenes and xanthophylls. BCO2 metabolites are potentially further metabolized *via* hydroxylation, as some hydroxylated compounds were detected earlier *in vivo* (Figure 9.1), though their origin (plant *vs*. *in vivo*) could not be clearly differentiated.[57,160,161] It also is possible that these compounds can then be further conjugated to form sulfated and glucuronidated metabolites, as glucuronidation has been shown for retinol.[162] This was demonstrated following the administration of isotopically labeled all-*trans* beta-carotene in rats, upon which the majority of radioactively labeled compounds were studied in bile, and 73–96% were water-soluble compounds that yielded retinol or retinoic acid following cleavage by glucuronidase.[163] Retinoyl-beta-glucuronide (RAG) was also found in the plasma of humans, though at rather low concentrations (*i.e.* ~2.4 ng mL^{-1}).[164] Further glucuronidated metabolites such as 9-*cis*-4-oxo RAG were identified in subjects treated with 9-*cis* retinoic acid.[165] These findings would also be in line with excretion of beta-carotene *via* urine, which was detected *via* isotopic studies (6.5% of the administered dose),[166] though the nature of the compounds was not determined at that time. However, it is apparent that BCO1 and BCO2 are among the most important carotenoid metabolism steps in humans.

In addition to further metabolism, carotenoids or their metabolites may also be re-secreted back into the intestinal lumen. This may happen *via*

cellular abrasion of the enterocytes or *via* shuttling carotenoids back *via* transporters. Whether, how and to what extent this happens is unclear. As highlighted in a review by Reboul,[30] SRB1 has been shown to be able to act as a transporter in both directions, and vitamin E[167] and D[168] efflux to the apical side was shown for Caco-2 cells, and a similar phenomenon could also be assumed for carotenoids. However, other transporters, including other ATP binding cassette subfamily proteins such as ABCG5,[169] ABCB1[23] and ABCG8,[170] may also play a role, as suggested for lutein and ABCG5 for beta-carotene[171] in human trials investigating genetic polymorphisms.

In summary, the further metabolism following a potential cleavage by BCO1/2 remains unclear, but is likely to include further reactions with phase II enzymes, such as glucuronidation and perhaps sulfation, though a number of other oxidation products such as the formation of keto-compounds[172,173] and penta-cyclic compounds[57] have also been suggested (Figure 9.1).

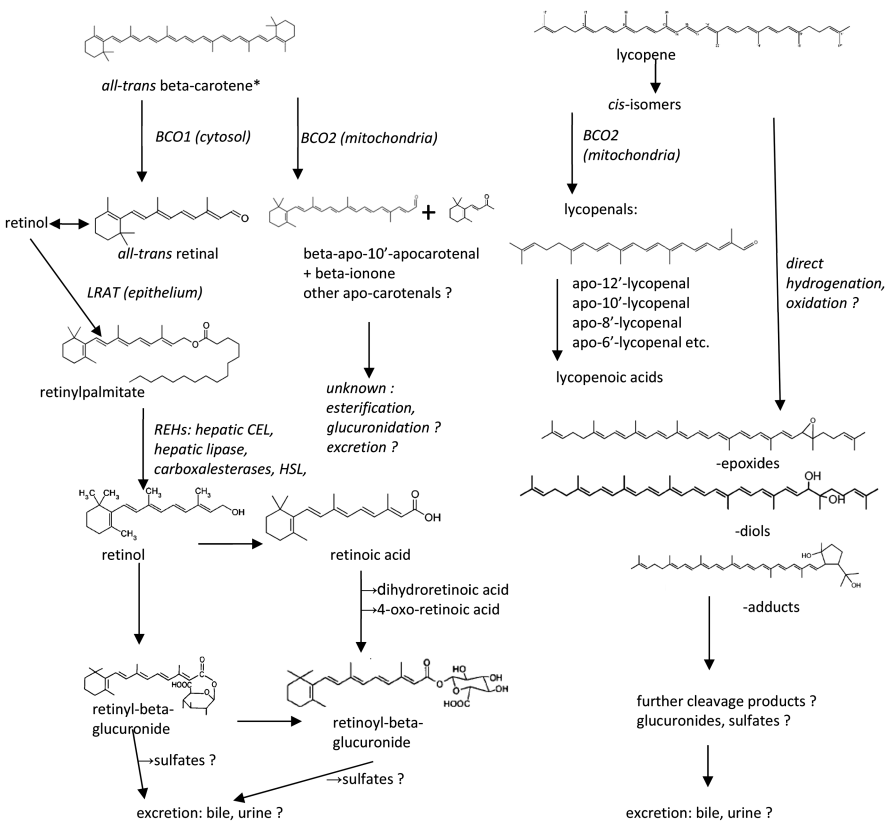

Figure 9.1 Simplified metabolic pathway of carotenoids – examples lycopene[57] and beta-carotene.[27,162,174] Main isomers would be 9-*cis*, 13-*cis* and 15-*cis* isomers for beta-carotene[62] and 9-*cis*, 13-*cis* and 15-*cis* isomers for lycopene.[62,175] FA: fatty acid; HSL: hormone-sensitive lipase. REH: retinyl ester hydrolysis enzymes. [a]*Cis–trans*-isomerization not considered here.

9.5 Colonic Fate of Carotenoids

All carotenoids that are not absorbed in the small intestine will eventually reach the colon, either in bioaccessible form (emulsified) or precipitated. In addition to non-absorbed carotenoids, carotenoids are also re-excreted *via* bile and the pancreas back into the intestinal lumen (*i.e.* following their original absorption), as shown by, for example, isotopic studies in salmon.[176] As absorption of carotenoids is only approximately 10–40%, the remaining 60–90% should reach the colon, not counting potential endogenous losses of carotenoids or metabolites. Several *in vitro* studies have shown that carotenoids, following colonic fermentation, are not completely recovered, but that they are degraded (*i.e.* fermented) by the microbiota. For example, in a study with fermented plum and cabbage carotenoids, only 4–25% of carotenoids were recovered following colonic fermentation.[177] In another study by Goni *et al.*, colonic recovery of lutein, lycopene and beta-carotene were 19/19%, 17/3% and 24/21%, respectively, for digested fruits/vegetables.[178] In another study by those authors, losses of pure beta-carotene standard were as high as 98%,[130] though no metabolites were reported (Table 9.5).

However, the nature of these microbiota-fermented carotenoids is not known. Unlike for polyphenols, which are known to undergo numerous reactions in the gut, including ring fission, deglycosylation, hydrolysis, deglucuronidation and demethylation, no literature exists on carotenoid products in the large intestine. Though it is possible that more polar degradation products are produced, this is speculative at present. Some carotenoid metabolites originating from the body, however, are also expected, as retinoylglucuronide excreted *via* the bile following beta-carotene administration may be de-glucuronidated.

Furthermore, it remains unknown whether carotenoids can be absorbed from the colon. In an earlier human trial, it was found that maximum carotenoid concentrations in colonic cells trailed behind that of blood plasma by 5–7 days, suggesting insignificant direct absorption from the luminal content,[179] but rather absorption *via* the small intestine followed by carotenoid distribution *via* the circulatory system. In an insightful rat study by Oshima *et al.*,[180] rats received intracolonic doses of lycopene, and low amounts were detected in serum and higher amounts in the liver, suggesting that colonic uptake does play a role. However, mixed micelles are present in the colon, and there is indirect evidence of absorption of other lipophilic compounds (*e.g.* of vitamin K with long side chains [phylloquinone, log P: 10.9, https://www.phenomenex.com/Compound?id=Vitamin+K]), as these were found in the livers of humans,[181] though their availability is possibly low as deduced following colorectal administration in rats.[182] Finally, as BCO1 has been detected in colonic cells, at least in the mouse,[183] carotenoid metabolism is also expected to occur in colonic epithelial cells. In summary, more research is warranted on potential colonic degradation products or possible colonic uptake of native carotenoids or their products, which may be low, but perhaps not zero.

