Review

Carotenoid Basics: From Food to Skin

Daniela Weber 1,2,3,* and Tilman Grune 1,2,3,4,5

Affiliations

1. Department of Molecular Toxicology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), 14558 Nuthetal, Germany
2. Food4Future (F4F), c/o Leibniz Institute of Vegetable and Ornamental Crops (IGZ), 14979 Grossbeeren, Germany
3. NutriAct Competence Cluster Nutrition Research Berlin-Potsdam, 14558 Nuthetal, Germany
4. Institute of Nutritional Science, University of Potsdam, 14469 Potsdam
5. Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria

* Correspondence: daniela.weber@dife.de

Abstract: The aim of this review is to provide a comprehensive and simple graphical overview on carotenoids. The review describes in four detailed figures the course of carotenoids from food to skin. Differences in chemical structures of the six most prominent carotenoids α-carotene, β-carotene, lutein, zeaxanthin, lycopene and β-cryptoxanthin and their seasonal variations in plasma concentrations resulting from different availability of fruits and vegetables are highlighted. Furthermore, factors affecting carotenoid status, sites of storage and how and where they can be assessed (dietary intake and in vivo) are discussed. The route of carotenoid transport after ingestion, transport across the enterocyte and receptors involved in carotenoid uptake in peripheral cells are shown followed by the routes of carotenoid delivery to the skin and reasons for variation in skin carotenoid status are visualized.

Keywords: carotenoids, dietary intake, skin carotenoids, intestinal absorption
Carotenoid Basics: Food Sources and Structures

Carotenoids are a class of lipophilic compounds synthesized by plants, algae, bacteria and fungi. More than 1,000 molecules belong to the carotenoid group (Yabuzaki, 2017), however, only six seem to be found in human plasma in relevant amounts and thus, have been studied in the context of different diseases including cancer, frailty, cataract, obesity, cardiovascular diseases and skin diseases (Krinsky and Johnson, 2005, Kochlik et al., 2019, Weber et al., 2020, Henning et al., 2023a, Linnewiel-Hermoni et al., 2015, Thies et al., 2012, Jacques et al., 2013, Cui et al., 2013). These six most prominent plasma carotenoids are α-carotene, β-carotene, lutein, zeaxanthin, lycopene and β-cryptoxanthin (Figure 1).

The structures of carotene and β-carotene as well as lutein and zeaxanthin, respectively, differ only in the position of one double-bond (orange frames in Figure 1A). The addition of one hydroxyl-group (blue arrows in Figure 1A) to α-carotene results in lutein whereas the addition to β-carotene gives the structure of zeaxanthin or β-cryptoxanthin, depending on which ring the hydroxyl group is located. Carotenoids containing a hydroxyl group are termed xanthophylls, those without hydroxyl group are called carotenes. Lycopene, in contrast to the other carotenoids, is an open structure that does not contain a ring.

Carotenoids are powerful antioxidants and are able to quench singlet oxygen and free radicals due to the structure containing conjugated double bonds (Fiedor and Burda, 2014). Given the long unsaturated aliphatic chain carotenoids are lipophilic compounds and as such, located in the lipid membranes and adipose tissue. The structure also allows UV absorption and protects the macula from oxidative stress (Arunkumar et al., 2020). In addition to macula protection, carotenoid supplements have been shown to increase UV protection in human skin (Ito et al., 2018, Groten et al., 2019, Baswan et al., 2020). Lutein and zeaxanthin are known as macular as they are present in the eye where they act as antioxidants and absorb blue light. Carotenoids can act as pro-oxidants under certain conditions (Edge and Truscott, 2018) so it is important to note, that especially in supplements containing high doses of carotenoids, adverse effects such as cancer can result (Goodman et al., 2004, Alpha-Tocopherol, 1994). Recent clinical trials have excluded participants with a history of lung cancer or describe cancer (unknown if previous or current) as an exclusion criterion (Zhou et al., 2020, Group et al., 2012). Besides the direct antioxidant effects, carotenoids are able to alter intracellular-signaling cascades as well as gene expression and interact with transduction cascades, such as...
NF-κB and Nrf2 translocation (Rubin et al., 2017). For instance, in cell culture studies, β-carotene was able to inhibit pro-inflammatory mediators, including NO, interleukins 1β and 6, and MCP-1, and suppress NF-κB (Lin et al., 2012). For a comprehensive review on the role of carotenoids in viral defense, especially against SARS-CoV-2 and associated symptoms see Khalil et al. (Khalil et al., 2021).

