

Distribution and metabolism of dietary carotenoids in humans as a criterion for development of nutritional supplements*

Frederick Khachik

Department of Chemistry and Biochemistry, Joint Institute for Food Safety and Applied Nutrition (JIFSAN), University of Maryland, College Park, 20742 MD, USA

Abstract: There are approximately 40–50 carotenoids in commonly consumed fruits and vegetables in a typical U.S. diet. These can be divided into carotenoid epoxides, mono- and dihydroxycarotenoids, hydrocarbon carotenoids, and carotenol acyl esters. However, among these, only a selected group of carotenoids are routinely found in human plasma, breast milk, major organs, and ocular tissues. In addition, several carotenoid metabolites have also been isolated and characterized from human plasma, tissues, and ocular tissues. The proposed metabolic transformation of carotenoids in humans will be discussed. Dietary carotenoids and their metabolites have been implicated in the prevention of cancer, cardiovascular disease, and age-related macular degeneration (AMD). An approach for the development of a nutritional supplement that is based on the distribution of carotenoids and their metabolites in humans will be discussed.

Keywords: food carotenoids; human plasma carotenoids; carotenoid metabolism; carotenoid oxidation products; HPLC analysis of carotenoids; multicarotenoid nutritional supplement.

INTRODUCTION

Although approximately 600 carotenoids have been isolated from natural sources and characterized [1], the number of dietary carotenoids in common fruits and vegetables consumed in the United States is in excess of 40 [2,3]. Only 13 *all-E*-carotenoids which belong to the class of hydroxy- and hydrocarbon carotenoids (carotenes) have been detected in human serum and milk [4,5]. In addition, eight metabolites resulting from two major dietary carotenoids, namely, (3*R*,3'*R*,6'*R*)-lutein, (3*R*,3'*R*)-zeaxanthin, and lycopene have also been characterized [2–9]. The vitamin A active carotenoids in human serum or plasma and milk are: α -carotene, β -carotene, β -cryptoxanthin, and γ -carotene. However, the nutritional significance of other non-vitamin A active carotenoids in prevention of cancer [10,11], heart disease [12,13], and age-related macular degeneration (AMD) [14–20] has also been realized. Here, the proposed metabolic transformations of non-vitamin A active carotenoids that play an important role in the biological activity of these compounds is discussed. Based on the correlation between dietary carotenoids and those found in human plasma and tissues, an approach for development of a multicarotenoid nutritional supplement is recommended. However, to address the impact of dietary carotenoids and their metabolites on human health, the distribution of these pigments in foods, human plasma, and tissues needs to be discussed first.

*Paper based on a presentation at the 14th International Symposium on Carotenoids, Edinburgh, Scotland, 17–22 July 2005. Other presentations are published in this issue, pp. 1477–1557.

DISTRIBUTION OF CAROTENOIDS IN FRUITS AND VEGETABLES

Extracts from fruits and vegetables are usually analyzed by high-performance liquid chromatography (HPLC) in order to separate and quantitate the various carotenoids [2]. The characterization of carotenoids is accomplished by combination of HPLC/UV/vis photodiode array detection, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and comparison of the HPLC/UV/vis profiles of unknowns with those of synthetic samples. Extensive HPLC analyses of fruits and vegetables to date have revealed that each of these foods has its own unique carotenoid distribution. However, these foods can be generally divided into three major categories [2,3]. These are: (1) the green, (2) yellow/red, and (3) yellow/orange fruits and vegetables.

