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Lutein, Lycopene, and Their Oxidative Metabolites in Chemoprevention of Cancer

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Abstract Numerous epidemiological studies have demonstrated that consuming large quantities of fruits and vegetables reduces the risk for several types of human cancers. Carotenoids are abundant in fruits and vegetables and have been extensively studied as cancer preventive agents. A proposed mechanism of action for the protective effect of carotenoids against cancer is based on their antioxidant capability. Recently, we have isolated and characterized 14 new carotenoids, including seven metabolites from the extracts of human serum/plasma. This brings the total number of identified blood carotenoids to 21. Lutein and lycopene, abundant in most fruits and vegetables as well as human serum, have been shown to possess strong antioxidant capability. Among the metabolites of lutein, four result from oxidation and two from non-enzymatic dehydration. The metabolite of lycopene has been identified as 5,6-dihydroxy-5,6-dihydrolycopene, which apparently results from oxidation of lycopene to an intermediate, lycopene epoxide. This intermediate may undergo metabolic reduction to form the lycopene metabolite. Although *in vivo* oxidation of lutein to its metabolites has been demonstrated based on data obtained from two human studies, *in vivo* oxidation of lycopene to its metabolite has not yet been established. Recent preliminary studies involving healthy subjects ingesting purified lutein and zeaxanthin (a dietary dihydroxycarotenoid isomeric to lutein) are presented. We propose a possible antioxidant mechanism of action for lutein and lycopene that leads to formation of the oxidation products of these promising chemopreventive agents.

Key words: Antioxidants, bioavailability, carotenoid oxidation, human studies, serum carotenoids, zeaxanthin

In the past decade several foods and food components have been studied for their inhibitory effect on carcinogenesis. Numerous epidemiological studies have concluded that high consumption of fruits and vegetables reduces the risk of cancer. Dietary prevention of cancer is based upon the mechanism of action (*i.e.*, antioxidant activity), toxicity, and efficacy of certain chemical compounds, or micronutrients [1]. Carotenoids are one class of micronutrients that have been studied for their preventive effect against cancer; they are also one of the most widespread groups of pigments found in the plant and animal kingdoms. To date approximately seven hundred naturally occurring carotenoids have been isolated and characterized. During the past decade, we have identified and quantified carotenoids from fruits and vegetables commonly consumed in the US [2–10]. The results indicate that up to 50 dietary carotenoids

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may be absorbed and metabolized by humans [8]. Recently, 14 new carotenoids, including seven metabolites found in human blood, have been isolated and characterized [8,11–13]. A comparison of dietary carotenoids and human serum carotenoids has revealed that only selected groups of carotenoids are permitted into the bloodstream. Among those found in both food and blood, α-carotene and β-carotene, along with few lesser carotenoids, contribute to vitamin A activity. However, the nutritional benefits and metabolic functions of non-vitamin A active carotenoids are unknown. Lutein, a dihydroxycarotenoid, and lycopene, a hydrocarbon carotenoid, are two non-vitamin A active carotenoids abundant in most fruits and vegetables as well as human serum. Lutein and lycopene possess exceptionally high antioxidant activity compared to other carotenoids and may therefore be useful in chemoprevention of cancer. This is based on epidemiological studies, the distribution of lutein and lycopene in fruits, vegetables, and human

TABLE I. Concentration (mg/100 g edible food) of Lutein and β-Carotene in Fruits and Vegetables from Cook, Fiji, and Tahiti Islands

FRUITS AND VEGETABLES	SOURCE	LUTEIN	β-CAROTENE
Amaranthus Leaves	Fiji	13.60	8.27
Beans, Green	Fiji	3.30	1.82
Beans-Long, Green	Tahiti	1.06	0.32
Chinese Cabbage, Wild	Cook	1.27	2.90
Chinese Cabbage, Pakchoy	Cook	9.50	13.79
Chinese Cabbage	Fiji	7.47	4.57
Swamp Cabbage	Fiji	7.90	5.37
Chinese Cabbage, White	Tahiti	1.55	0.76
Chinese Cabbage, Green	Tahiti	1.47	1.11
Drumstick Leaves	Fiji	12.16	10.17
Fafa	Tahiti	9.46	7.40
Wild Fern	Fiji	4.86	1.74
Ham Soy	Tahiti	3.22	2.09
Hibiscus, Ruka Viti	Cook	4.30	5.66
Hibiscus Leaves	Fiji	8.89	5.70
Lettuce, Salate Verte	Tahiti	1.56	1.23
Mustard Green	Fiji	7.41	4.89
Silverbeet Leaves	Cook	3.53	3.54
Creeping Spinach	Fiji	5.28	4.36
Taro Leaves	Cook	3.63	4.58
Taro Leaves	Fiji	8.66	4.21
Watercress	Tahiti	1.16	Trace