Plasma carotenoids are an excellent measure for fruit and vegetable intake (Al-Delaimy et al., 2005, Al-Delaimy et al., 2004, Campbell et al., 1994). The most common sources are shown in Figure 1B. Lycopene is found mostly in tomatoes, tomato products such as sauces, ketchup and tomato juice, grapefruits and watermelons. β-cryptoxanthin is most prominent carotenoid in oranges, orange juice, tangerines and mandarins. Lutein and zeaxanthin are present in green leafy vegetables such as kale, brussels sprouts, spinach, salads and in corn. α- and β-carotene are generalists and as such, present in many orange, yellow and green fruits and vegetables including carrots, squash, sweet potato, peppers, peaches, papaya, juices. There are various carotenoids databases available for different countries (Dias et al., 2018, Holden et al., 1999), some contain detailed values for several carotenoids while databases of other countries, including Germany, contain only data on β-carotene (Bundeslebensmittelschlüssel). Plasma concentrations of carotenoids vary depending on their dietary intake and subsequently underly seasonal variations (Figure 1C). Further factors that have an impact on carotenoid status are described in Figure 2. The carotenoids with the highest plasma concentration are lycopene and β-carotene (around 1 µmol/L). Lycopene is highest in the late summer months and fall whereas β-carotene is highest in the early summer months. β-Cryptoxanthin increases during winter, most likely because citrus fruits are more available in Europe in winter. Lutein/zeaxanthin and α-carotene do not show significant seasonal variation. It should be noted, that seasonal variation is different in other regions (Cantilena et al., 1992, Al-Delaimy et al., 2004).

**Carotenoids Absorption, Status and their Assessment**

Carotenoids are dietary components and as such depend on an intact digestive tract, among other factors. After absorption, they are circulated (see Figure 3 for details on “Carotenoid Transport and Cellular Uptake”) and subsequently stored in tissues where they can be
measured. Besides measurement in tissues, dietary intake can also be assessed to evaluate carotenoid status.

The food matrix, preparation and fat content impact absorption (Figure 2A). For instance, cooked foodstuffs provide better bioavailability which is further increased by addition of fats, due to the lipophilic nature of carotenoids. Some carotenoids lower the chylomicron response of other carotenoids when ingested simultaneously, but this did not impact plasma concentrations after a 3-week study (Tyssandier et al., 2002). Ester-linkage of xanthophylls may improve absorption (Breithaupt et al., 2002), as well as the isomeric state: all-trans-β-carotene showed a higher absorption rate compared to the cis-form (During et al., 2002). Some literature exists showing that age may impair bioavailability of lycopene but not of the other carotenoids (Cardinault et al., 2003, Stuetz et al., 2016). Host-related factors such as lipid metabolism, inflammation and genetics can furthermore influence carotenoid status in terms of impaired absorption and lead to lower carotenoid bioavailability. For more details see excellent review by von Lintig et al. (von Lintig et al., 2020). Whether or not redox stress in the gastrointestinal tract has an impact on the absorption and/or bioavailability of carotenoids is unknown. The enzyme that converts β-carotene (β-carotene oxygenase 2, BCO2, see Figure 3B) to apocarotenoids is expressed as oxidative-stress inducible gene (Babino et al., 2015). It has been hypothesized, that oxidative stress, as in chronic diseases and in excess consumption of carotenoids, can induce BCO2 and thus, carotenoid breakdown in tissues and circulation (Babino et al., 2015).

