Carotenoids and Their Metabolites in Human Serum, Breast Milk, Major Organs, and Ocular Tissues

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1. Carotenoids in Human Serum and Breast Milk

Carotenoids in human serum and breast milk originate from consumption of fruits and vegetables that are one the major dietary sources of these compounds. Carotenoids in fruits and vegetables can be classified as: 1) hydrocarbon carotenoids or carotenes, 2) monohydroxycarotenoids, 3) dihydroxycarotenoids, 4) carotenol acyl esters, and 5) carotenoid epoxides. Among these classes, only carotenes, monohydroxy- and dihydroxycarotenoids are found in the human serum/plasma and milk [1, 2]. Carotenol acyl esters apparently undergo hydrolysis in the presence of pancreatic secretions to regenerate their parent hydroxycarotenoids that are then absorbed. Although carotenoid epoxides have not been detected in human serum/plasma or tissues and their fate is uncertain at present, an *in vivo* bioavailability study with lycopene involving rats indicates that this class of carotenoids may be handled and modified by the liver [3]. Detailed isolation and identification of carotenoids in human plasma and serum has been previously published [1, 2]. This has been accomplished by simultaneously monitoring the separation of carotenoids by HPLC-UV/Vis-MS as well as comparison of the HPLC-UV/Vis–MS profiles of unknowns with those of known synthetic or isolated carotenoids. As shown in Table 1, as many as 21 carotenoids are typically found in human serum.

Table 1. A list of human serum carotenoids originating from foods as well their metabolites identified in human serum and breast milk.

	Serum & Breast Milk Carotenoids	**(Dietary Source)**
	Dihydroxycarotenoids	(greens, yellow/orange fruits and vegetables, pasta foods)
1	(*all-E, 3R,3´R,6´R*)-lutein + (*Z*)-stereoisomers: (*9Z*), (*9´Z*), (*13Z*), (*13´Z*), (*13Z,13´Z*)	
2	(*all-E, 3R,3´R*)-zeaxanthin + (*Z*)-stereoisomerrs: (*9Z*), (*9´Z*), (*13Z*), (*15Z*)	
3	(*3S,6S,3´S,6´S*)-ε,ε-carotene-3,3´-diol (lactucaxanthin)	
	Monohydroxycarotenoids	(yellow/orange fruits and vegetables)
4	β,ε-caroten-3-ol (α-cryptoxanthin)	
5[a]	3-hydroxy-β-carotene (β-cryptoxanthin)	
	Hydrocarbon Carotenoids	(yellow/orange, red fruits and vegetables)
6	(*all-E*)-ψ,ψ-carotene (lycopene) + (*Z*)-stereoisomrs	
7	7,8-dihydro-ψ,ψ-carotene (neurosporene)	
8[a]	β,ψ-carotene (γ-carotene)	
9	ζ-carotene + (*Z*)-stereoisomer	
10	(*all-E*)-phytofluene + (*15Z*)-stereoisomer	
11	(*all-E*)-phytoene + (*15Z*)-stereoisomer	
12[a]	(*6´R*)-α-carotene	(greens, yellow/orange, red fruits and vegetables)
13[a]	(*all-E*)-β-carotene + (*Z*)-stereoisomers: (*9Z*), (*13Z*)	

	Carotenoid Metabolites	**Possible Metabolic Source**
1	(*all-E, 3R,3´S,6´R*)-lutein (3´-epilutein)	(*3R,3´R,6´R*)-lutein
2	ε,ε-carotene-3,3´-dione	(*3R,3´R,6´R*)-lutein and/or
3	3´-hydroxy-ε,ε-caroten-3-one	(*3R,3´R*)-zeaxanthin
4	3-hydroxy-β,ε-caroten-3´-one + (*Z*)-stereoisomer	
5	3-hydroxy-3´,4´-didehydro-β,γ-carotene (anhydrolutein I)	(*3R,3´R,6´R*)-lutein
6	3-hydroxy-2´,3´-didehydro-β,ε-carotene (anhydrolutein II)	
7	2,6-cyclolycopene-1,5-diol I	lycopene
8	2,6-cyclolycopene-1,5-diol II	

[a]Refers to carotenoids with vitamin A activity.

These are 13 dietary and 8 carotenoid metabolites. The structures of dihydroxy- and monocarotenoids are shown in Figure 1. The structures of the hydrocarbon carotenoids and carotenoid metabolites that are present in human serum are shown in Figures 2 and 3, respectively. The HPLC analyses of extracts from breast milk of three lactating mothers have also revealed that the qualitative carotenoid profiles in milk are quite similar to that of serum [2]. However, depending on the nature of carotenoids, the concentrations of these compounds in milk are much lower than those in serum.