TABLE II. Concentration of Lutein and β-Carotene in Vegetables (100 mg/100 g edible food) Common in US Diet

FRUITS AND VEGETABLES	LUTEIN	β-CAROTENE	REFERENCES
Broccoli	2.83	2.33	2,9
Brussel Sprouts	1.59	0.53	2
Cabbage	0.31	0.08	2
Green Beans	0.59	0.47	2,9
Kale	39.55	14.60	2
Spinach	12.50	8.90	2,9
Winter Squash	7.00	0.99	4

serum/plasma, and *in vitro* and *in vivo* studies of lutein, lycopene, and other carotenoids.

Recent preliminary studies involved healthy subjects who consumed purified lutein and zeaxanthin (a dietary dihydroxycarotenoid isomeric to lutein) isolated from natural sources. We found possible *in vivo* antioxidant mechanisms of action of lutein and lycopene and metabolic reactions leading to formation of the oxidation products of these promising chemopreventive agents.

EPIDEMIOLOGICAL STUDIES AND DISTRIBUTION OF LUTEIN AND LYCOPENE IN FRUITS AND VEGETABLES

Using recently generated food composition data for carotenoids, researchers at the Cancer Research Center of Hawaii have reanalyzed a population-based, case-controlled study of diet and lung cancer conducted in Hawaii in 1983–1985 [14]. After adjusting for smoking and other co-variates, they reported comparable dose-dependent inverse associations for dietary β-carotene, α-carotene, or lutein. When subjects were cross-classified by their total intakes of β-carotene, α-carotene, and lutein, those with high intake for all three carotenoids had the lowest risk of lung cancer. Similar epidemiological studies in the past decade indicated that the incidence of several types of cancers among the populations of South Pacific Island nations (*e.g.*, Cook, Fiji, Tahiti) are unusually low, which has been attributed to dietary habits. The qualitative and quantitative distribution of carotenoids in several fruits and vegetables commonly consumed in the South Pacific Islands has also been analyzed by the authors. The results indicate that lutein and β-carotene are the major carotenoid constituents of these fruits and vegetables as shown in Table I [15]. Similarly, the concentration of lutein and β-carotene in several green vegetables commonly consumed in the US are shown in Table II. All foods listed in Tables I and II have higher concentrations of lutein than β-carotene. Lutein is also abundant in yellow/orange fruits and vegetables including mango, papaya, peaches, prunes, acorn squash, winter squash, and oranges [4–6].

Lycopene is the most abundant carotenoid, found nearly exclusively in tomatoes and tomato-based food products such as tomato paste, tomato sauce, and tomato-based soups. Other dietary carotenoids such as 5,6-dihydroxy-5,6-dihydrolycopene, lycopene 5,6-epoxide, γ-carotene, neurosporene, ζ-carotene, β-carotene, phytofluene, and phytoene are also present in tomato-based products, but at very low concentrations [9,16]. In addition, we recently isolated and characterized two minor carotenoids from extracts of tomatoes and tomato-based products, tentatively identified as 5,6-dimethoxy-5,6-dihydrolycopene and dimethoxy-prolycopene [16]. In numerous epidemiological studies, including the study of the eating habits of the inhabitants of the South Pacific Island nations, the low incidence of several types of cancers was attributed to intake of

foods with high concentrations of β-carotene. Similar assumptions have resulted in numerous human studies employing supplemental β-carotene. However, since the dietary intake of lutein and lycopene is usually higher than that of β-carotene for most people, the results from epidemiological and human studies can be better interpreted if bioavailability, metabolism, and potential cancer preventive effects of these major dietary carotenoids are also studied.

DISTRIBUTION OF CAROTENOIDS AND THEIR METABOLITES IN HUMAN SERUM

Although there are more than 40 dietary carotenoids, only 14 carotenoids and seven metabolites have been detected in human blood [8,11–13]. These carotenoids and their dietary sources are listed in Table III. Analytical techniques separate and quantify all 21 of these carotenoids by high performance liquid chromatography (HPLC). These HPLC techniques can also simultaneously detect vitamin A, vitamin E (γ- and α-tocopherol) and several other non-vitamin dietary components including caffeine and piperine (major component of black pepper).