Adipose tissue is the major storage site of carotenoids in the body and there are regional differences (Chung et al., 2009) (Figure 2B). Abdominal fat exhibits higher concentrations than buttocks and thigh (Chung et al., 2009). The skin and liver are further sites of carotenoid storage. Transporters on the target cells allow to selectively uptake carotenoids (see Figure 3C) such as lutein and zeaxanthin in the eye (retinal pigment epithelium) (Arunkumar et al., 2020), and lycopene in the prostate (Grainger et al., 2015). Plasma is not per se a site of storage but also contains a considerable amount since plasma volume is around 2.5-3L and total plasma carotenoids are around 3-4 µmol/L (Henning et al., 2023b). Concentrations in adipose tissue range around 3.5 (thigh) to 6 (buttocks) nmol/mg of total carotenoids (Chung et al., 2009).

The most prominent way to measure carotenoids status is via blood analyses (Figure 2C). Carotenoids can be measured in plasma samples that have been stored in biobanks for a very
long time (up to 20 years) since they are very stable in plasma at -80°C (Comstock, 1993). The advantage is that single carotenoids are detected and these can be explained by intake of certain fruits and vegetables (and their products). In recent years, the measurement of carotenoids in skin has become more prevalent due to devices that can be used by study nurses and allow quick data acquisition. Skin biopsies are invasive and therefore not applicable in large-scale human studies. The measurement of skin carotenoids by resonance Raman spectroscopy and reflectance spectroscopy (Darvin et al., 2011) has increased in the last years, not only due to the convenient, non-invasive and fast bedside measurement but also due to the high correlation with plasma levels. In contrast to plasma measurements, the discrimination of single carotenoids in skin is still under development.

Most common ways to assess dietary intake include carotenoids by questionnaires, hand-written records and apps (Figure 2D). These non-invasive techniques allow screening of large cohorts without the burden of taking blood samples. Food frequency questionnaires can be electronically evaluated making them very convenient for large cohorts. They allow the categorization of dietary habits (mostly regarding the last 12 months) but not the assessment of single (micro-)nutrients or other dietary constituents. 24h recalls are interviews that are usually conducted by trained personnel and the detailed intake of single micro- and macronutrients can be calculated. 24h recalls only represent the nutrient intake of the previous day which is not representative for the usual dietary habit or nutrient intake of a person. The dietary record, usually conducted over 3 days, has a high participant burden and also requires manually entered data. The advantage is that the intake of single micro- and macronutrients can be calculated. New technologies to assess dietary nutrient intakes includes the use of apps. Numerous apps exist, some based on photos that the participants take of their meals (including a reference card for size of portions). The data usage from apps can be quite valuable since the apps can be linked to nutrient databases and results can be immediately integrated/evaluated by scientists. However, all of the described tools, questionnaires, hand-written records and apps can be subject to bias.

Carotenoids can be measured in biological matrices, mainly plasma and serum, by HPLC (Figure 2E). According to the chemical structures, those carotenoids with a similar structure are eluted very close together on a reversed-phase column (see lutein and zeaxanthin as well as α- and β-carotene) in the HPLC chromatogram. Lycopene is eluted as a “mountain range” and usually results of total lycopene are given as a sum of the three peaks. This results from
different isomers (cis and trans) that can be present on different of the double-bonds within lycopene molecules.

**Carotenoid Transport and Cellular Uptake**

Carotenoids in the chyme are transported along the small intestine where absorption takes place. In an optimal scenario, the carotenoids are emulsified in lipid droplets, the intestine is intact and enzymes and bile salts are present at optimal concentrations.

Carotenoids are transported in the small intestine along with other food constituents (Figure 3A). They are emulsified into lipid droplets, then incorporated into mixed micelles together with gastrointestinal enzymes and taken up by the enterocytes (brush border cells). After being packed into chylomicrons, they are secreted and transported via the lymph to the capillaries and further to the peripheral target cells. Chylomicron remnants continue to the liver where carotenoids can be stored or metabolized, excreted or secreted (Desmarchelier and Borel, 2017). For secretion, carotenoids are packaged into VLDL in the liver and further distributed to target tissues in the circulation along with lipid transport. Little is known on the impact of intestinal microbiota on absorption and bioavailability of carotenoids (Eroglu et al., 2023). A large proportion of dietary carotenoids passes through the small intestine and continues to the colon. There they can act via different actions which include anti-inflammatory and antioxidant (see section on Carotenoid Basics: Food Sources and Structures) which can potentially (beneficially) alter gut microbiota composition. In addition, they can act as substrate for beneficial bacteria (pre-biotic function), increase tight junction integrity and mucus secretion (barrier function) and impact the immune system (the most abundant antibody isotype IgA was significantly increased in mice after β-carotene supplementation, similar to the effect of retinol (Nishiyama et al., 2011))(Eroglu et al., 2023). The recent review by Eroglu highlights that the interaction of gut microbiota and carotenoids is clearly understudied (Eroglu et al., 2023).