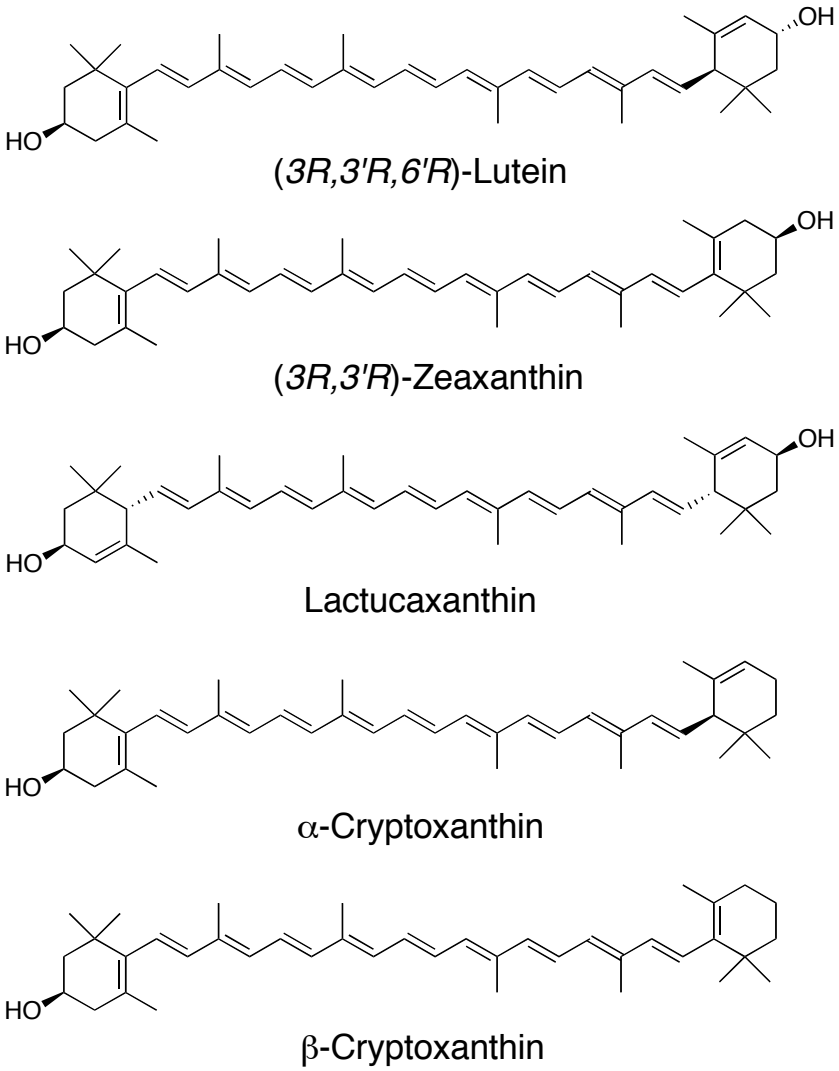

Fig. 1. The chemical structures of dihydrox- and monohydroxycarotenoids identified in human serum and breast milk.