Table 9.5 Losses during carotenoid digestion in the gastrointestinal tract, including the colon when studied.[a]

Carotenoid(s) studied	System	Meal	Losses/degradation	Other observations	Reference
Small intestine					
Beta-carotene	*In vitro* (static)	Carotenoids in sunflower oil, sugar and phospholipids	40% losses after intestinal phase	No oxidation products, no isomers observed	113
Lutein	*In vitro* (static)	Carotenoids in sunflower oil, sugar and phospholipids	40% losses after intestinal phase	No oxidation products, no isomers observed	113
Lycopene	*In vitro* (static)	Carotenoids in sunflower oil, sugar and phospholipids	20% losses after intestinal phase	No oxidation products, no isomers observed	113
Zeaxanthin	*In vitro* (TIM)	Tomatoes and beadlets	No losses	No *trans–cis* isomerization during digestion	114
Lutein	*In vitro* (TIM)	Tomatoes and beadlets	No losses	No *trans–cis* isomerization during digestion	114
Beta-carotene	*In vitro* (TIM)	Tomatoes and beadlets	30% losses after intestinal phase	No *trans–cis* isomerization during digestion	114
Lycopene	*In vitro* (TIM)	Tomatoes and beadlets	20% losses after intestinal phase	No *trans–cis* isomerization during digestion	114
Colon					
Total carotenoids (beta-carotene + lutein)	*In vitro* (static)	*S. oleracea*, *S. americanum* and *C. aconitifolius* carotenoids	92%, 96% and 23% losses during colonic fermentation	No degradation products investigated	130
Lutein	*In vitro* (static)	*S. oleracea*, *S. americanum* and *C. aconitifolius* carotenoids	92.5%, 95.5% and 23.0% losses during colonic fermentation	No degradation products investigated	130
Beta-carotene	*In vitro* (static)	*S. oleracea*, *S. americanum* and *C. aconitifolius* carotenoids	98.5%, 97.0% and 23.0% losses during colonic fermentation	No degradation products investigated	130
Total carotenoids (beta-carotene, lutein)	*In vitro* (static)	Various plum and cabbage varieties	4–25% of after colonic fermentation	No degradation products investigated	131

[a]TIM: TNO gastrointestinal model (dynamic multi-compartmental model).

9.6 Further Metabolism of Carotenoids Following Cellular Uptake

9.6.1 Conversion into Vitamin A

Clearly, the fraction of provitamin A carotenoids that can be absorbed and then be cleaved and turned into vitamin A active compounds, a term also called "bioconversion," is very variable, depending, as emphasized above, on the food matrix and factors influencing the absorption of beta-carotene, and finally host factors such as BCO1 cleavage activity. In a study by Leung *et al.*,[184] it was shown that bioconversion differed between variants of BCO1, with a double mutant of the SNP R267S:rs12934922 and A379V:rs7501331 showing 57% reduced catalytic cleavage activity. In a human clinical study by Lietz *et al.*,[185] three polymorphisms of the *BCO1* gene (rs6420424, rs11645428 and rs6564851) hampered BCO1 catalytic activity by 59%, 51% and 48%, respectively. The expression of BCO1 appears to be regulated by the intestine-specific homeobox transcription factor (ISX), as retinoic acids appear to stimulate its expression, which in turn reduces expression of BCO1, limiting vitamin A formation in the intestinal cell.[186] Thus, the fate of beta-carotene (*i.e.* its conversion to retinal) is influenced by genetic factors, which is possibly the cause of some people being rather high converters of beta-carotene to vitamin A, while others (~45% in Westernized countries) are low converters[187]. However, for more detailed reviews on carotenoids as sources of vitamin A, the reader is referred to other articles[188–190]

9.6.2 Intracellular Transport and Sequestration of Carotenoids into Chylomicrons

Following carotenoid cellular uptake, potential cleavage and/or further re-esterification, carotenoids are secreted *via* chylomicrons into the lymphatic system. This appears to be the case for all compounds with a log $P > 5$ (which would include all major dietary carotenoids), below which compounds can be further transported *via* the portal vein.[191] As apo-carotenoids also have a log *P* value of approximately 5 or slightly higher (http://foodb.ca/compounds/FDB022576), these are also assumed to be sequestered *via* the portal pathway, at least to some extent, though they have not been detected so far. In a study by Kopec *et al.* with orally administered ^{13}C-beta-carotene, ^{13}C-retinol was observed in whole plasma but not in the TRL fraction, while no non-vitamin A active ^{13}C-beta-apo-carotenals, ^{13}C-beta-apo-carotenols or ^{13}C-beta-apo-carotenoic acids were observed in the plasma or TRL samples. Thus, at least for beta-carotene metabolism, apo-carotenoids are not secreted *via* chylomicrons to a measurable extent.[192]

The details of chylomicron sequestration in the enterocyte are not completely elucidated, but possibly include binding of carotenoids to some intracellular transport proteins (due to their otherwise low cytosolic solubility) and their carrying to the endoplasmic reticulum, followed by the Golgi

apparatus for sequestration into chylomicrons. Regarding the binding proteins, potential candidates include fatty acid binding protein (FABP), and also BCO1 itself, as reviewed previously.[6] A human postprandial study found an association between a SNP in FABP and lycopene in the plasma TRL fraction,[193] supporting a potential role for its participation in intracellular transport.

However, carotenoids are eventually assembled, by the aid of apolipoprotein A1 (APOA1),[194] into chylomicrons. They are then released *via* exocytosis to the lacteals and the lymphatic system. Another route has been speculated upon, which includes secretion of carotenoids to the basolateral site directly *via* APOA1, by lipid transfer to high-density lipoprotein (HDL) from the membrane protein ABCA1. This is supported by a study showing direct transport of xanthophylls *via* ABCA1 to APOA1 in hamsters.[195]

It is interesting to note that after consumption of a carotenoid-rich meal, carotenoids appear to be stored for a certain time in the enterocytes and are sequestered following the consumption of a later meal rich in lipids, which appears to foster the formation and secretion of carotenoid-loaded chylomicrons,[22,24] and thus follow-up meals could influence the kinetics, if not the bioavailability, of carotenoids.

9.7 Carotenoid Biodistribution, Target Tissues and Excretion

Following their sequestration into chylomicrons and transport in the lymph, carotenoids reach the bloodstream and are transported to the liver. Chylomicrons are partly stripped of their triglycerides along the way to react with chylomicron remnants, not affecting carotenoids. While the liver appears partly to constitute a storage organ for carotenoids, and more so for vitamin A, carotenoids are also redistributed into various lipoproteins. While xanthophylls appear to be distributed about evenly between HDL and low-density lipoprotein (LDL), carotenes appear to be associated primarily with the LDL fraction.[196]

Carotenoids are then released *via* the lipoproteins to different tissues. Carotenoids have been detected in many different tissues and, based on the concentrations encountered (Table 9.1), there does not appear to exist a dominating primary target tissue, although the adipose tissue appears to be an important if not the major storage organ for carotenoids, and liver concentrations are typically higher than those of other organs. It is plausible that tissues expressing a larger number of especially LDL receptors are candidates for storing higher concentrations of carotenoids, such as adipose tissue,[197] prostate tissue[60] or adrenal tissue,[62] which appears to be the case (Table 9.1), at least for adipose tissue and adrenal tissue. As many tissues contain both BCO1 and BCO2,[27] similar metabolites as in the enterocytes may be formed, though few data are available on tissue metabolites. Because of the relation of carotenoids to AMD, the macula constitutes an important target tissue for lutein, zeaxanthin and *meso*-zeaxanthin (a stereoisomer

of zeaxanthin produced *in vivo* from lutein and present to some extent in seafood) – for further information, please consult further reviews.[198] Also, a non-enzymatic oxidation product of lutein, 3′-oxolutein, has been related to the human retina.[199]

Following administration of isotopically labeled phytoene (^{13}C-labeled), Moran *et al.*[97] developed a compartment model (Figure 9.2) highlighting major body pools of phytoene. A similar model was developed for ^{13}C-labeled lycopene,[97] also highlighting ongoing *trans* → *cis* isomerization in the human body. These studies also demonstrated that the largest pools were slow-turnover pools, possibly representing various body tissues of slow exchanges such as the adipose tissue, followed by fast-turnover pools, which may include liver metabolism.