Chemical structure and source of the seven carotenoid metabolites are shown in Figure 1. As evident from their chemical structures, six of these metabolites result from dietary lutein and zeaxanthin and one from dietary lycopene. Zeaxanthin is a dihydroxycarotenoid isomeric to lutein. In contrast to lutein, dietary zeaxanthin is only present at modest concentrations in corn, squash, apricots, peaches, and oranges.

TABLE III. Dietary Carotenoids Isolated and Characterized in Extracts from Human Serum[a]

ENTRY	CAROTENOIDS[b,c]	DIETARY SOURCE
1	Lutein	2–6,10–24
2	Zeaxanthin	16,10–22
3	α-Cryptoxanthin	22
4	β-Cryptoxanthin[c]	13,15–16,18,21–22
5	5,6-Dimethoxy-5,6-dihydrolycopene[b,d]	25
6	Dimethoxy-prolycopene[b,d]	25
7	Lycopene	1,9,25
8	α-Carotene[c]	2–3,8,17–19,21
9	β-Carotene[c]	1–25
10	Neurosporene[b]	25
11	γ-Carotene[b,c]	1,25
12	ζ-Carotene[b]	1,7–9,13,15–16,19,22,25
13	Phytofluene[b]	1,7,9,13,15–16,19,22,25
14	Phytoene[b]	1,7,9,13,15–16,19,22, 25

KEYS: 1) apricot, 2) beans (green), 3) lima beans, 4) broccoli, 5) brussel sprouts, 6) cabbage, 7) cantaloupe, 8) carrots, 9) pink grapefruit, 10) kale, 11) kiwi, 12) lettuce, 13) mango, 14) muskmelon (honeydew), 15) papaya, 16) peaches, 17) peas (green), 18) prunes, 19) pumpkin, 20) squash (acorn), 21) squash (winter), 22) oranges, 23) sweet potato, 24) spinach, 25) tomatoes and tomato-based products.

[a][8,11–13]; [b]indicates newly characterized carotenoids in human serum; [c]indicates vitamin A active carotenoids; [d]detailed identification will be published elsewhere.

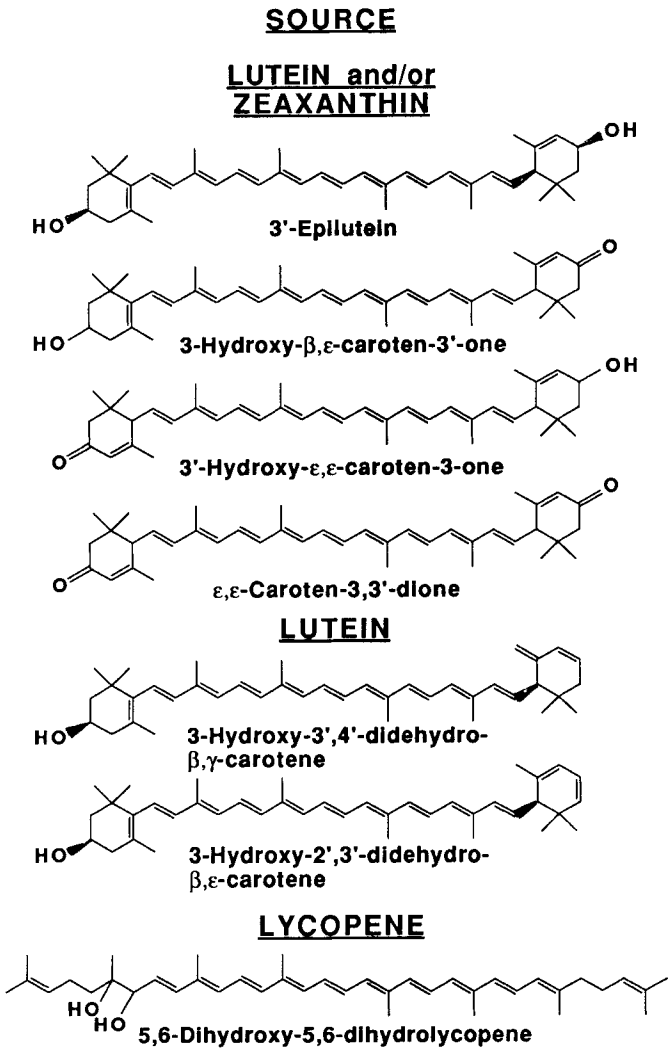

Fig. 1. Carotenoid metabolites in human serum.