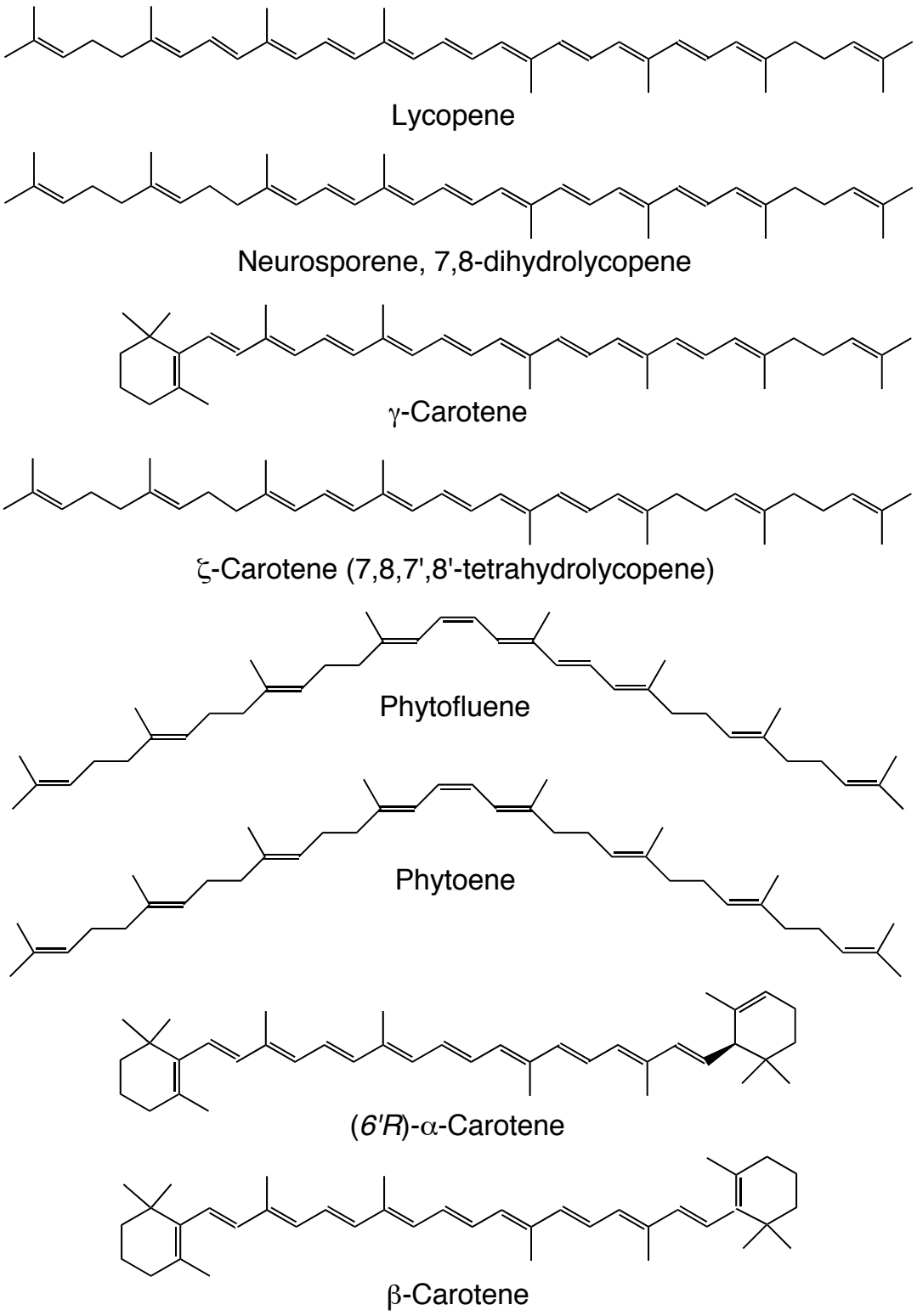

Fig. 2. The chemical structures of hydrocarbon carotenoids identified in human serum and breast milk

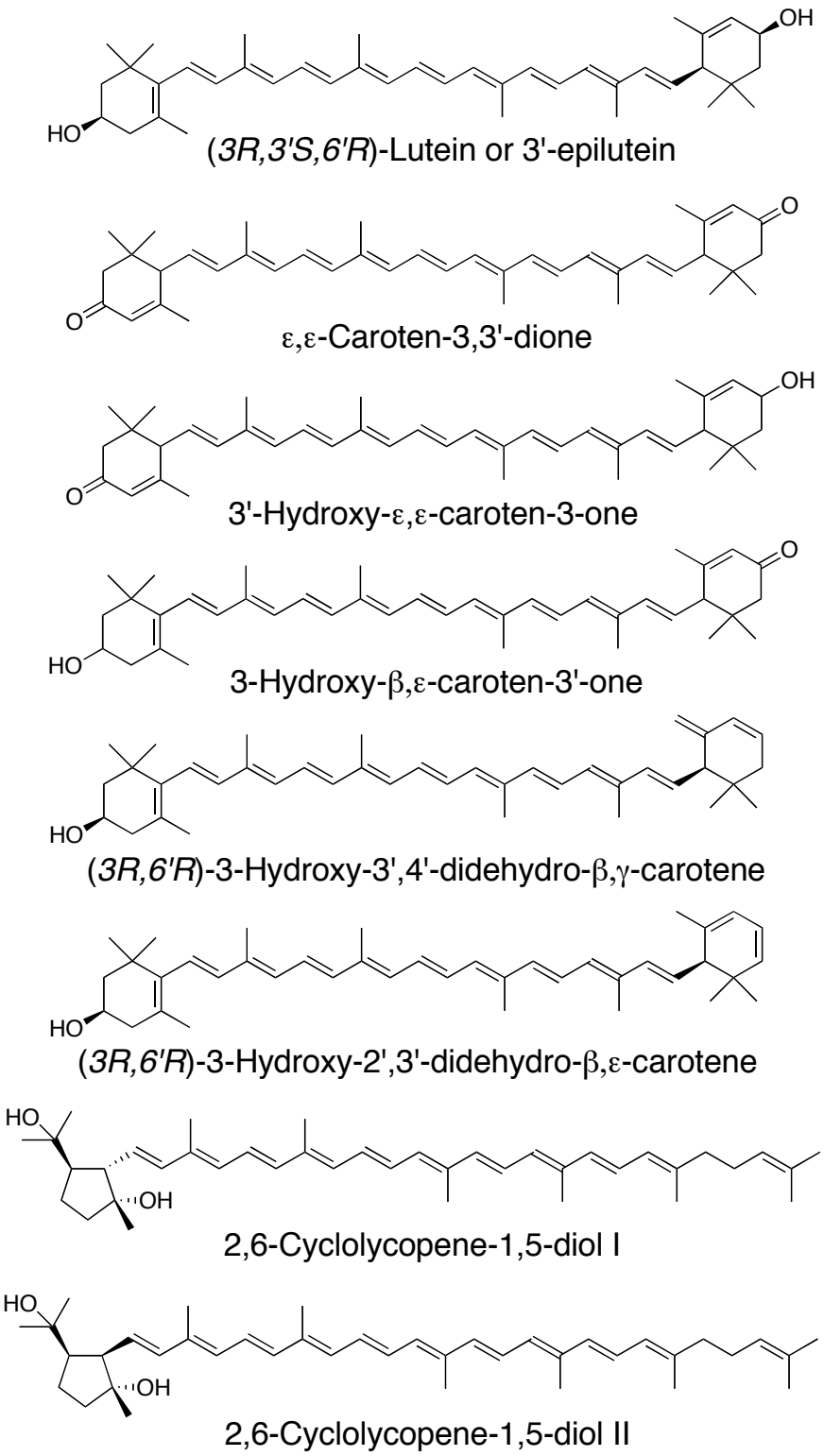

Fig. 3. The chemical structures of carotenoid metabolites identified in human serum and breast milk