Hardly any data are available regarding carotenoid excretion pathways. As Zile *et al.* found that the majority of vitamin A active compounds following administration of isotopic ATRA to rats[163] were present in the water-soluble fraction in the bile as retinol and retinoic acid glucuronides (in all-*trans* and 13-*cis* forms), it can be assumed that, at least for beta-carotene, biliary

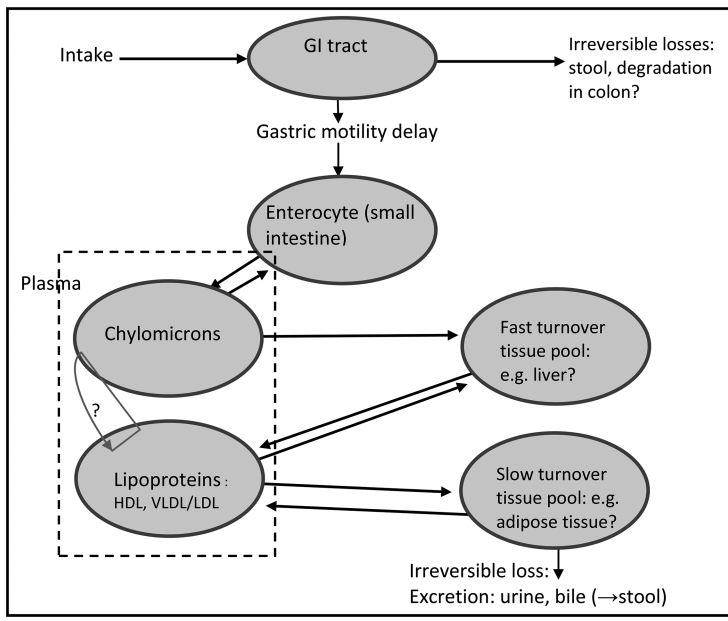

Figure 9.2 Human compartmental kinetic model of non-provitamin A carotenoids based on measurements in plasma following dosings as carried out by Diwadkar-Navsariwala[144] and stable isotope administration by Moran *et al.*[97,202] for lycopene and phytofluene. Arrows represent the direction of exchange between the different pools and ovals are compartments (kinetically homogenous pools). Gastric motility delay likely represents GI transit time. More details on beta-carotene compartment models can be found in the thesis by Park.[203]

excretion as glucuronides is one main pathway of excretion. As urinary excretion was also demonstrated following the radioactive administration of ^{14}C-beta-carotene,[166] it could be speculated that glucuronides or other polar metabolites such as apo-carotenoids are partly secreted in the urine. In the study of Ho *et al.*,[166] approximately 6.5% (range 1.7–15.8%) of the orally administered dose was excreted in urine within 30 days. Interestingly, mono- and di-glucuronides of crocetin were also found in plasma of rats following oral administration at higher concentrations than the free molecule, also suggesting that glucuronidation could be a fate of apo-carotenoids.[200]

By contrast, the cumulative dose excreted in feces ranged between 28% and 100% (mean 56%) within 14 days.[166] Similar results were found by Hickenbottom *et al.*[201] following administration of a small dose of radiolabeled beta-carotene, demonstrating cumulative recoveries in urine and stool of 4% and 45%, respectively. As the latter constituted mostly non-absorbed beta-carotene detected within the first 2 days of test meal intake, and only 1% in addition was recovered later due to endogenous losses, urinary excretion appeared to be the major route of excretion in this trial.

9.8 Interrelation of Carotenoid Degradation Products, Metabolites and Health Aspects

9.8.1 Health Benefits Associated with Native Carotenoids

It ought to be emphasized that our current gaps in knowledge regarding further carotenoid metabolism coincide with our lacking a grasp on carotenoid health-beneficial aspects, and this failure to characterize the true bioactive constituents of carotenoid-rich food items, if any, will impede any further meaningful intervention trials aimed at ameliorating ailments related to low intake and circulating carotenoids.

While direct antioxidant effects of the native carotenoids clearly have been shown *in vitro*, including quenching of singlet oxygen,[204,205] protection against UV light as reviewed previously[206] and quenching of peroxyl radicals originating, for example, from lipid oxidation,[207] it is unclear whether these effects can be translated as such to the *in vivo* situation. However, oxidative stress measured by the dichlorofluorescein method in cell culture (Caco-2) following exposure to H_2O_2 and astaxanthin at 10 μM reduced oxidative stress, indicating that such high but intestinally reachable concentrations of native carotenoids can have beneficial effects.[208] As the cleavage rate into derivatives (*via* BCO1/2) is slow, it could be assumed that carotenoids may also protect the cell membranes from oxidative stress *in vivo*. In this respect, carotenes are likely to be situated in the more apolar area of the fluid mosaic model of the cell membrane (*i.e.* in the middle part[209]), while xanthophylls may span the membrane, occupying the outer domains with the polar parts of their molecules.[210]

9.8.2 Health Benefits Associated with Carotenoid *In Vivo* Metabolites

As most studies did not investigate carotenoid degradation products and their concentrations in tissues following *in vivo* studies, it is difficult to ascribe any observed effects of carotenoids to either the native compounds or their metabolites. Several studies have pointed out that carotenoid metabolites (*e.g.* the resulting apo-carotenals) have a higher affinity to interact with transcription factors in the cytosol due to their higher solubility and higher electrophilicity. In a study by Linnewiel *et al.*,[37] several apo-carotenals obtained from the oxidation of lycopene with potassium permanganate were tested in several transfected cell lines (MCF-7 breast cancer cells, T47D mammary cancer cells and LNCaP prostate cancer cells) regarding their interaction with Nrf2. It was found that: (1) aldehydes, not acids, were most active; (2) the activity of the compounds depended on the relative position of the methyl group 3 to the terminal aldehyde; and (3) the optimal length of the dialdehyde derivative was 12 carbons in the main chain. In another study by the same authors,[36] the authors investigated the effect of synthetic apo-carotenals on T47D, HOS and MC3T3-E1 osteoblast-like cells transfected with an NF-κB reporter gene. Again, while native lycopene was found to be rather ineffective, apo-carotenals strongly influenced NF-κB activation, possibly by binding to cysteine residues of NF-κB *via* the reaction of α,β-unsaturated carbonyls with nucleophilic protein thiols to a Michael adduct. Similarly, in a rat study with lutein,[211] photooxidative degradation products reduced lipopolysaccharide (LPS)-triggered inflammation, including NF-κB downstream products such as malondialdehyde, prostaglandin E2, TNF-α and IL-6, more strongly than lutein in serum.