RECENT ABSORPTION AND METABOLIC STUDIES WITH LUTEIN AND ZEAXANTHIN IN HUMANS

Recent preliminary absorption and metabolic studies involved healthy subjects with purified lutein and zeaxanthin.

Isolation of Lutein and Zeaxanthin

Although dark green vegetables are excellent dietary sources of lutein, the isolation and purification of this compound in large quantities from green vegetables is time-consuming and costly; several additional purification steps are required to separate lutein from the large quantities of chlorophylls, β-carotene, and carotenoid epoxides. On the other hand, extracts from the petals of marigold flowers (*Tagetes erecta*, var. *orangeade*) are an excellent source of lutein in large quantities and contain no significant levels of other carotenoids. The only contaminating carotenoid is 2–3% of dietary zeaxanthin. The detailed procedure for isolation and purification of lutein for human consumption from extracts of marigold flowers has recently been patented [17]. Zeaxanthin was similarly isolated from Lycium Chinese Mill, a Chinese fruit known as

"Guji." The purity of the isolated lutein and zeaxanthin were confirmed by UV/visible spectrophotometric measurements, nuclear magnetic resonance (NMR) spectroscopy, and combined HPLC/mass spectrometry (MS).

Preparation of Lutein and Zeaxanthin Dose For Oral Supplementation

Carotenoids are fat-soluble nutrients usually associated with the lipoprotein fractions of human blood. Their absorption and bioavailability significantly increases if these compounds are orally ingested with small amounts of oil or foods that contain lipids. Therefore lutein and zeaxanthin doses were prepared as a suspension in olive oil in the following manner in small batches consisting of 125 aliquots, each containing 10 mg of lutein in 4 ml of olive oil. Zeaxanthin was prepared similarly.

Purified lutein from marigold flower extract (2.1 g; 97% lutein/3% zeaxanthin) was added to a solution of DL-α-tocopherol (56 mg) in absolute alcohol (25 ml). Food-grade polysorbate-80 (an emulsifier, 4 g) was added and the mixture was sonicated for 10 minutes. The addition of DL-α-tocopherol (0.01% by weight/volume) stabilized lutein and prevented oxidation during storage. This suspension was then mixed with *ca.* 470 ml of light, mild olive oil (saturated fat/polyunsaturated fat = 2/1) until the total volume of the solution was 500 ml. The mixture was sonicated for 5 min to produce an olive oil suspension of lutein stored under nitrogen at 5°C. A 4 ml aliquot of this suspension contained 10 mg of lutein by spectrophotometric analysis. Aliquots of the olive oil containing 10 mg lutein were spread on a bagel and eaten. At various intervals, stability and purity of the lutein or zeaxanthin olive oil suspension was determined by spectrophotometric analysis; lutein and zeaxanthin stored at 5°C were stable for one month.

Toxicity Criteria

The officials of the Food and Drug Administration (FDA) have indicated that animal toxicology data will not be required for short-term studies involving food-source carotenoids, *e.g.*, lutein and zeaxanthin, if the daily dose of these compounds is within several fold of the average daily dietary levels of these compounds in humans. The 10 mg/day dose of lutein or zeaxanthin ingested by subjects was not expected to cause toxicity or side effects since these dose levels are only several fold higher than the average dietary concentration of these carotenoids in common fruits and vegetables. For example, daily ingestion of 25 g of a fresh, dark green vegetable such as kale would theoretically provide 10 mg of lutein (Table II). However, the bioavailability and absorption of carotenoids from foods is not as high as supplementation with pure carotenoids.

Design and Duration of Human Studies

Lutein The authors, three healthy Caucasian males (nonsmokers) between the ages of 42–59, were the subjects of this study. During this experiment, the diet of the subjects excluded green and yellow-orange fruits and vegetables containing significant quantities of lutein (*i.e.*, broccoli, spinach, kale, green beans, green peas, lettuce, pumpkin, squash, peaches, oranges, and citrus fruits). Otherwise, the subjects consumed self-selected diets and kept complete daily dietary records. Baseline concentrations of plasma carotenoids were determined 25 and 11 days prior to lutein dosage (three data points, days –25, –11, and 0). At the start of the study (day 0) and for 18 days, the subjects ingested the 10 mg/day dose of purified lutein. Plasma carotenoids were extracted and monitored by HPLC on days 0, 2,