The presence of mono- and dihydroxycarotenoids, and hydrocarbon carotenoids in human serum is due to the consumption of greens, yellow/orange, and red fruits and vegetables (Table 1). The presence of a wide spectrum of carotenoids and their metabolites in human serum and breast milk suggest that these essential nutrients most likely function in concert to promote the health of the mother and infant. Therefore, it appears prudent that diets of lactating mothers should include a variety of fruits and vegetables to supply wide spectrum of bioavailable carotenoids to mother and infant. However in many instances, because of certain health related problems, mothers are unable to breast-feed and rely on infant formula to provide adequate nutrition for their child. Currently, most infant formulas are fortified with vitamins, nutrients, and in some cases with β-carotene. Since most of the prominent carotenoids found in human serum and milk are currently available from natural sources, the infant formula can be modified to include as many dietary carotenoids as possible. Alternatively, lactating mothers who breast-feed their infants can be supplemented with a carefully designed mixture of carotenoids that closely resembles the relative distribution of these compounds in fruits, vegetables, and human serum [4].

The proposed metabolic transformation of lutein and zeaxanthin in humans involve a series of oxidation-reduction and double bond isomerization reactions according to the pathways shown in Figure 4. Human bioavailability and metabolic studies have supported the possibility of *in vivo* oxidation of these carotenoids in humans [5-7].

Two dehydation products of lutein, 3-hydroxy-3',4'-didehydro-β,γ-carotene and 3-hydroxy-2',3'-didehydro-β,ε-carotene, that have been identified in human plasma are not of dietary origin. These carotenoids are apparently formed in the presence of acids by the non-enzymatic dehydration of lutein in the human stomach [8].

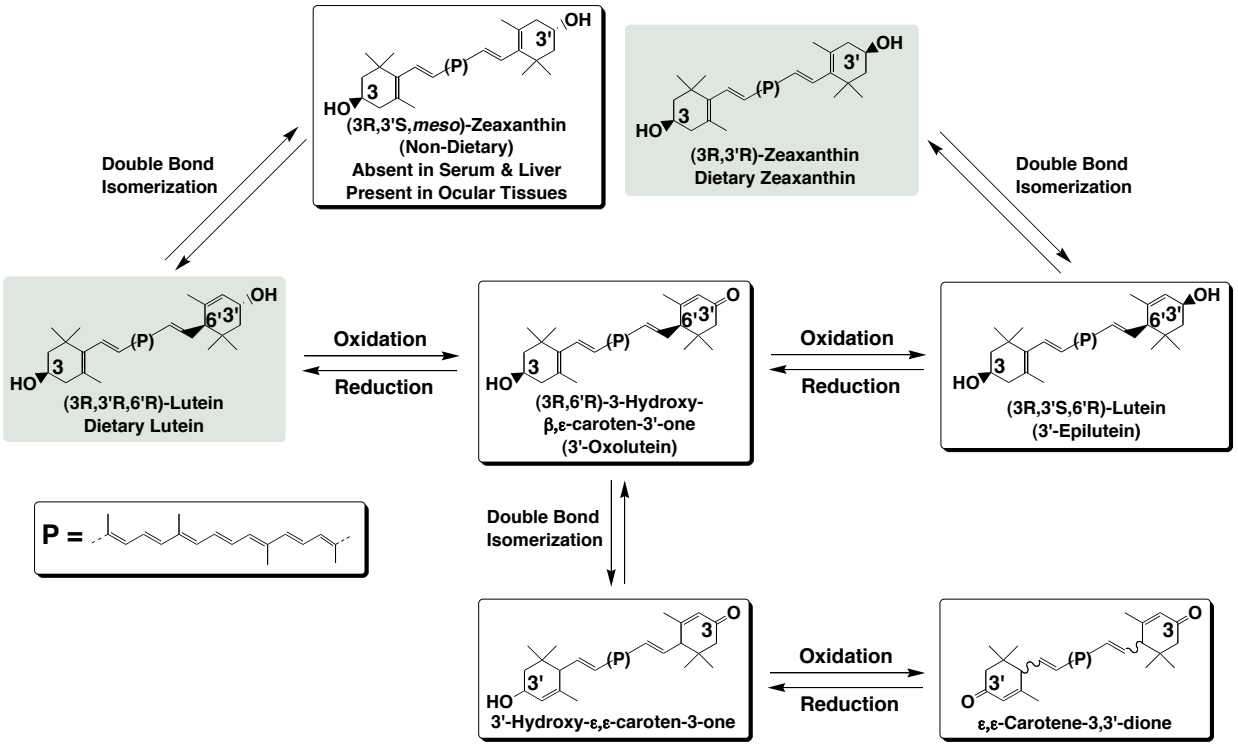

Fig. 4. Proposed metabolic transformation of dietary lutein and zeaxanthin in humans.

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 as shown in Figure 5 [5-7, 9].