In several studies, it was suggested that lycopene metabolites could interact with RXR and RAR, which are important for regulating immune system functions, among others. In a recent study by Aydemir *et al.*,[39] it was shown that lycopene fed to mice regulated genes affected by vitamin A deficiency, bringing their expression back to levels seen in rats without vitamin A deficiency. This was ascribed – though it was not directly detected – to lycopene metabolites such as to apo-15′-lycopenoid acid,[212] highlighting the potency of these metabolites, suggesting that these have vitamin A-like properties.

In a recent review by Bonet *et al.*,[213] it has been proposed that apo-carotenals of beta-carotene and possibly other carotenoids influence peroxisome proliferator-activated receptors (by acting as agonists or antagonists) in adipose tissue, thereby regulating proliferation of adipose tissue. In support of this, several human studies have found that obese subjects typically have lower levels of circulating carotenoids,[214,215] though it is not clear whether this is due to rather low intake of carotenoids, increased storage of carotenoids in adipose tissue or increased turnover in other tissues.

Finally, several plant-derived apo-carotenoids have been shown to be bioactive in humans. For example, the plant hormone abscisic acid has been shown to ameliorate experimental IBD in an animal model,[216] to act as a cytokine in humans, positively influencing systemic sclerosis markers *in vitro*,[35]

and to improve glucose tolerance in humans in small quantities (0.5–1 μg kg bodyweight^{-1}),[34] possibly *via* enhancing insulin production and GLUT4-related glucose cellular uptake.

In summary, though more confirmation is needed, many studies point out that carotenoid metabolites (such as those produced by BCO1/2) can be very bioactive and may be more potent than their native precursors, effecting many inflammation, oxidative stress and cell proliferation pathways *via* interacting with transcription factors and nuclear receptors. Thus, understanding the metabolism of carotenoids is key to our understanding of their health-related benefits or risks, and more research in this domain is needed. At present, research regarding carotenoid metabolites is limited, at least in part due to methodological limitations. Firstly, not many carotenoid metabolites are commercially available, and even if they are available, they are quite expensive. The same is true for isotopically labeled carotenoids for following the metabolism of orally administered tracers. Secondly, their sensitive and selective analysis requires sophisticated and likewise expensive analytical instrumentation (*i.e.* chromatographic separation techniques coupled to mass-spectrometry, such as atmospheric-pressure-ionization based liquid chromatography-mass spectrometry ([APCI-]LC-MS-MS), which many laboratories do not have access to, even though this technique has become more available in recent years).

9.9 Conclusions

The metabolic fate of carotenoids depends on several factors (Figure 9.3). When carotenoids can be released from the matrix and are bioaccessible, they may enter the body pool of carotenoids, while their colonic fate remains unknown. Their release from the diet is dependent on both the food matrix and food constituents, foremost the presence of lipids, and host factors such as the availability of sufficient bile and pancreatin. Following their cellular uptake, which depends on the expression of active transporters (SRB1, CD36, NPC1L1 and ABCA1) related to SNPs, provitamin A carotenoids may then be preferably cleaved by BCO1 into retinal, while non-provitamin A carotenoids may be preferentially cleaved by BCO2 into several apo-carotenals, if at all.

In what follows, carotenoids and their metabolites and retinyl esters (produced from retinol) will be transported to the liver, where they may be stored or further distributed in the body by HDL and very-low-density lipoprotein/LDL. Apo-carotenoids may likewise be transported to target tissues, though their transport is not understood and the transport of the free form in the bloodstream appears limited. Though no single specific target tissue of carotenoids exists, the liver appears to constitute a major storage tissue, especially for vitamin A, while a large part of carotenoids is stored in adipose tissue. The macula of the retina is a target for lutein and zeaxanthin. Further metabolism in tissues expressing BCO1/2 appears likely. The resulting apo-carotenoids are expected to interact with transcription factors in the cytosol of the cell, including NF-κB, Nrf2 and some metabolites such as retinal, and some lycopeneoids may interact with the nuclear receptors RAR and RXR. In the process that follows this, it is likely that apo-carotenoids

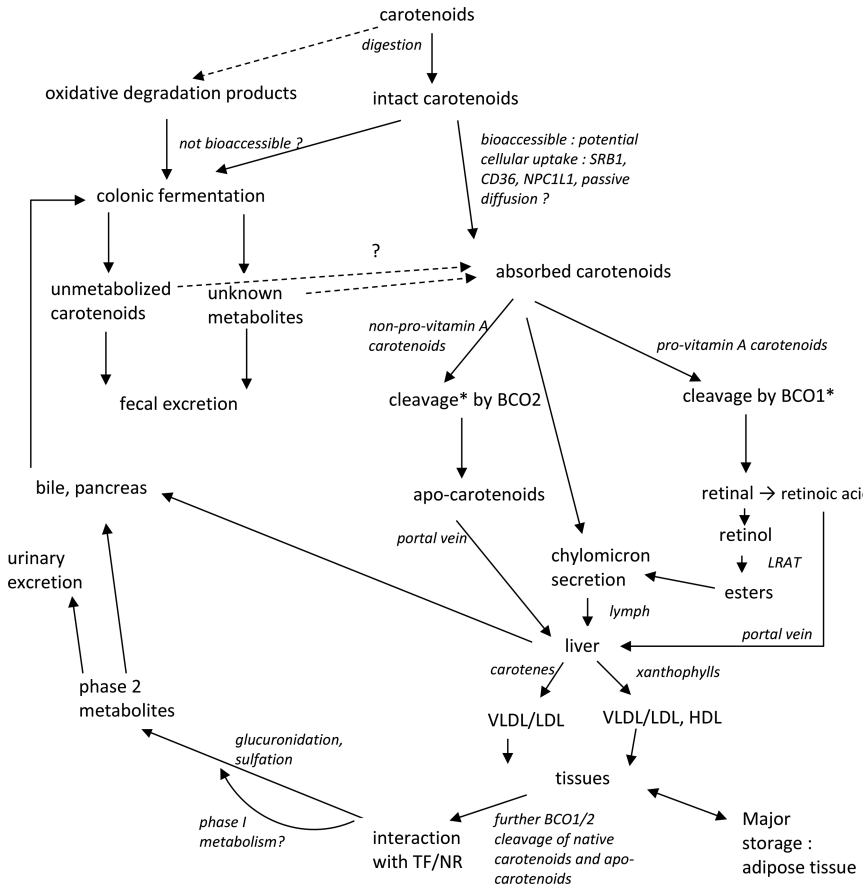

Figure 9.3 Simplified overview on the metabolic fate of dietary-derived carotenoids in the human body. TF: transcription factor; NR: nuclear receptors. [a]Preferred cleavage pathway, though provitamin A may also be cleaved by BCO2 and non-provitamin A carotenoids by BCO1.

are eventually metabolized by phase II enzymes (sulfotransferases and UDP-glucuronosyltransferases). The resulting sulfates and glucuronides are more water soluble and can be excreted *via* the kidneys, while native carotenoids may be secreted back into the gut *via* bile.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

The insights obtained from the EU-COST actions POSITIVE (FA-1403) and EUROCAROTEN (CA-15136) are greatly appreciated.