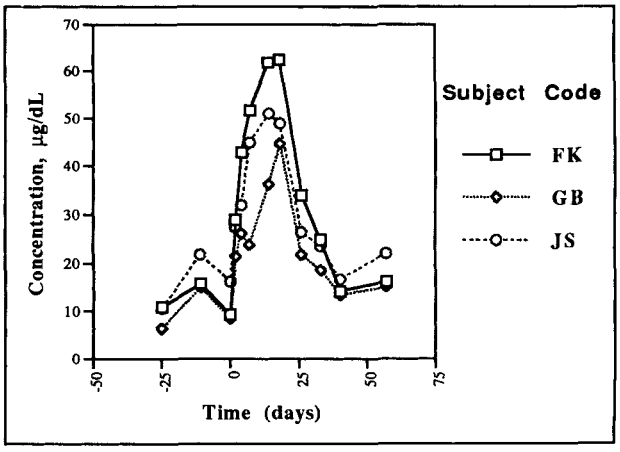

Fig. 2. Plasma concentration of lutein in three male subjects before and during ingestion of 10 mg/day pure lutein.

4, 7, 14, 18, 26, 33, 40, and 57. Detailed procedures for extraction, structural elucidation, and analysis of carotenoids and their metabolites by HPLC have been previously published [11–13].

Zeaxanthin The same subjects described above participated in this experiment. The protocol was identical except that foods containing zeaxanthin (*i.e.*, apricot, peaches, squash, oranges, and citrus fruits) were avoided. Baseline concentrations of plasma carotenoids were determined 6 days prior to zeaxanthin dosage (two data points, days –6 and 0). At the start of the study (day 0) and for 21 days, the subjects ingested the 10 mg/day dose of purified zeaxanthin. Plasma carotenoids were extracted and analyzed by HPLC on days 0, 1, 2, 4, 7, 14, 22, 30, 36, 43, 58, 87, and 101 [11–13].

RESULTS

The blood levels of lutein increased 4–5 fold after one week of supplementation (Fig. 2). Specifically, the average lutein concentration in human serum is 280 nM, which readily increased to 1,400 nM after one week of supplementation and did not plateau. Similarly, ingestion of lutein resulted in an increase in concentration of zeaxanthin and lutein oxidation products, *i.e.*, monoketocarotenoids and diketocarotenoids (data not shown). However, the plasma concentration of 3'-epilutein did not increase significantly. In the study with zeaxanthin, the plasma levels of this compound increased by four-fold after one week of supplementation as shown in Figure 3. In addition, the plasma levels of lutein, 3'-epilutein, and the oxidation products of these compounds (ketocarotenoids) increased significantly. In this study, an inconsistency appeared in the baseline plasma levels of zeaxanthin before and after the subjects were supplemented with this compound. However, plasma zeaxanthin concentration in all three subjects, monitored for nearly three months following the study, suggest that the baseline plasma concentrations of this compound determined on days –6 and 0 of this study were inaccurate. This may be attributed to analytical variations associated with extraction and analysis of carotenoids by HPLC, which can normally be overcome by increasing the number of subjects and data points. The data generated from these studies demonstrated, for the first time, that *in vivo* oxidation of lutein and zeaxanthin is a key reaction in the metabolism of these non-vitamin A active dihydroxycarotenoids.

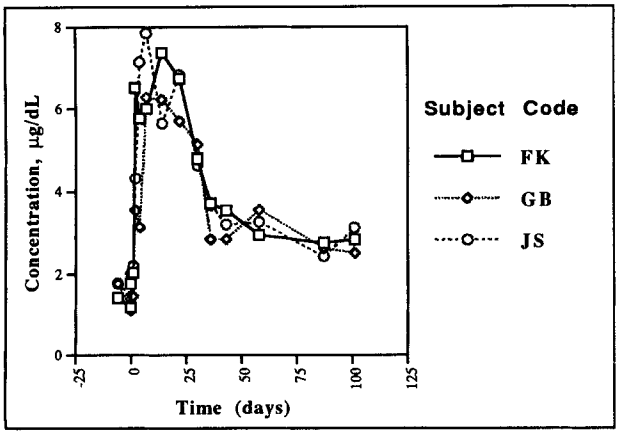

Fig. 3. Plasma concentration of zeaxanthin in three male subjects before and during ingestion of 10 mg/day pure zeaxanthin.