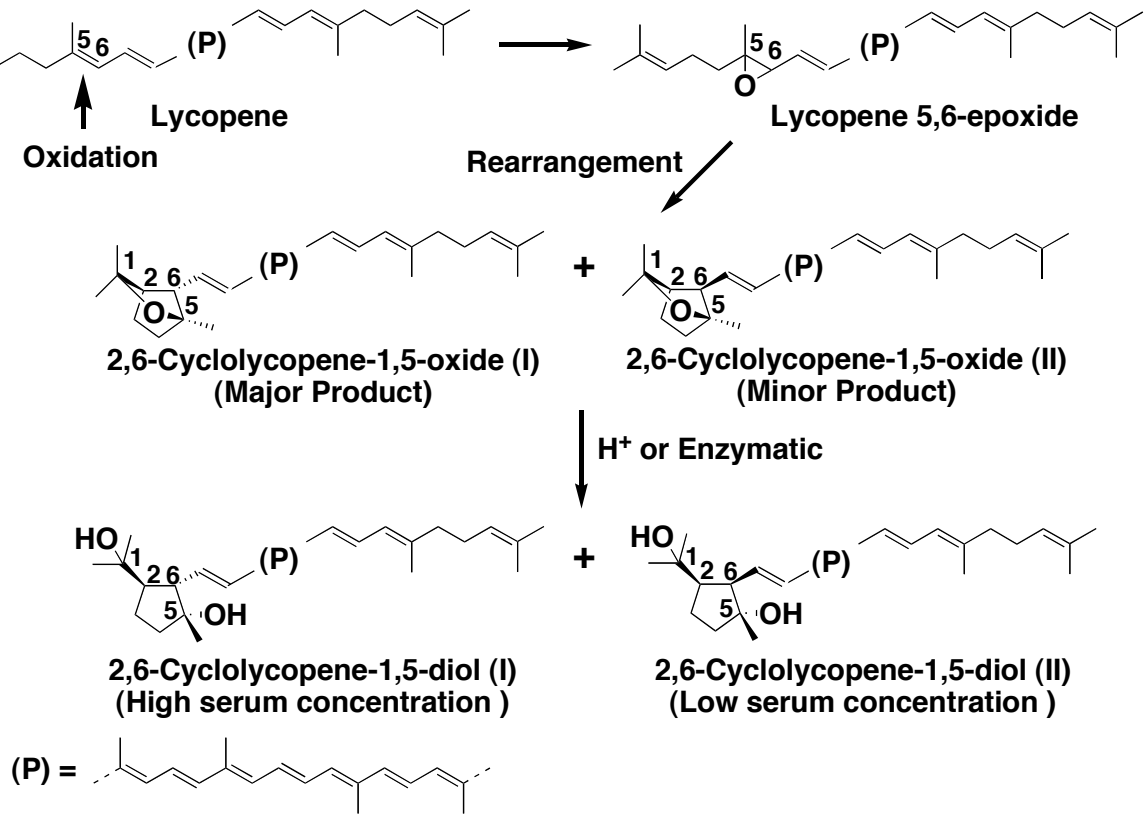

Fig. 5. Proposed metabolic transformation of lycopene in humans; only the relative but not the absolute configuration of 2,6-cyclolycopene-1,5-diols I and II at C-2, C-5 and C-6 is known.

2. Carotenoids in Major Organs

Detailed analysis of extracts from several human organs and tissues including liver, lung, breast, and cervix has revealed the presence of the same prominent carotenoids and their metabolites that are found in human serum [10]. These carotenoids are: lutein, zeaxanthin, anhydrolutein, α- and β-cryptoxanthin, lycopene and its metabolite, 2,6-cyclolycopene-1,5-diol, ζ-carotene, α- and β-carotene, phytofluene, and phytoene. The human lung tissue has been shown to accumulate relatively high concentrations of lutein, β-cryptoxanthin, lycopene, β-carotene, phytofluene, and particularly phytoene. As mentioned earlier, the major dietary sources of lycopene, ζ-carotene, phytofluene, and phytoene are tomatoes and tomato products [11].

3. Ocular Carotenoids and Their Metabolic Pathways

In 1945, Wald tentatively identified the yellow pigment in the human macula as a carotenoid belonging to the xantophyll families in green leaves [12]. In 1985, Bone et al. presented preliminary evidence that the human macular pigment is a mixture of lutein and zeaxanthin [13]. Finally, in 1993 Bone et al. established the complete identification and stereochemistry of the human macular pigment as lutein [(*3R,3′R,6′R*)-β,ε-carotene-3,3′-diol], zeaxanthin [(*3R,3′R*)-β,β-carotene-3,3′-diol], and *meso*-zeaxanthin [(*3R,3′S*)-β,β-carotene-3,3′-diol] [14]. It has been hypothesized that lutein and zeaxanthin protect the macula against photooxidative damage that can cause age-related macular degeneration (AMD) which is the leading cause of blindness in persons aged 60 years or older. Two mechanisms for the protective role of lutein and zeaxanthin against AMD have been proposed, these are: 1) by functioning as antioxidants and/or 2) as optical filters [15, 16]. This is further supported by the fact that these carotenoids have also been found in the rod outer segment (ROS) of the human eye [17]. ROS is the region of the retina most exposed to oxidation due to its relatively high oxygen tension and its high concentration of long–chain polyunsaturated fatty acids. Because polyunsaturated fatty acids are readily susceptible to oxidation, the presence of a high concentration of lutein and zeaxanthin would be expected to suppress such oxidative processes and provide protection to the ROS by an antioxidant mechanism of action.