Different transporters are responsible for uptake of mixed micelles: SR-B1 (scavenger-receptor B1), NPC1L1 (Niemann-Pick C1-like protein 1), and CD36 (cluster of differentiation 36) (Figure 3B). In addition, passive diffusion along a concentration gradient may also occur. Within the enterocyte, provitamin-A-carotenoids can be converted to retinal (central cleavage via cytosolic BCO1, β-carotene oxygenase 1) and retinol (via retinol dehydrogenases),
however, the majority of conversion to retinal takes place in the retina. The carotenoids that can undergo conversion to retinol via cleavage to retinaldehyde and retinol (Sahu and Maeda, 2016, Weber and Grune, 2012) are termed provitamin-A carotenoids and include α-carotene, β-carotene and β-cryptoxanthin. They can contribute around 35% to retinol supply (Weber and Grune, 2012). Other carotenoids can be converted to apocarotenoids via eccentric cleavage by BCO2 (β-carotene oxygenase 2) which is located at the inner mitochondrial membrane. Carotenoids and their remnants are packed into chylomicrons in the endoplasmic reticulum (ER) and processed in the Golgi apparatus. For excellent review see (Desmarchelier and Borel, 2017).

Peripheral cells possess specific receptors that allow chylomicrons, VDLD, LDL and HDL to release carotenoids and lipids into the target cells (Figure 3C). These membrane proteins are: Lipoprotein lipase (LPL), C36, LDL receptor and scavenger-receptor B1. Carotenoids from LDL are released and taken up into target cells by receptor-mediated endocytosis. Those from HDL are taken up via interaction with SR-B1, while chylomicrons release carotenoids and lipids after interaction with LPL and CD36.

**Skin Carotenoids**

To understand why skin can be a good medium to measure carotenoid status one must know the layers of human skin (Figure 4A). The stratum corneum is the outer layer of the skin. Below it, there is the epidermis containing keratinocytes. Capillaries, hair follicles, nerves and sweat glands are present in the dermis, the layer between the epidermis and the subcutaneous fatty tissue. Keratinocytes migration to the surface takes around six weeks, stratum corneum renewal takes two to three weeks (Darvin et al., 2008).

The secretion via sweat glands (Route 1 in Figure 4B) similar to vitamin E is one of the two main routes of carotenoid delivery to the skin (Darvin et al., 2009, Darvin et al., 2011). Through this process it can take 1-3 days for carotenoids ingested via supplements to occur on the skin surface. The other main route is diffusion from the adipose tissue, blood and lymph through the dermis and epidermis to the stratum corneum (Route 2). Keratinocyte migration (Route 3) is the natural route of maturation and can also transport carotenoids. And finally topical application of products containing carotenoids (Route 4) can also lead to increase in skin levels (Darvin et al., 2011). The main routes are diffusion and secretion. It has been shown that
Carotenoid levels in skin increase with increased dietary intake of carotenoids (Darvin et al., 2008, Henning et al., 2023b) and that this correlates well with total plasma carotenoids. In supplementation studies, it has been shown that it takes around four weeks to see significant increases in skin carotenoid content (Stahl et al., 1998) and six weeks to observe an effect in photoprotection (Stahl and Sies, 2012).