References

1. J. Paniagua-Michel, J. Olmos-Soto and M. A. Ruiz, *Methods Mol. Biol.*, 2012, **892**, 1–12.
2. Y. Yang, R. Yatsunami and A. Ando, *et al.*, *J. Bacteriol.*, 2015, **197**, 1614–1623.
3. Y. S. Kim and D. K. Oh, *Appl. Microbiol. Biotechnol.*, 2010, **88**, 807–816.
4. J. C. Beltran and C. Stange, *Subcell. Biochem.*, 2016, **79**, 239–272.
5. C. Desmarchelier and P. Borel, *Trends Food Sci. Technol.*, 2017, **69**, 270–280.
6. T. Bohn, C. Desmarchelier and L. O. Dragsted, *et al.*, *Mol. Nutr. Food Res.*, 2017, **61**, DOI: 10.1002/mnfr.201600685.
7. E. T. Leermakers, S. K. Darweesh and C. P. Baena, *et al.*, *Am. J. Clin. Nutr.*, 2016, **103**, 481–494.
8. J. Chen, W. Jiang and L. Shao, *et al.*, *Int. J. Food Sci. Nutr.*, 2016, **67**, 744–753.
9. N. Yu, X. Su and Z. Wang, *et al.*, *Nutrients*, 2015, **7**, 9309–9324.
10. I. Kompauer, J. Heinrich and G. Wolfram, *et al.*, *Public Health Nutr.*, 2006, **9**, 472–479.
11. L. G. Wood, M. L. Garg and R. J. Blake, *et al.*, *J. Am. Coll. Nutr.*, 2005, **24**, 448–455.
12. M. S. Donaldson, *Nutrients*, 2011, **3**, 1003–1022.
13. L. Ma, R. Liu and J. H. Du, *et al.*, *Nutrients*, 2016, **8**, 426.
14. N. I. Krinsky and E. J. Johnson, *Mol. Aspects Med.*, 2005, **26**, 459–516.
15. W. Stahl and H. Sies, *Am. J. Clin. Nutr.*, 2012, **96**, 1179s–1184s.
16. A. Kaulmann and T. Bohn, *Nutr. Res.*, 2014, **34**, 907–929.
17. Y. Sharoni, K. Linnewiel-Hermoni and M. Khanin, *et al.*, *Mol. Nutr. Food Res.*, 2012, **56**, 259–269.
18. T. Bohn, *Int. J. Vitam. Nutr. Res.*, 2016, **10**, 1–5.
19. G. Bjelakovic, D. Nikolova and L. L. Gluud, *et al.*, *Cochrane Database Syst. Rev.*, 2012, **14**, Cd007176.
20. D. Albanes, O. P. Heinonen and J. K. Huttunen, *et al.*, *Am. J. Clin. Nutr.*, 1995, **62**, 1427s–1430s.
21. G. S. Omenn, G. E. Goodman and M. D. Thornquist, *et al.*, *N. Engl. J. Med.*, 1996, **334**, 1150–1155.
22. T. Bohn, M. Blackwood and D. Francis, *et al.*, *Nutr. Cancer*, 2013, **65**, 919–929.
23. P. Borel, C. Desmarchelier and M. Nowicki, *et al.*, *Free Radical Biol. Med.*, 2015, **83**, 238–244.
24. N. Z. Unlu, T. Bohn and S. K. Clinton, *et al.*, *J. Nutr.*, 2005, **135**, 431–436.
25. C. Sy, B. Gleize and O. Dangles, *et al.*, *Mol. Nutr. Food Res.*, 2012, **56**, 1385–1397.
26. T. Bohn, *Curr. Nutr. Food Sci.*, 2008, **4**, 53–72.
27. A. Eroglu and E. H. Harrison, *J. Lipid Res.*, 2013, **54**, 1719–1730.
28. A. Eroglu, D. P. Hruszkewycz and C. dela Sena, *et al.*, *J. Biol. Chem.*, 2012, **287**, 15886–15895.

29. G. P. Lobo, J. Amengual and G. Palczewski, *et al.*, *Biochim. Biophys. Acta*, 2012, **1821**, 78–87.
30. E. Reboul, *Nutrients*, 2013, **5**, 3563–3581.
31. E. H. Harrison, *Biochim. Biophys. Acta*, 2012, **1821**, 70–77.
32. N. Umigai, K. Murakami and M. V. Ulit, *et al.*, *Phytomedicine*, 2011, **18**, 575–578.
33. L. W. Levy, E. Regalado and S. Navarrete, *et al.*, *Analyst*, 1997, **122**, 977–980.
34. M. Magnone, P. Ameri and A. Salis, *et al.*, *FASEB J.*, 2015, **29**, 4783–4793.
35. S. Bruzzone, F. Battaglia and E. Mannino, *et al.*, *Biochem. Biophys. Res. Commun.*, 2012, **422**, 70–74.
36. K. Linnewiel-Hermoni, Y. Motro and Y. Miller, *et al.*, *Free Radical Biol. Med.*, 2014, **75**, 105–120.
37. K. Linnewiel, H. Ernst and C. Caris-Veyrat, *et al.*, *Free Radical Biol. Med.*, 2009, **47**, 659–667.
38. X. D. Wang, *Am. J. Clin. Nutr.*, 2012, **96**, 1214s–1222s.
39. G. Aydemir, Y. Kasiri and E. M. Bartok, *et al.*, *Mol. Nutr. Food Res.*, 2016, **60**, 2413–2420.
40. G. Aydemir, Y. Kasiri and E. Birta, *et al.*, *Mol. Nutr. Food Res.*, 2013, **57**, 739–747.
41. M. Lucarini, S. Lanzi, L. D'Evoli, L. D'Evoli and A. Aguzzi, *et al.*, *Int. J. Vitam. Nutr. Res.*, 2006, **76**, 103–109.
42. M. Porrini and P. Riso, *J. Nutr.*, 2005, **135**, 2042s–2045s.
43. E. Biehler, A. Alkerwi and L. Hoffmann, *et al.*, *J. Food Compos. Anal.*, 2012, **25**, 56–65.
44. M. E. O'Neill, Y. Carroll and B. Corridan, *et al.*, *Br. J. Nutr.*, 2001, **85**, 499–507.
45. M. M. Murphy, L. M. Barraj and D. Herman, *et al.*, *J. Acad. Nutr. Diet.*, 2012, **112**, 222–229.
46. E. N. Story, R. E. Kopec and S. J. Schwartz, *et al.*, *Annu. Rev. Food Sci. Technol.*, 2010, **1**, 189–210.
47. USDA, *National Nutrient Database for Standard Reference Release 28*, 2015, http://ndb.nal.usda.gov/ndb/foods/show, accessed 05.11.2015: USDA.
48. A. Kaulmann, M. C. Jonville and Y. J. Schneider, *et al.*, *Food Chem.*, 2014, **155**, 240–250.
49. C. Stange-Klein and M. Rodriguez-Conception, Carotenoids in carrots, in *Pigments in Fruits and Vegetables*, ed. C. Chen, Springer, Heidelberg, 2015.
50. F. Gurmu, S. Hussein and M. Laing, *Int. J. Vitam. Nutr. Res.*, 2014, **84**, 65–78.
51. Y. M. Peng, Y. S. Peng and Y. Lin, *et al.*, *Nutr. Cancer*, 1995, **23**, 233–246.
52. S. Scarmo, B. Cartmel and H. Lin, *et al.*, *Arch. Biochem. Biophys.*, 2010, **504**, 34–39.
53. H. H. Schmitz, C. L. Poor and R. B. Wellman, *et al.*, *J. Nutr.*, 1991, **121**, 1613–1621.