In 1997, Khachik et al. provided preliminary evidence for the possible antioxidant role of lutein and zeaxanthin in the retina by identifying and quantifying lutein, zeaxanthin, and their oxidative metabolites in human and monkey retinas [18]. These metabolites were: (*3R,3´S,6´R*)-lutein (3´-epilutein), 3-hydroxy-β,ε-carotene-3´-one (3´-oxolutein), 3´-hydroxy-ε,ε-caroten-3-one, and ε,ε-carotene-3,3´-dione. An oxidation product of lycopene, 2,6-cyclolycopene-1,5-diol

was also identified in an extract from human retina. In addition, the most common geometrical isomers of lutein and zeaxanthin, i.e. 9*Z*-luttein, 9´*Z*-lutein, 13*Z*-lutein, 13´*Z*-lutein, 9*Z*-zeaxanthin, and 13Z-zeaxanthin, normally present in human serum, were also detected at low concentrations in retina.

In two separate human supplementation studies with lutein and zeaxanthin, Khachik et al. have shown that supplementation with 10 mg/day of either lutein or zeaxanthin for 3 weeks result in an increase in the plasma concentrations of these carotenoids as well as their oxidative metabolites [5]; these were the same metabolites that were also found in the human retina as described above [18].

The metabolic pathways that have been postulated for the transformation of dietary lutein and zeaxanthin to their metabolites are shown in Figure 6. According to these pathways, non-dietary 3'-epilutein and 3'-oxolutein in human ocular tissues could be formed as a result of a series of oxidation-reduction reactions from dietary lutein and/or zeaxanthin. The formation of (*3R,3'R*; *meso*)-zeaxanthin that was first identified in the human macula by Bone et al. [14] was explained by double isomerization of dietary (*3R,3'R,6'R*)-lutein [18]. In a more recent study by Khachik et al., (*3R,3'R*; *meso*)-zeaxanthin has been shown to be absent in the human serum and liver while relatively high concentrations of this carotenoid metabolite were found in all ocular tissues [retina, macula, retinal pigment epithelium (RPE)/choroid, ciliary body, iris, lens] examined [19].

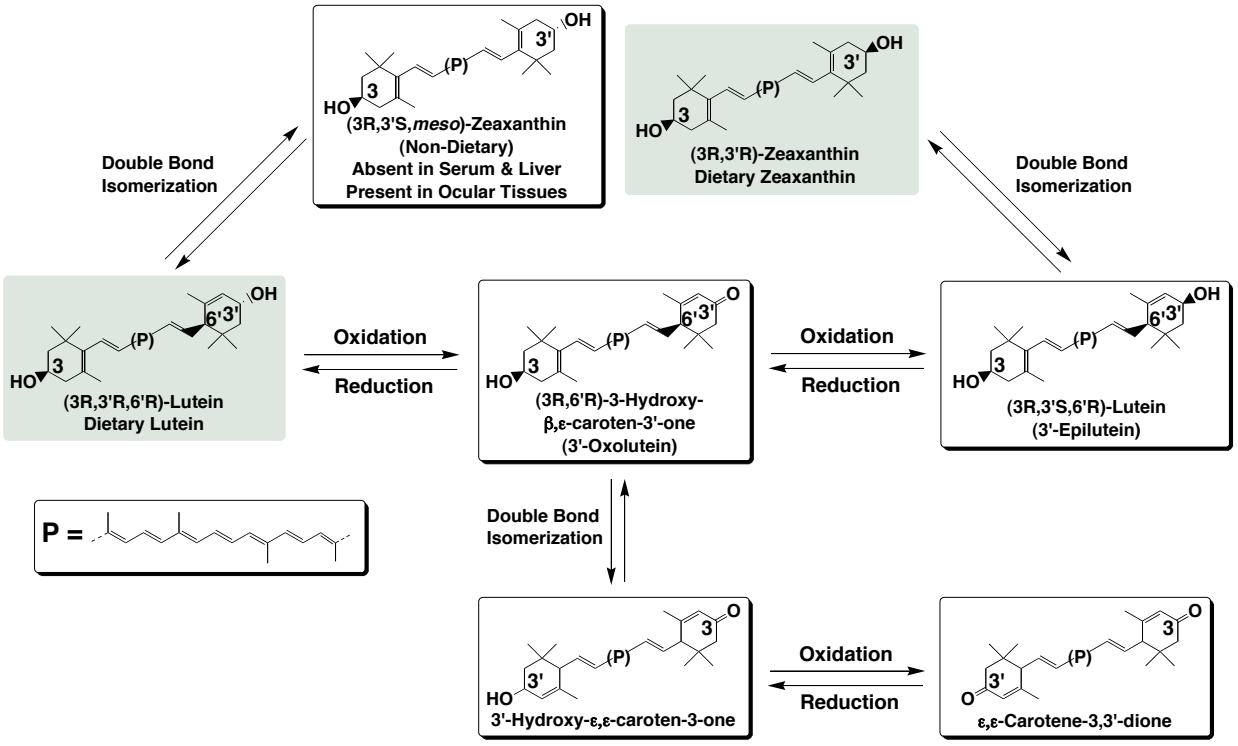

Fig. 6. Proposed metabolic transformation of dietary lutein and zeaxanthin in human retina. The metabolites of lutein and zeaxanthin that are absent in foods but present in human plasma and ocular tissues are shown in boxes.