The green fruits and vegetables usually consist of the same carotenoids, mainly neoxanthin, violaxanthin, and lutein epoxide as well as lutein, α -carotene, and β -carotene, but at varying concentrations [21]. Since there is a well-established correlation between the concentration of chlorophylls and carotenoids, the darker green the fruits and vegetables are, the higher is the chlorophyll and carotenoid content. The most commonly consumed green fruits and vegetables in the United States are: green beans, lima beans, broccoli, brussels sprouts, cabbage, kale, kiwi, lettuce, muskmelon (honeydew), green peas, and spinach [2]. There are some subtle differences among the various greens. For example, a certain variety of lettuce, e.g., romaine lettuce (*Lactuca sativa*, variety Rommana) in addition to the above carotenoids, also contains lactucaxanthin [(3*S*,6*S*,3'*S*,6'*S*)- ϵ,ϵ -carotene-3,3'-diol] which is often detected in human serum at very low concentrations [4]. In certain green vegetables (i.e., green beans, lima beans, and green peas), the concentration of α -carotene is much higher than the concentration of this compound in other greens such as broccoli, brussels sprouts, kale, and spinach. In the latter green vegetables, only very low concentrations of α -carotene, particularly relative to β -carotene, can be detected. The green fruits and vegetables are excellent dietary sources of lutein, which is often present at much higher concentrations than β -carotene in these foods [21,22]. Quantitative data with regard to dietary (3*R*,3'*R*,6'*R*)-lutein, (3*R*,3'*R*)-zeaxanthin, and their (*E/Z*)-geometrical isomers are scarce and, in most cases, only the combined concentrations of these two carotenoids in foods are reported. However, recently the qualitative and quantitative distribution of lutein, zeaxanthin, and their (*E/Z*)-isomers in the extracts from some of the most commonly consumed fruits, vegetables, and pasta products have been determined by HPLC employing a silica-based nitrile-bonded column [23]. Lutein and zeaxanthin also accumulate in the human macula, and the high dietary intake of these carotenoids has been inversely correlated with a reduction in the incidence of AMD that is the leading cause of blindness in the Western world [14]. The distribution of lutein, zeaxanthin, and other dietary carotenoids as well as their metabolites in human ocular tissues will be discussed later. Detailed carotenoid analyses of extracts from plasma of human subjects consuming raw green fruits and vegetables have not detected carotenoid epoxides, whereas high concentrations of lutein, α -carotene, and β -carotene are normally present [4–6].

The yellow/red fruits and vegetables contain mostly hydrocarbon carotenoids (carotenes). The common yellow ones are apricot, cantaloupe, carrot, pumpkin, and sweet potato that are the primary sources of α -carotene and β -carotene [24,25]. The typical red fruits (i.e., tomato, pink grapefruit, and watermelon) are major dietary sources of carotenoids such as lycopene, ζ -carotene, phytofluene, phytoene, and to a lesser extent also contain neurosporene, γ -carotene, and β -carotene [22,25,26]. All of the hydrocarbon carotenoids of the yellow/red fruits and vegetables are absorbed by humans [4–6]. However, lycopene is one of the most important carotenoids in this group since tomatoes and tomato-based food products, which are the main source of this compound, constitute a large proportion of fruit consumed in the Western diet [26]. Consequently, a high concentration of lycopene is normally found in human serum. Several studies have associated a high intake of foods rich in lycopene with a lower risk for the incidence of prostate cancer [27–29].

The yellow/orange fruits and vegetables (e.g., mango, papaya, peaches, prunes, acorn and winter squash, and oranges) each have unique and complex carotenoid profiles [2]. This is because these foods, in addition to the carotenoids found in green and yellow fruits and vegetables described above, contain

a number of hydroxycarotenoids and epoxides which are esterified with straight-chain fatty acid esters such as lauric, myristic, and palmitic acids [2,3,25,30,31]. The major carotenoids in yellow/orange fruits and vegetables are: lutein, zeaxanthin, α -cryptoxanthin, β -cryptoxanthin, α -carotene, and β -carotene. The esterified hydroxycarotenoids have not been detected in human serum and appear to undergo hydrolysis in the presence of pancreatic secretions to regenerate their parent hydroxycarotenoids that are then absorbed into the blood stream.

DISTRIBUTION OF CAROTENIDS AND THEIR METABOLITES IN HUMANS

Carotenoids in human serum and milk originate from consumption of fruits and vegetables that are one the major dietary sources of these compounds. Comparison of the qualitative profile of carotenoids in foods with those of human serum and milk has revealed that, with the exception of carotenoid epoxides, all of the carotenoids described earlier are all absorbed by humans [4,5]. Pro-vitamin A carotenoids such as α -carotene, β -carotene, β -cryptoxanthin, and γ -carotene are in part converted to vitamin A and are also absorbed into the blood and tissues intact. Non-vitamin A active carotenoids (e.g., α -cryptoxanthin, neurosporene, ζ -carotene, phytofluene, and phytoene) appear to be absorbed intact. At present, there is no evidence to suggest that these carotenoids undergo metabolic transformation.