54. C. Manach, G. Williamson and C. Morand, *et al.*, *Am. J. Clin. Nutr.*, 2005, **81**, 230S–242S.
55. B. J. Burri, T. R. Neidlinger and A. J. Clifford, *J. Nutr.*, 2001, **131**, 2096–2100.
56. W. K. Al-Delaimy, P. Ferrari and N. Slimani, *et al.*, *Eur. J. Clin. Nutr.*, 2005, **59**, 1387–1396.
57. F. Khachik, C. J. Spangler and J. C. Smith Jr, *et al.*, *Anal. Chem.*, 1997, **69**, 1873–1881.
58. G. J. Handelman, E. A. Dratz and C. C. Reay, *et al.*, *Invest. Ophthalmol. Visual Sci.*, 1988, **29**, 850–855.
59. S. Alaluf, U. Heinrich and W. Stahl, *et al.*, *J. Nutr.*, 2002, **132**, 399–403.
60. S. K. Clinton, C. Emenhiser and S. J. Schwartz, *et al.*, *Cancer Epidemiol., Biomarkers Prev.*, 1996, **5**, 823–833.
61. R. Vishwanathan, M. J. Kuchan and S. Sen, *et al.*, *J. Pediatr. Gastroenterol. Nutr.*, 2014, **59**, 659–665.
62. W. Stahl, W. Schwarz and A. R. Sundquist, *et al.*, *Arch. Biochem. Biophys.*, 1992, **294**, 173–177.
63. R. S. Parker, *Eur. J. Clin. Nutr.*, 1997, **51**(suppl. 1), S86–S90.
64. O. Hernell, J. E. Staggers and M. C. Carey, *Biochemistry*, 1990, **29**, 2041–2056.
65. P. Borel, P. Grolier and M. Armand, *et al.*, *J. Lipid Res.*, 1996, **37**, 250–261.
66. R. E. Kopec and M. Failla, *J. Food Compost. Anal.*, 2017, DOI: 10.1016/j.jfca.2017.1006.1008.
67. W. Xu, P. Ling and T. Zhang, *J. Drug Delivery*, 2013, **2013**, 340315.
68. E. Reboul, M. Richelle and E. Perrot, *et al.*, *J. Agric. Food Chem.*, 2006, **54**, 8749–8755.
69. V. Tyssandier, E. Reboul and J. F. Dumas, *et al.*, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2003, **284**, G913–G923.
70. E. Biehler and T. Bohn, *Curr. Nutr. Food Sci.*, 2010, **6**, 44–69.
71. C. E. West and J. J. Castenmiller, *Int. J. Vitam. Nutr. Res.*, 1998, **68**, 371–377.
72. P. Borel, *Clin. Chem. Lab. Med.*, 2003, **41**, 979–994.
73. H. Palafox-Carlos, J. F. Ayala-Zavala and G. A. Gonzalez-Aguilar, *J. Food Sci.*, 2011, **76**, R6–R15.
74. T. Bohn, Provitamin A carotenoids - occurrence, intake, and bioavailability, in *Vitamin a and Carotenoids: Chemistry, Analysis, Function and Effects (Food and Nutritional Components in Focus)*, ed. V. Preedy, RSC Publishing, London, 2012.
75. E. Biehler, L. Hoffmann and E. Krause, *et al.*, *J. Nutr.*, 2011, **141**, 1769–1776.
76. P. Borel, C. Desmarchelier and U. Dumont, *et al.*, *Br. J. Nutr.*, 2016, **116**, 2091–2096.
77. J. Corte-Real, C. Guignard and M. Gantenbein, *et al.*, *Br. J. Nutr.*, 2017, **117**, 1560–1569.
78. E. Biehler, A. Kaulmann and L. Hoffmann, *et al.*, *Food Chem.*, 2011, **125**, 1328–1334.

79. D. M. Fahy, Y. C. O'Callaghan and N. M. O'Brien, *Food Addit. Contam.*, 2004, **21**, 42–51.
80. M. Richelle, M. Enslen and C. Hager, *et al.*, *Am. J. Clin. Nutr.*, 2004, **80**, 171–177.
81. F. Granado-Lorencio, E. Donoso-Navarro and L. M. Sanchez-Siles, *et al.*, *J. Agric. Food Chem.*, 2011, **59**, 11819–11824.
82. S. Baumgartner, R. T. Ras and E. A. Trautwein, *et al.*, *Eur. J. Nutr.*, 2017, **56**, 909–923.
83. S. H. Hageman, L. She and H. C. Furr, *et al.*, *Lipids*, 1999, **34**, 627–631.
84. J. Corte-Real, E. Richling and L. Hoffmann, *et al.*, *Nutr. Res.*, 2014, **34**, 1101–1110.
85. J. F. Zayas, *Functionality of Proteins in Food*, Springer, Heidelberg, 1997.
86. C. Soukoulis and T. Bohn, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**, 1–36.
87. T. Bohn, *Curr. Nutr. Food Sci*, 2008, **4**, 240–258.
88. R. E. Kopec, J. L. Cooperstone and R. M. Schweiggert, *et al.*, *J. Nutr.*, 2014, **144**, 1158–1166.
89. K. H. van het Hof, I. A. Brouwer and C. E. West, *et al.*, *Am. J. Clin. Nutr.*, 1999, **70**, 261–268.
90. R. M. Schweiggert and R. Carle, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 1807–1830.
91. P. Mapelli-Brahm, J. Corte-Real and A. J. Melendez-Martinez, *et al.*, *Food Chem.*, 2017, **229**, 304–311.
92. M. L. Failla, C. Chitchumroonchokchai and B. K. Ishida, *J. Nutr.*, 2008, **138**, 482–486.
93. N. Z. Unlu, T. Bohn and D. Francis, *et al.*, *J. Agric. Food Chem.*, 2007, **55**, 1597–1603.
94. A. C. Boileau, N. R. Merchen and K. Wasson, *et al.*, *J. Nutr.*, 1999, **129**, 1176–1181.
95. T. W. Boileau, A. C. Boileau and J. W. Erdman Jr, *Exp. Biol. Med. (Maywood)*, 2002, **227**, 914–919.
96. A. J. Melendez-Martinez, P. Mapelli-Brahm and A. Benitez-Gonzalez, *et al.*, *Arch. Biochem. Biophys.*, 2015, **572**, 188–200.
97. N. E. Moran, J. A. Novotny and M. J. Cichon, *et al.*, *J. Nutr.*, 2016, **146**, 368–376.
98. J. L. Cooperstone, R. A. Ralston and K. M. Riedl, *et al.*, *Mol. Nutr. Food Res.*, 2015, **59**, 658–669.
99. M. J. Rodriguez-Roque, B. de Ancos and R. Sanchez-Vega, *et al.*, *Food Funct.*, 2016, **7**, 380–389.
100. N. Z. Unlu, T. Bohn and D. M. Francis, *et al.*, *Br. J. Nutr.*, 2007, **98**, 140–146.
101. E. A. Tydeman, M. L. Parker and M. S. Wickham, *et al.*, *J. Agric. Food Chem.*, 2010, **58**, 9847–9854.
102. O. Livny, R. Reifen and I. Levy, *et al.*, *Eur. J. Nutr.*, 2003, **42**, 338–345.
103. P. Palmero, A. Panozzo and I. Colle, *et al.*, *Food Chem.*, 2016, **199**, 423–432.
104. K. R. Moelants, L. Lemmens and M. Vandebroeck, *et al.*, *J. Agric. Food Chem.*, 2012, **60**, 11995–12003.