Lutein, zeaxanthin, and their geometrical isomers as well as their oxidative metabolites have also been identified and quantified in all ocular structures of the human eye with the exception of vitreous, cornea, and sclera [20]. This study revealed the presence of monohydroxycarotenoids (α-cryptoxanthin, β-cryptoxanthin) and hydrocarbon carotenoids (neurosporene, γ-carotene, lycopene, α-carotene, β-carotene) in the human ciliary body while only lycopene, α-carotene, and β-carotene were detected in human RPE/choroids. Uveal structures (iris, ciliary body, and RPE/choroids) were shown to account for approximately 50% of the eye's total carotenoids and nearly 30% of lutein and zeaxanthin [20]. *all-trans*-Lycopene and 5-*cis*-lycopene were also identified and quantified in all human ocular tissues with the exception of neural retina and lens.

Carotenoids and their metabolites including (3R,3´S; *meso*)-zeaxanthin in plasma, liver, and ocular tissues of humans as well as those of two non-primate animal models, quails and frogs have also been identified [19].

(*3R,3´S*; *meso*)-Zeaxanthin has also been identified and quantified in other tissues of the human eye at varying concentrations but has not been detected in human liver [19]. Establishing the constitution of zeaxanthin in ocular tissues versus plasma and liver is highly significant because this can provide an insight into the transformation of lutein and zeaxanthin in the human eye. This is because one of the major difficulties in elucidating the metabolism of carotenoids in the eye is due to the fact that the same carotenoid metabolites that are observed in the human serum are also observed in the human ocular tissues. Therefore, metabolic studies must be able to distinguish between those carotenoid metabolites formed locally in the eye and the ones that may be simply transported to the eye tissues from the circulating blood. However, (*3R,3´S*; *meso*)-zeaxanthin is an exception since this non-dietary carotenoid is absent in the plasma and liver and is most likely formed in the ocular tissues from double bond isomerization of dietary lutein as proposed in Figure 4. Johnson et al. have recently provided unequivocal and definitive proof for the metabolic transformation of lutein to (*3R,3´S*; *meso*)-zeaxanthin [21]. These authors have clearly demonstrated that (*3R,3´S*; *meso*)-zeaxanthin that was absent in the retinas of xanthophylls-free and (*3R,3´R*)-zeaxanthin-fed primates, was present in the retinas of xanthophylls-free primates after supplementation with (*3R,3´R,6´R*)-lutein.

4. Comparative Profiles of Carotenoids in Foods and Human Serum

Comparison of the qualitative profile of carotenoids in foods with those of human serum is shown in Figure 7. The first group is vitamin A active carotenoids such as α-carotene, β-carotene, β-cryptoxanthin, and γ-carotene that in addition to converting to vitamin A are also

absorbed into human serum, organs, and tissues. The second group: α-cryptoxanthin, neurosporene, ζ-carotene, phytofluene, and phytoene, have no vitamin A activity and appear to be absorbed intact. At present there is no evidence to suggest that these carotenoids undergo metabolic transformation. As discussed earlier, several oxidative metabolites of lycopene, lutein, zeaxanthin, and lactucaxanthin in human serum and milk have been isolated and characterized [1, 2]. Human bioavailability and metabolic studies have supported the possibility of *in vivo* oxidation of lutein and zeaxanthin in humans [5, 6]. The metabolic transformation of these carotenoids involves a series of oxidation-reduction reactions similar to those shown in Figures 4 and 6. There are also two metabolites of lutein that are apparently formed in the presence of acids by non-enzymatic dehydration of this compound in the human digestive system. A human supplementation study with lycopene has also provided preliminary evidence for the *in vivo* oxidation of this carotenoid to 2,6-cyclolycopene-1,5-diols I and II according to the proposed pathways shown in Figure 5 [9].

Carotenol acyl esters that are abundant in many fruits and vegetables have not been detected in human serum or milk [1, 2]. Only two monohydroxycarotenoids, namely, α-cryptoxanthin and β-cryptoxanthin have been detected in common fruits and vegetables as well as human serum and milk. Similarly, of all the dihydroxycarotenoids isolated from various natural sources, only lutein, zeaxanthin, and lactucaxanthin have been found in foods, human serum and milk. The concentration of lactucaxanthin in human serum is normally very low since the dietary source of this compound is limited to Romaine lettuce, *Lactuca sativa* [22].