Several metabolites of lutein, zeaxanthin, and lycopene in human serum and milk have also been isolated and characterized [4,5]. The proposed metabolic transformation of lutein and zeaxanthin in humans involves a series of oxidation–reduction and double-bond isomerization reactions. These pathways lead to the formation of (3*R*,3'*S*,6'*R*)-lutein (3'-epilutein), (3*R*,6'*R*)-3-hydroxy- β , ϵ -caroten-3'-one, (3*R*,6'*S*)-3-hydroxy β , ϵ -caroten-3'-one, 3'-hydroxy- ϵ , ϵ -caroten-3-one, (6*R*,6'*S*)- ϵ , ϵ -carotene-3,3'-dione, (6*S*,6'*S*)- ϵ , ϵ -carotene-3,3'-dione, (6*R*,6'*S*)- ϵ , ϵ -carotene-3,3'-dione, and (6*R*,6'*R*)- ϵ , ϵ -carotene-3,3'-dione. These transformations are shown in Fig. 1.

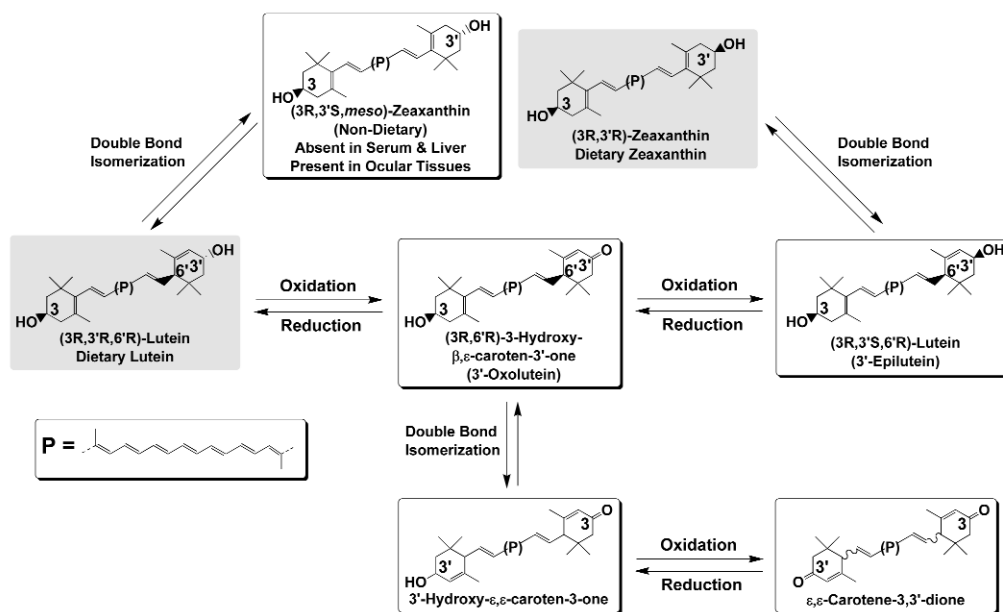


Fig. 1 Proposed metabolic transformation of dietary lutein and zeaxanthin in humans; (3*R*,3'*S*,*meso*)-zeaxanthin is absent in human serum and liver but present in human ocular tissues [35].

Human bioavailability and metabolic studies have supported the possibility of *in vivo* oxidation of these carotenoids in humans [9,32,33]. Similar pathways for transformation of lutein and zeaxanthin to their metabolites identified in human ocular tissues have also been proposed [34,35]. One of the metabolites of lutein that has been identified in human ocular tissues despite its absence in human liver and plasma is (3*R*,3'*S*; *meso*)-zeaxanthin [16,35]. This carotenoid is most likely formed from double-bond isomerization of dietary (3*R*,3'*R*,6'*R*)-lutein. The presence of the oxidation products of lutein and zeaxanthin in human plasma and ocular tissues is in agreement with the role and function of these carotenoids as antioxidants.

Two dehydration products of lutein, 3-hydroxy-3',4'-didehydro- β , γ -carotene (anhydrolutein I), and 3-hydroxy-2',3'-didehydro- β , ϵ -carotene (anhydrolutein II) that are not of dietary origin have also been identified in human plasma [4,5,8] (Fig. 2). These compounds are apparently formed in the presence of acids by the nonenzymatic dehydration of lutein in the human stomach.

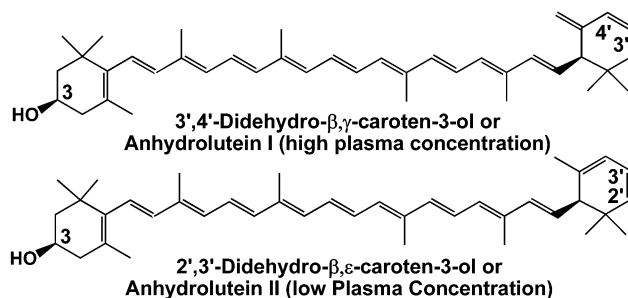


Fig. 2 Dehydration products of lutein identified in human plasma.