PATHWAYS LEADING TO THE FORMATION OF LUTEIN AND ZEAXANTHIN METABOLITES IN HUMANS

Food-derived carotenoids should be transported via the circulating blood to affect tumors at sites other than the gastrointestinal tract. One hypothesis regarding a potential role of carotenoids as cancer preventive agents is based on their antioxidant capability to quench singlet oxygen and other oxidizing species, inhibit lipid peroxidation, and prevent further promotion and replication in the neoplastic cell. If a free radical mechanism is involved in the initiation and promotion of carcinogenesis, carotenoids such as lutein, zeaxanthin, and lycopene may participate in quenching peroxides and protecting cells from oxidative damage. As a result of this proposed antioxidant activity, several oxidative metabolites of these carotenoids should be formed. In metabolic reactions of non-vitamin A active carotenoids, the general skeleton of the polyene chain of the carotenoids remains intact. However, specific chemical transformations of the carotenoid endgroup can result in the formation of many metabolites. With the exception of the lycopene metabolite to be discussed later, metabolites of lutein and zeaxanthin can result from four types of

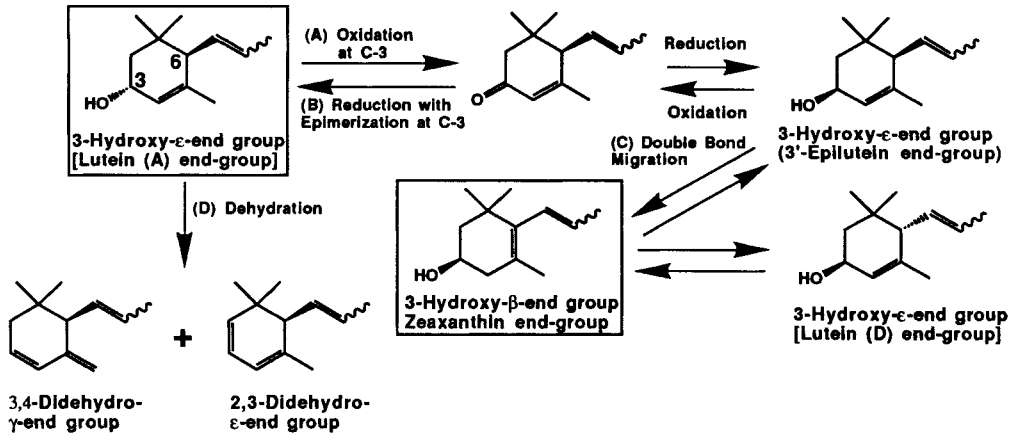

Fig. 4. Metabolic reactions of carotenoids in humans; chemical transformation of carotenoid end-groups. Pathway A: Allylic oxidation of 3-hydroxy-ε end-group at C-3; Pathway B: Reduction of the resulting 3-keto-ε-end-group accompanied by epimerization at C-3; Pathway C: Reversible double bond migration in 3-hydroxy-β-end-group to form a 3-hydroxy-ε end-group; Pathway D: Acid-catalyzed dehydration of 3-hydroxy-ε-end-group.

reactions involving the end-groups of these carotenoids as shown in Figure 4.

The formation of lutein and zeaxanthin metabolites is accompanied by one or a combination of several of these reactions. For example, dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin can exist in an equilibrium involving an intermediate carotenoid known as 3'-epilutein as shown in Figure 5. Allylic oxidation of natural lutein at C-3 (path A) results in the formation of oxolutein B, which can exist in an equilibrium with lutein and 3'-epilutein through reduction reactions (path B). 3'-Epilutein [(3R,3'S,6'R)-lutein] and zeaxanthin can also exist in an equilibrium through reversible double bond migration (path C). Therefore, the presence of 3'-epilutein in human serum/plasma [13] may be due to metabolic conversion of both lutein and/or zeaxanthin to this compound. The migration of the double bond in the 3-hydroxy-β end-group of zeaxanthin activates the neighboring allylic hydroxyl group, making this substituent readily susceptible to oxidation (path A) and reduction (path B) reactions. As a result, monoketocarotenoids such as oxoluteins B and D are formed from lutein A or B and D, respectively (see Fig. 5).

Further double bond migration followed by allylic oxidation of oxoluteins B and D eventually results in the formation of diketocarotenoids as shown in Figure 6. These metabolic pathways agree with results obtained from the two human studies with lutein and zeaxanthin described earlier. In these studies, as a result of lutein and zeaxanthin ingestion, serum levels of monoketocarotenoids and diketocarotenoids in all three subjects increased substantially. Furthermore, these types of reactions have been quite common in the metabolism of carotenoids in animals [18]. Lutein and zeaxanthin oxidation products have been isolated from hen's egg yolk by several researchers. Schiedt *et al.* [19] and Matsuno *et al.* [20] have reported possible metabolic pathways for the formation of several mono- and diketocarotenoids from lutein and zeaxanthin.