105. W. Sriwichai *Déterminants de la bioaccessibilité des caroténoides et tocophérols de légumes feuilles: comparaison variétale et influence du prodéde*, GAIA, 2016.
106. R. M. Schweiggert, D. Mezger and F. Schimpf, *et al.*, *Food Chem.*, 2012, **135**, 2736–2742.
107. J. Riedl, J. Linseisen and J. Hoffmann, *et al.*, *J. Nutr.*, 1999, **129**, 2170–2176.
108. L. Yonekura and A. Nagao, *Biosci., Biotechnol., Biochem.*, 2009, **73**, 196–199.
109. X. Liu, J. Bi and H. Xiao, *et al.*, *J. Agric. Food Chem.*, 2015, **63**, 8534–8543.
110. J. Corte-Real, M. Bertucci and C. Soukoulis, *et al.*, *Food Funct.*, 2017, **8**, 1008–1019.
111. J. Corte-Real, M. Iddir and C. Soukoulis, *et al.*, *Food Chem.*, 2016, **197**, 546–553.
112. D. B. Rodriguez-Amaya, Effects of processing and storage, in *Food Carotenoids*, John Wiley & Sons, Ltd, 2015, pp. 132–173.
113. R. E. Kopec, B. Gleize and P. Borel, *et al.*, *Food Funct.*, 2017, **8**, 1494–1503.
114. S. Blanquet-Diot, M. Soufi and M. Rambeau, *et al.*, *J. Nutr.*, 2009, **139**, 876–883.
115. J. Courraud, J. Berger and J. P. Cristol, *et al.*, *Food Chem.*, 2013, **136**, 871–877.
116. C. Chitchumroonchokchai, S. J. Schwartz and M. L. Failla, *J. Nutr.*, 2004, **134**, 2280–2286.
117. F. Granado-Lorencio, B. Olmedilla-Alonso and C. Herrero-Barbudo, *et al.*, *J. Agric. Food Chem.*, 2007, **55**, 6387–6394.
118. M. G. Ferruzzi, J. L. Lumpkin and S. J. Schwartz, *et al.*, *J. Agric. Food Chem.*, 2006, **54**, 2780–2785.
119. M. Richelle, B. Sanchez and I. Tavazzi, *et al.*, *Br. J. Nutr.*, 2010, **103**, 1800–1807.
120. C. Sy, O. Dangles and P. Borel, *et al.*, *Free Radical Biol. Med.*, 2013, **63**, 195–206.
121. M. Alminger, A. M. Aura and T. Bohn, *et al.*, *Compr. Rev. Food Sci. Food Saf.*, 2014, **13**, 413–436.
122. J. Corte-Real, C. Desmarchelier and P. Borel, *et al.*, *Food Chem.*, 2017, **239**, 751–759.
123. A. Asai, L. Yonekura and A. Nagao, *Br. J. Nutr.*, 2008, **100**, 273–277.
124. A. B. Barua and J. A. Olson, *J. Nutr.*, 2001, **131**, 3212–3215.
125. B. O. Durojaye, K. M. Riedl and R. W. Curley, *et al.*, *FASEB J.*, 2017, **31**, 635.18.
126. J. Chung, K. Koo and F. Lian, *et al.*, *J. Nutr.*, 2012, **142**, 405–410.
127. A. Asai, M. Terasaki and A. Nagao, *J. Nutr.*, 2004, **134**, 2237–2243.
128. F. Khachik, G. R. Beecher and N. F. Whittaker, *J. Agric. Food Chem.*, 1986, **34**, 603–616.
129. C. Sy, O. Dangles and P. Borel, *et al.*, *Arch. Biochem. Biophys.*, 2015, **572**, 89–100.

130. J. Serrano, I. Goni and F. Saura-Calixto, *J. Agric. Food Chem.*, 2005, **53**, 2936–2940.
131. A. Kaulmann, C. M. Andre and Y. J. Schneider, *et al.*, *Food Chem.*, 2015, **197**, 325–332.
132. M. S. Ward, D. Y. Zhao and P. S. Bernstein, *J. Ocul. Biol. Dis. Inf.*, 2008, **1**, 12–18.
133. P. Layer and J. Keller, *J. Clin. Gastroenterol.*, 1999, **28**, 3–10.
134. J. Meier and A. Sturm, *Dig. Dis.*, 2009, **27**, 240–245.
135. S. D'Silva, X. Xiao and M. E. Lowe, *J. Lipid Res.*, 2007, **48**, 2478–2484.
136. D. A. Garrett, M. L. Failla and R. J. Sarama, *J. Agric. Food Chem.*, 1999, **47**, 4301–4309.
137. M. J. Periago, S. Bravo and F. J. Garcia-Alonso, *et al.*, *J. Agric. Food Chem.*, 2013, **61**, 3859–3867.
138. D. Y. Hui and P. N. Howles, *J. Lipid Res.*, 2002, **43**, 2017–2030.
139. T. Imai, M. Imoto and H. Sakamoto, *et al.*, *Drug Metab. Dispos.*, 2005, **33**, 1185–1190.
140. C. Chitchumroonchokchai and M. L. Failla, *J. Nutr.*, 2006, **136**, 588–594.
141. F. Granado, B. Olmedilla and E. Gil-Martinez, *et al.*, *Br. J. Nutr.*, 1998, **80**, 445–449.
142. D. E. Breithaupt, A. Bamedi and U. Wirt, *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.*, 2002, **132**, 721–728.
143. D. M. Gustin, K. A. Rodvold and J. A. Sosman, *et al.*, *Cancer Epidemiol., Biomarkers Prev.*, 2004, **13**, 850–860.
144. V. Diwadkar-Navsariwala, J. A. Novotny and D. M. Gustin, *et al.*, *J. Lipid Res.*, 2003, **44**, 1927–1939.
145. A. During, M. M. Hussain and D. W. Morel, *et al.*, *J. Lipid Res.*, 2002, **43**, 1086–1095.
146. A. Kaulmann, T. Serchi and J. Renaut, *et al.*, *Br. J. Nutr.*, 2012, **108**, 963–973.
147. L. O'Sullivan, S. A. Aisling and N. M. O'Brien, *Int. J. Vitam. Nutr. Res.*, 2009, **79**, 337–347.
148. H. S. Ribeiro, J. M. Guerrero and K. Briviba, *et al.*, *J. Agric. Food Chem.*, 2006, **54**, 9366–9369.
149. L. O'Sullivan, L. Ryan and N. O'Brien, *Br. J. Nutr.*, 2007, **98**, 38–44.
150. E. Reboul and P. Borel, *Prog. Lipid Res.*, 2011, **50**, 388–402.
151. Y. Yamanashi, T. Takada and R. Kurauchi, *et al.*, *J. Atheroscler. Thromb.*, 2017, **24**, 347–359.
152. G. Palczewski, J. Amengual and C. L. Hoppel, *et al.*, *FASEB J.*, 2014, **28**, 4457–4469.
153. J. Amengual, G. P. Lobo and M. Golczak, *et al.*, *FASEB J.*, 2011, **25**, 948–959.
154. J. Amengual, M. A. Widjaja-Adhi and S. Rodriguez-Santiago, *et al.*, *J. Biol. Chem.*, 2013, **288**, 34081–34096.
155. K. Q. Hu, C. Liu and H. Ernst, *et al.*, *J. Biol. Chem.*, 2006, **281**, 19327–19338.
156. M. Gajic, S. Zaripheh and F. Sun, *et al.*, *J. Nutr.*, 2006, **136**, 1552–1557.
157. C. dela Sena, S. Narayanasamy and K. M. Riedl, *et al.*, *J. Biol. Chem.*, 2013, **288**, 37094–37103.