Metabolism of lycopene in humans involves epoxidation at the 5,6-position, followed by rearrangement and ring opening to yield 2,6-cyclolycopene-1,5-diols I and II whose structures have been confirmed by partial and total synthesis [36,37] (Fig. 3).

a mixture of these compounds (multicarotenoid) appear to be a more logical strategy. One approach in developing such a multicarotenoid supplement is to formulate its composition similar to the distribution of carotenoids in the serum of healthy humans. In a 1997 publication, we reported the relative distribution of major dietary carotenoids in the serum of 10 healthy human subjects with a high intake of fruits and vegetables [40]. The average distributions (% weight) of some of the major carotenoids in the serum of these subjects were: lutein (20 %), zeaxanthin (3 %), α -cryptoxanthin (4 %), β -cryptoxanthin (8 %), anhydroluteins I & II (3 %), α -carotene (6 %), β -carotene (10 %), lycopene (20 %), ζ -carotene (10 %), phytofluene (8 %), phytoene (4 %), γ -carotene (2 %), and neurosporene (2 %). Supplementation studies with this mixture could serve as a starting point in fine-tuning the composition of the multicarotenoid. In these studies, it would be desirable to proportionally increase the plasma concentration of all the carotenoids without causing interaction and/or competitive absorption between these compounds. While there is a large inter-individual variability in the serum carotenoid profiles of human subjects who are on self-selecting diets, this variability is significantly reduced in subjects supplemented with carotenoids. As a result, the serum concentration of these compounds can be closely correlated with the composition of the supplemental multicarotenoid. Because the family of dietary carotenoids has been implicated in the prevention of several disabling diseases, supplementation of humans with multicarotenoid is well justified.

REFERENCES

1. H. Pfander. In *Key to Carotenoids*, M. Gerspacher, M. Rychener, R. Schwabe (Eds.), pp. 11–218, Birkhäuser, Basel (1987).
2. F. Khachik, G. R. Beecher, M. B. Goli, W. R. Lusby. *Pure Appl. Chem.* **63**, 71 (1991).
3. F. Khachik, G. R. Beecher, M. B. Goli, W. R. Lusby. In *Methods in Enzymology*, Vol. 213, L. Packer (Ed.), pp. 347–359, Academic Press, New York (1992).
4. F. Khachik, G. R. Beecher, M. B. Goli, W. R. Lusby, J. C. Smith Jr. *Anal. Chem.* **64**, 2111 (1992).
5. F. Khachik, C. J. Spangler, J. C. Smith Jr., L. M. Canfield, A. Steck, H. Pfander. *Anal. Chem.* **69**, 1873 (1997).
6. F. Khachik, G. R. Beecher, M. B. Goli, W. R. Lusby, C. E. Daitch. In *Methods in Enzymology*, Vol. 213A, L. Packer (Ed.), pp. 205–219, Academic Press, New York (1992).
7. F. Khachik, G. Englert, C. E. Daitch, G. R. Beecher, W. R. Lusby, L. H. Tonucci. *J. Chromatogr. Biomed. Appl.* **582**, 153 (1992).
8. F. Khachik, G. Englert, G. R. Beecher. *J. Chromatogr. Biomed. Appl.* **670**, 219 (1995).
9. F. Khachik, A. Steck, H. Pfander. In *Food Factors for Cancer Prevention*, H. Ohigashi, T. Osawa, J. Terao, S. Watanabe, T. Yoshikawa (Eds.), pp. 542–547, Springer Verlag, Tokyo (1997).
10. M. S. Micozzi. In *Nutrition and Cancer Prevention*, M. S. Micozzi (Ed.), pp. 213–241, Marcel Dekker, New York (1989).
11. G. van Poppel. *Eur. J. Cancer* **29A**, 1335 (1993).
12. D. L. Morris, S. B. Kritchevsky, C. E. Davis. *J. Am. Med. Assoc.* **272**, 1439 (1994).
13. J. M. Gaziano, J. E. Manson, L. G. Branch, G. A. Colditz, W. C. Willet, J. E. Buring. *Ann. Epidemiol.* **5**, 255 (1995).
14. J. M. Seddon, U. A. Ajani, R. D. Sperduto, R. Hiller, N. Blair, T. C. Burton, M. D. Farber, E. S. Gragoudas, J. Haller, D. T. Miller, L. A. Yannuzzi, W. Willet. *J. Am. Med. Assoc.* **272**, 1413 (1994).
15. R. A. Bone, J. T. Landrum, S. L. Tarsis. *Vision Res.* **25**, 1531 (1985).
16. R. A. Bone, J. T. Landrum, G. W. Hime, A. Cains, J. Zamor. *Invest. Ophthalmol. Visual Sci.* **34**, 2033 (1993).
17. W. Schalch. In *Free Radicals and Aging*, I. Emerit, B. Chance (Eds.), pp. 280–298, Birkhauser, Basel (1992).
18. D. M. Snodderly. *Am. J. Clin. Nutr.* **62** (Suppl.), 1448S (1995).