Acid catalyzed dehydration is another metabolic reaction of carotenoids with 3-hydroxy-ε-end groups which results in the formation of two isomeric carotenoids (path D, Fig. 4). Among the dietary carotenoids that appear in human serum, lutein is believed to undergo dehydration in the acidic stomach to form two dehydration products, 3-hydroxy-3',4'-didehydro-β,γ-carotene (high plasma concentration) and 3-hydroxy-2',3'-didehydro-β,ε-carotene (low plasma concentration). These compounds have been isolated from extracts of human serum and characterized by various spectroscopic techniques as well as organic synthesis [Khachik F, unpublished results].

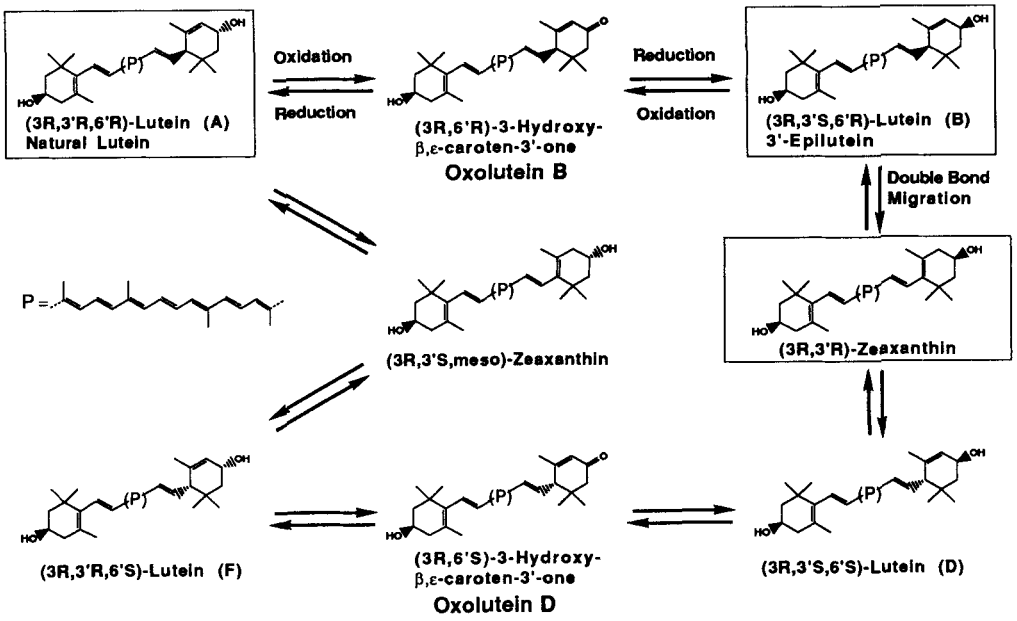

Fig. 5. Equilibrium between lutein, 3'-epilutein, and zeaxanthin in human serum.