158. L. Wu, X. Guo and W. Wang, *et al.*, *Exp. Biol. Med. (Maywood)*, 2016, **241**, 1879–1887.
159. B. Li, P. P. Vachali and A. Gorusupudi, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 10173–10178.
160. F. Khachik, L. Carvalho and P. S. Bernstein, *et al.*, *Exp. Biol. Med. (Maywood)*, 2002, **227**, 845–851.
161. F. Khachik, F. F. de Moura and D. Y. Zhao, *et al.*, *Invest. Ophthalmol. Visual Sci.*, 2002, **43**, 3383–3392.
162. A. B. Barua and N. Sidell, *J. Nutr.*, 2004, **134**, 286s–289s.
163. M. H. Zile, R. C. Inhorn and H. F. DeLuca, *J. Biol. Chem.*, 1982, **257**, 3537–3543.
164. A. B. Barua and J. A. Olson, *Am. J. Clin. Nutr.*, 1986, **43**, 481–485.
165. J. O. Sass, E. Masgrau and J. H. Saurat, *et al.*, *Drug Metab. Dispos.*, 1995, **23**, 887–891.
166. C. C. Ho, F. F. de Moura and S. H. Kim, *et al.*, *J. Nutr.*, 2009, **139**, 1480–1486.
167. E. Reboul, A. Klein and F. Bietrix, *et al.*, *J. Biol. Chem.*, 2006, **281**, 4739–4745.
168. E. Reboul, A. Goncalves and C. Comera, *et al.*, *Mol. Nutr. Food Res.*, 2011, **55**, 691–702.
169. K. L. Herron, M. M. McGrane and D. Waters, *et al.*, *J. Nutr.*, 2006, **136**, 1161–1165.
170. P. Borel, F. S. de Edelenyi and S. Vincent-Baudry, *et al.*, *Ann. Med.*, 2011, **43**, 47–59.
171. P. Borel, C. Desmarchelier and M. Nowicki, *et al.*, *J. Nutr.*, 2015, **145**, 1740–1747.
172. S. Picone, A. Ritieni and A. Fabiano, *et al.*, *Clin. Biochem.*, 2012, **45**, 1558–1563.
173. E. Kotake-Nara and A. Nagao, *Mar. Drugs*, 2011, **9**, 1024–1037.
174. D. N. D'Ambrosio, R. D. Clugston and W. S. Blaner, *Nutrients*, 2011, **3**, 63–103.
175. C. W. Hadley, S. K. Clinton and S. J. Schwartz, *J. Nutr.*, 2003, **133**, 727–732.
176. O. J. Torrissen and K. Ingebrigtsen, *Aquaculture*, 1992, **108**, 381–385.
177. A. Kaulmann, C. M. Andre and Y. J. Schneider, *et al.*, *Food Chem.*, 2016, **197**, 325–332.
178. I. Goni, J. Serrano and F. Saura-Calixto, *J. Agric. Food Chem.*, 2006, **54**, 5382–5387.
179. P. P. Nair, A. Lohani and E. P. Norkus, *et al.*, *Cancer Epidemiol., Biomarkers Prev.*, 1996, **5**, 913–916.
180. S. Oshima, T. Inakuma and T. Narisawa, *J. Nutr. Sci. Vitaminol. (Tokyo)*, 1999, **45**, 129–134.
181. M. J. Shearer, A. Bach and M. Kohlmeier, *J. Nutr.*, 1996, **126**, 1181s–1186s.
182. M. M. Groenen-van Dooren, J. E. Ronden and B. A. Soute, *et al.*, *Biochem. Pharmacol.*, 1995, **50**, 797–801.

183. A. Lindqvist, Y. G. He and S. Andersson, *J. Histochem. Cytochem.*, 2005, **53**, 1403–1412.
184. W. C. Leung, S. Hessel and C. Meplan, *et al.*, *FASEB J.*, 2009, **23**, 1041–1053.
185. G. Lietz, A. Oxley and W. Leung, *et al.*, *J. Nutr.*, 2012, **142**, 161s–165s.
186. G. P. Lobo, J. Amengual and D. Baus, *et al.*, *J. Biol. Chem.*, 2013, **288**, 9017–9027.
187. S. J. Hickenbottom, J. R. Follett and Y. Lin, *et al.*, *Am. J. Clin. Nutr.*, 2002, **75**, 900–907.
188. G. Lietz, A. Oxley and C. Boesch-Saadatmandi, *et al.*, *Mol. Nutr. Food Res.*, 2012, **56**, 241–250.
189. A. M. Priyadarshani, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 1710–1717.
190. S. A. Tanumihardjo, N. Palacios and K. V. Pixley, *Int. J. Vitam. Nutr. Res.*, 2010, **80**, 336–350.
191. W. N. A. Charman and V. J. Stella, *Int. J. Pharm.*, 1986, **33**, 165–172.
192. R. E. Kopec, C. Caris-Veyrat and M. Nowicki, *et al.*, *FASEB J.*, 2017, **31**, 32–35.
193. P. Borel, M. Moussa and E. Reboul, *et al.*, *J. Nutr.*, 2007, **137**, 2653–2659.
194. S. Dash, C. Xiao and C. Morgantini, *et al.*, *Annu. Rev. Nutr.*, 2015, **35**, 265–294.
195. E. J. Niesor, E. Chaput and J. L. Mary, *et al.*, *Lipids*, 2014, **49**, 1233–1243.
196. H. C. Furr, *J. Nutr.*, 2004, **134**, 281s–285s.
197. H. Y. Chung, A. L. Ferreira and S. Epstein, *et al.*, *Am. J. Clin. Nutr.*, 2009, **90**, 533–539.
198. P. S. Bernstein, B. Li and P. P. Vachali, *et al.*, *Prog. Retinal Eye Res.*, 2016, **50**, 34–66.
199. P. Bhosale and P. S. Bernstein, *Anal. Biochem.*, 2005, **345**, 296–301.
200. A. Asai, T. Nakano and M. Takahashi, *et al.*, *J. Agric. Food Chem.*, 2005, **53**, 7302–7306.
201. S. J. Hickenbottom, S. L. Lemke and S. R. Dueker, *et al.*, *Eur. J. Nutr.*, 2002, **41**, 141–147.
202. N. E. Moran, M. J. Cichon and K. M. Riedl, *et al.*, *Am. J. Clin. Nutr.*, 2015, **102**, 1436–1449.
203. H. Park, Model-based compartmental analysis of the kinetics of retinol and beta-carotene in humans: statistical considerations in designing and building models for retinol plus expanded models for beta-carotene, PhD, Pennsylvania State University, 2011.
204. F. Bohm, R. Edge and T. G. Truscott, *Acta Biochim. Pol.*, 2012, **59**, 27–30.
205. T. Heymann, P. Heinz and M. A. Glomb, *J. Agric. Food Chem.*, 2015, **63**, 3279–3287.
206. E. Fernandez-Garcia, *Food Funct.*, 2014, **5**, 1994–2003.
207. A. A. Woodall, G. Britton and M. J. Jackson, *Biochim. Biophys. Acta*, 1997, **1336**, 575–586.
208. Z. Li, X. Dong and H. Liu, *et al.*, *Mol. Vis.*, 2013, **19**, 1656–1666.
209. J. Fiedor and K. Burda, *Nutrients*, 2014, **6**, 466–488.

210. F. J. Pashkow, D. G. Watumull and C. L. Campbell, *Am. J. Cardiol.*, 2008, **101**, 58d–68d.
211. B. Nidhi, G. Sharavana and T. R. Ramaprasad, *et al.*, *Food Funct.*, 2015, **6**, 450–460.
212. C. Caris-Veyrat, A. L. Garcia and E. Reynaud, *et al.*, *Int. J. Vitam. Nutr. Res.*, 2016, in press.
213. M. L. Bonet, J. A. Canas and J. Ribot, *et al.*, *Subcell. Biochem.*, 2016, **79**, 377–414.
214. Y. Kitamura, K. Tanaka and C. Kiyohara, *et al.*, *Int. J. Epidemiol.*, 1997, **26**, 307–314.
215. L. Wang, J. M. Gaziano and E. P. Norkus, *et al.*, *Am. J. Clin. Nutr.*, 2008, **88**, 747–754.
216. A. J. Guri, R. Hontecillas and H. Si, *et al.*, *Clin. Nutr.*, 2007, **26**, 107–116.