19. L. M. Rapp, S. S. Maple, J. H. Choi. *Invest. Ophthalmol. Visual Sci.* **41**, 1200 (2000).
20. M. Neuringer, M. M. Sandstorm, E. J. Johnson, D. M. Snodderly. *Invest. Ophthalmol. Visual Sci.* **45**, 3234 (2004).
21. F. Khachik, G. R. Beecher, N. F. Wittaker. *J. Agric. Food Chem.* **34**, 603 (1986).
22. F. Khachik, M. B. Goli, G. R. Beecher, J. Holden, W. R. Lusby, M. D. Tenorio, M. R. Barrera. *J. Agric. Food Chem.*, **40**, 390 (1992).
23. J. M. Humphries, F. Khachik. *J. Agric. Food Chem.* **51**, 1322 (2003).
24. F. Khachik, G. R. Beecher. *J. Agric. Food Chem.* **35**, 732 (1987).
25. F. Khachik, G. R. Beecher, W. R. Lusby. *J. Agric. Food Chem.* **37**, 1465 (1989).
26. L. H. Tonucci, J. M. Holden, G. R. Beecher, F. Khachik, C. S. Davis, G. Mulokozi. *J. Agric. Food Chem.* **43**, 579 (1995).
27. P. H. Gann, J. Ma, E. Giovannucci, W. Willett, F. M. Sacks, C. H. Hennekens, M. J. Stampfer. *Cancer Res.* **59**, 1225 (1999).
28. E. Giovannucci, E. B. Rimm, Y. Liu, M. J. Stampfer, W. C. Willett. *J. Natl. Cancer Inst.* **94**, 391 (2002).
29. T. W. Boileau, Z. Liao, S. Kim, S. Lemeshow, J. W. Erdman Jr., S. K. Clinton. *J. Natl. Cancer Inst.* **95**, 1578 (2003).
30. F. Khachik, G. R. Beecher. *J. Agric. Food Chem.* **36**, 929 (1988).
31. F. Khachik, G. R. Beecher, W. R. Lusby. *J. Agric. Food Chem.* **36**, 938 (1988).
32. F. Khachik, G. R. Beecher, J. C. Smith Jr. *J. Cell. Biochem.* **22**, 236 (1995).
33. F. Khachik, J. S. Bertram, M. T. Huang, J. W. Fahey, P. Talalay. In *Antioxidant Food Supplements in Human Health*, L. Packer, M. Hiramatsu, T. Yoshikawa (Eds.), pp. 203–229, Academic Press, Tokyo (1999).
34. F. Khachik, P. Bernstein, D. L. Garland. *Invest. Ophthalmol. Visual Sci.* **38**, 1802 (1997).
35. F. Khachik, F. F. Moura, D. Y. Zhao, C. P. Aebischer, P. S. Bernstein. *Invest. Ophthalmol. Visual Sci.* **43**, 3383 (2002).
36. F. Khachik, A. Steck, U. A. Niggli, H. Pfander. *J. Agric. Food Chem.* **46**, 4874 (1998).
37. B. Traber, H. Pfander. *Tetrahedron* **54**, 9011 (1998).
38. P. H. Gann, F. Khachik. *J. Natl. Cancer Inst.* **95**, 1563 (2003).
39. P. S. Bernstein, F. Khachik, L. S. Carvalho, G. J. Muir, D. Y. Zhao, N. B. Katz. *Exp. Eye Res.* **72**, 215 (2001).
40. F. Khachik, Z. Nir, R. L. Ausich, A. Steck, H. Pfander. In *Food Factors for Cancer Prevention*, H. Ohgashi, T. Osawa, J. Terao, S. Watanabe, T. Yoshikawa (Eds.), pp. 204–208, Springer Verlag, Tokyo (1997).