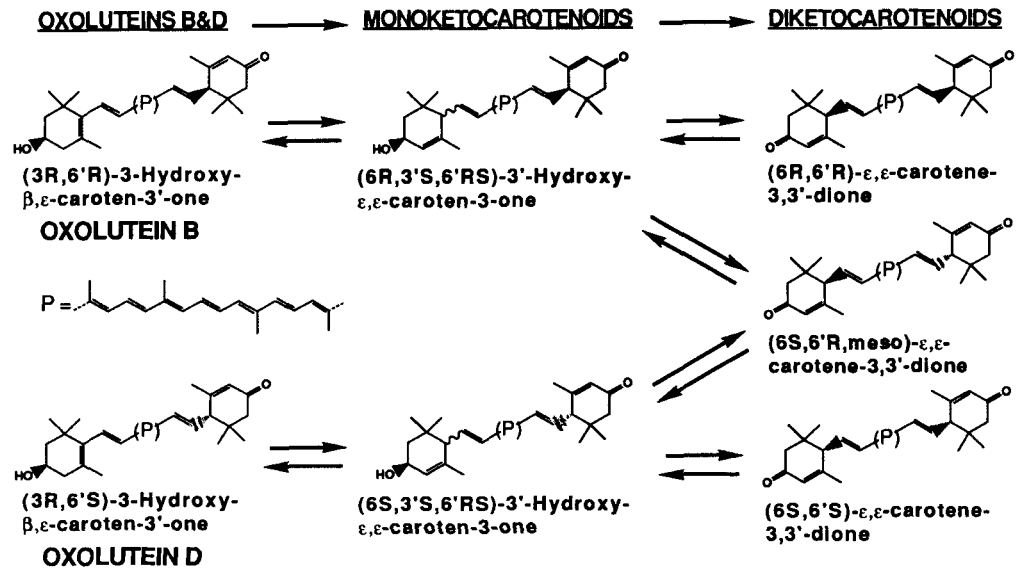

Fig. 6. Chemical transformation between lutein oxidation products in human serum; formation of diketocarotenoids from oxoluteins B & D.

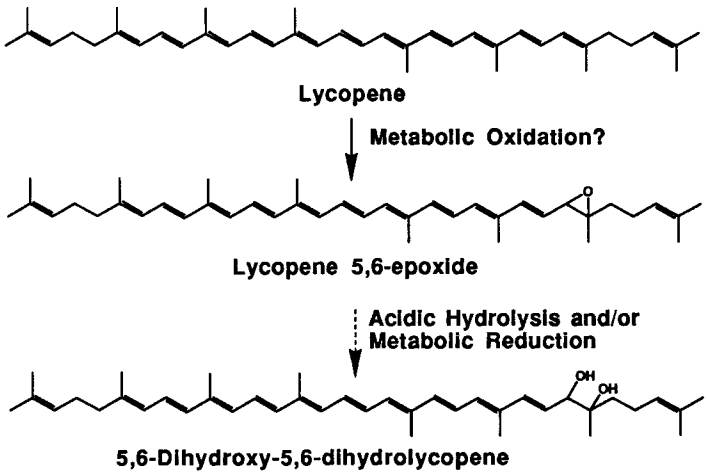

Fig. 7. Possible metabolic pathways for oxidation of lycopene in humans.

POSSIBLE METABOLIC PATHWAYS LEADING TO THE FORMATION OF LYCOPENE METABOLITE

The presence of 5,6-dihydroxy-5,6-dihydrolycopene in human serum is interesting since we have recently shown that tomatoes and tomato-based food products are major dietary sources of this compound [9,16]. In addition, tomato-based food products also contain lycopene 5,6-epoxide, which in strong acidic media similar to that of the human digestive system, may undergo hydrolysis to form 5,6-dihydroxy-5,6-dihydrolycopene as shown in Figure 7. However, the dietary levels of both of these compounds are far too low to account for the concentration of the 5,6-dihydroxy-5,6-dihydrolycopene observed in human serum. Alternatively, the metabolism of lycopene in humans may involve *in vivo* oxidation of this compound to form lycopene 5,6-epoxide, which may undergo metabolic reduction to 5,6-dihydroxy-5,6-dihydrolycopene. This mechanism is consistent with the chemical oxidation of lycopene by metachloroperbenzoic acid which results predominantly in oxidation of this compound at the 5,6-position to form lycopene 5,6-epoxide. The absence of lycopene 5,6-epoxide in human serum may be because only selected groups of carotenoids are permitted into the bloodstream; as a result, dietary carotenoid epoxides, abundant in most fruits and vegetables, have not been detected in human serum to date.

SUMMARY

Although there are more than 40 dietary carotenoids, only 14 have been identified in human plasma and tissues. These carotenoids belong to the selected classes of hydrocarbon carotenoids, hydroxycarotenoids, and their derivatives. For example, carotenoid epoxides, abundant in most green fruits and vegetables, have not been detected in human plasma. In addition to the dietary carotenoids identified in plasma, six metabolites of lutein and one of lycopene have been isolated and characterized. Among the metabolites of lutein, four result from oxidation and two from non-enzymatic dehydration. The metabolite of lycopene found in plasma, 5,6-dihydroxy-5,6-dihydrolycopene, may be the product of an *in vivo* oxidation of lycopene. Human studies using purified lutein and zeaxanthin (a regioisomer of lutein) confirm *in vivo* oxidation of these compounds to their metabolites. Based on these findings, lutein and lycopene, the most abundant carotenoids in the diet as well as in human plasma, are believed to possess strong antioxidant potentials. Although 21 plasma carotenoids have now been identified, only β-carotene has been studied extensively. Other dietary carotenoids,

including lutein and lycopene as well as their metabolites, need further investigation as potential chemopreventive agents.

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