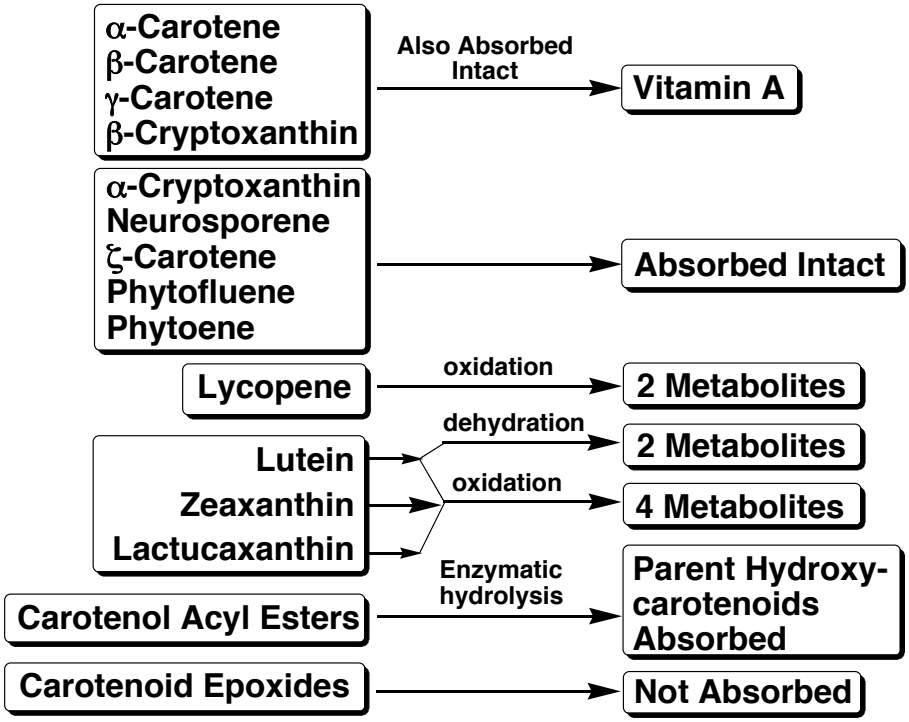

Fig. 7. Comparison between dietary and serum carotenoids and their transformation in humans.

A detailed knowledge of qualitative distribution of carotenoid in foods in comparison with that of human serum and tissues not only can distinguish between dietary carotenoids and their metabolites but can also provide valuable information with regard to the nature of the metabolic transformation. One of the best examples of this is 3-hydroxy-β,ε-caroten-3´-one (3´-oxolutein) that has not been detected in common fruits and vegetables but is found in human serum and ocular tissues (Fig. 24). Comparing the structural features of this carotenoid with carotenoids that have been identified in fruits and vegetables, clearly indicates that the most likely precursor of this carotenoid is dietary (*3R,3´R,6´R*)-lutein. This is because among all the possible candidates, only a minor modification of the chemical structure of lutein can yield 3'-oxolutein. The next step is to examine whether such a structural modification of lutein would be biologically reasonable. In this particular case, the transformation would require allylic oxidation of the hydroxyl group in the ε-end group of lutein that according to known biological processes

that involve oxido-reductase enzymes, seems quite feasible. Unfortunately, these types of speculative arguments can only provide a possible road map to the metabolism of carotenoids in humans. Consequently, the definitive metabolic pathways of carotenoids can only be unequivocally established by carefully designed supplementation studies with isotopically labeled compounds involving an appropriate animal model.

5. Nutritional Significance of Carotenoids in Disease Prevention

The majority of epidemiological and experimental studies to date have associated the high consumption of carotenoid-rich fruits and vegetables to a lower risk for several chronic diseases such as cancer, cardiovascular disease, and age-related macular degeneration. As described earlier, fruits and vegetables contain approximately 40-50 carotenoids with wide ranging structures. Among these 13 dietary carotenoids and 8 carotenoid metabolites are consistently present at relatively high concentrations in human serum and breast milk (Table 1). Food-derived carotenoids are also transported *via* the circulating blood to various organs and tissues (i.e. liver, lung, lung, breast, prostate, cervix, and etc.). In addition, lutein and zeaxanthin are also accumulated in all human ocular tissues (retina, macula, RPE-choroid, ciliary body, iris, lens) while a wide spectrum of dietary carotenoids are present in human ciliary body (α-cryptoxanthin, β-cryptoxanthin, neurosporene, γ-carotene, lycopene, α-carotene, β-carotene) and RPE-choroid (lycopene, α-carotene, and β-carotene). Although the presence of various classes of carotenoids in human serum, organs, and tissues does not unequivocally indicate their health benefits, per se, numerous *in vitro* and *in vivo* studies strongly suggest that carotenoids exhibit wide ranging biological activities. These include: antioxidant/anti-inflammatory properties [97], upregulation-enhancement of cellular communication [7, 23, 24], and induction of the activity of detoxication enzymes [7]. In addition, the *in vivo* anti-tumor activity of several carotenoids

against colon carcinogenesis in a rodent model has been reported [25]. Therefore, based on all of the experimental data available to date, it appears that dietary carotenoids can collectively serve as excellent chemoprotective agents in disease prevention. It is imperative to note that this protective effect should not be attributed to a single carotenoid even though in some cases a direct and strong correlation may exist for the protective role of certain carotenoids. This can be exemplified with the protective role of high consumption of tomatoes and tomato-based products in lowering the risk of prostate cancer that has been largely attributed to lycopene while these foods also contain other carotenoids such ζ-carotene, phytofluene, phytoene, neurosporene, γ-carotene. Therefore, the most logical approach is to consider all of the dietary carotenoids that are present in human serum (Table 1) as essential nutrients for human health. The structural diversity of dietary carotenoids also appears to play an important role in dictating the pathways by which these compounds function as antioxidants. For example, an acyclic hydrocarbon carotenoid such as lycopene is oxidized at the terminally conjugated double bond (5,6-bond, Fig. 5) while the oxidation of the dihydroxycarotenoid lutein, takes place on the allylic hydroxyl group in the ε-end group of this carotenoid (Figs. 4 & 6). Because of their well-demonstrated antioxidant ability, carotenoids are readily susceptible to *in vivo* oxidation in humans and therefore may serve as scavengers of the reactive electrophiles to provide protection to DNA against oxidative damage. Much of the definitive details concerning the biological activities, role, and function of carotenoids in humans still remain unexplored. Future studies should concentrate on elucidating the bioavailability, metabolism, function, interaction, and efficacy of the spectrum of dietary carotenoids as well as their metabolites.

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