There are regional differences, thus it is of importance which body region is chosen for measurement in human studies (Figure 4C). Usually, the palm of the hand is chosen since it is convenient and does not require eye protection in contrast to the forehead (depending on the device used). Areas with high numbers of sweat glands also exhibit higher skin carotenoid levels (Darvin et al., 2008). The levels are affected by season: High levels are observed in Summer and Fall, lower levels are observed in Winter (Darvin et al., 2008). The same study also showed that acute infection and stress are able to negatively impact skin carotenoid levels within days (Darvin et al., 2008). In a one-year study Darvin et al. demonstrated how lifestyle-factors such as illness, fatigue, stress, alcohol consumption, and smoking have a direct negative impact on skin carotenoids (Darvin et al., 2008). It has been reported in studies from the 1970s and 1980s, that 10% of patients with Diabetes tend to develop hypercarotenaemia with the normal intake of carotenoid-rich foods (Gouterman and Sibrack, 1980, Hoerer et al., 1975) resulting in yellow pigmentation of the skin. Newer studies suggest that the yellow color of the skin can be attributed to glycosylated proteins (Sasaki et al., 2022) in the skin, rather than carotenoids. Sasaki et al. demonstrated that levels of skin carotenoids in patients with diabetes compared to control subjects were similar (Sasaki et al., 2022). In addition, it has been shown that the impaired conversion of carotenoids to retinol in the diabetic liver may also lead to carotenoid accumulation in the skin (Gouterman and Sibrack, 1980, Lin, 2006, Guliani et al., 2021). Renal disease, hypothyroidism and hyperlipidemia may also contribute to reduced clearance of carotenoids (Lin, 2006). Even with normal intakes some patients could still exhibit carotenoderma (Priyadarshani, 2018, Sehgal et al., 2011). For excellent review on skin carotenoids see Melendez-Martinez et al. (Melendez-Martinez et al., 2019). The correlation between skin, plasma and dietary carotenoids was recently evaluated in a study from our group (Henning et al., 2023b). The correlation between total plasma carotenoids and reflection spectroscopy (r=0.81), Raman spectroscopy (r=0.72) and app-based dietary record (r=0.65) (measured in n=21 middle-aged participants on 3 visits, 4 weeks apart each). Thus, measuring carotenoids in skin is a reliable alternative to evaluate carotenoid intake and
plasma status without taking blood samples. This might be a great option for vulnerable populations, large-scale studies requiring high throughput measurement, studies with little budget and those in field settings without laboratory equipment.

Many aspects of carotenoids have been extensively researched, such as their molecular structure, their content in food and associations with human health and disease. Some fields, however, remain to be elucidated, including the effect of oxidative stress, inflammation and/or disease on absorption of carotenoids, the cross-talk between carotenoids and pathogens, their role in gut microbiota regulation and skin diseases. These understudied areas clearly demonstrate that carotenoid research continues to be relevant, even almost 200 years after their discovery.
Figure Legends

Figure 1. A. Chemical structure of the six most prominent carotenoids in plasma and in foodstuffs. B. Sources of carotenoids shown according to their seasonal availability in Europe. C. Seasonal variation in plasma concentration. Data shows mean, untransformed plasma carotenoid concentration from the European MARK-AGE Project (n=2,118): winter (n=499), spring (n=675), summer (n=496), fall (n=448) (Stuetz et al., 2016).

Figure 2. A. Factors that affect carotenoid absorption and status. B. Storage of carotenoids in human tissues. C. Blood and tissue measurements. Further details on skin carotenoids are shown in Figure 4. D. Ways to assess carotenoid intake. E. HPLC chromatogram of carotenoids in the UV channel (450 nm).

Figure 3. A. Carotenoid transport after ingestion. B. Carotenoid transport across the enterocyte. SR-B1 (scavenger-receptor B1), NPC1L1 (Niemann-Pick C1-like protein 1), CD36 (cluster of differentiation 36). BCO1 (β-carotene oxygenase 1), BCO2 (β-carotene oxygenase 2), ER (endoplasmic reticulum). C. Receptors involved in carotenoid uptake in peripheral cells.

Figure 4. A. Layers of the skin. B. Routes of carotenoid delivery to the skin. B. Routes of carotenoid delivery to the skin. C. Reasons for variation in skin carotenoid status.
**Figure Preparation:** Parts of the figures were prepared using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

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