Factors That Influence the Bioavailablity of Xanthophylls[1,2]

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Introduction

Epidemiologic and animal studies have demonstrated that carotenoid-rich diets are associated with a number of health benefits (1). The potential health benefits of carotenoids, such as their ability to act as antioxidants, immunoenhancers and inhibitors of premalignant lesions, (2) have stimulated investigators' interest. One of the most biologically plausible roles for carotenoids is the potential effect of dietary lutein and zeaxanthin for protection of the macula from degeneration (3,4). These possible health benefits seem to relate to the unique geometry of carotenoids.

Carotenoids are nonpolar compounds, which are divided into two subclasses, i.e., more polar compounds called xanthophylls, or oxycarotenoids, and the nonpolar hydrocarbon carotenes. Both classes have at least nine conjugated double bonds, which absorb specific wavelengths of visible light and thus provide carotenoids their characteristic colors. At the end of the polyene chain, at least one unsubstituted B-ionone ring must be present to have provitamin A activity (5). Xanthophylls have ring structures at the end of the conjugated double-bond chain with polar functions, such as hydroxyl or keto groups (6). Examples of xanthophylls include lutein, zeaxanthin, capsanthin, canthaxanthin, astaxanthin, echionine and β-cryptoxanthin. Small quantities of mono- and diester forms of lutein and zeaxanthin have been identified in foods (7), and some dietary lutein supplements are sold in the diester form. Papaya, peaches, prunes and squash all contain lutein diesters, whereas squash also contains lutein monoesters and peaches also contain zeaxanthin diesters (7). When fed at physiologic levels, mono- or diester forms must be deesterified before lutein or zeaxanthin absorption. Hydrocarbon carotenes include β-carotene, α-carotene, γ-carotene and lycopene. (See **Fig. 1**.)

Due to the large vitamin A–deficient population throughout the world, most carotenoid research has focused on hydrocarbon carotenes, particularly the provitamin A carotenes. In recent years, the focus has shifted to other carotenoids, particularly the oxycarotenoids (2). Xanthophylls of current interest include lutein, zeaxanthin and canthaxanthin. Lutein and zeaxanthin have been the primary focus because they are found specifically sequestered in the macula of the eye. In addition, there is evidence to suggest that lutein and zeaxanthin may also play a role in the prevention of epithelial cancers (8), cataract and other blinding disorders. Canthaxanthin has been used as a food-coloring agent and as part of tanning tablets (9).

The focus of this paper will be to review the current research on the bioavailability of xanthophylls with particular attention to the differential absorption of food and supplemental sources of xanthophylls. Although there is strong evidence to show that carotenoids have limited or poor absorption from foods, many supplemental forms of carotenoids prove to have higher bioavailability. Deming and Erdman (10) recently reviewed the factors affecting the bioavailability of carotenes.

Uptake and absorption of xanthophylls

The process of nutrient absorption requires movement of the digested food components into the mucosal cells of the intestinal wall. Uptake occurs when the xanthophyll or its metabolites enter the intestinal mucosal cells. Absorption is achieved with the movement of the xanthophyll or its bioactive metabolite through the mucosal cells into the portal or lymphatic system. Xanthophyll bioavailability can be defined as the proportion of the ingested xanthophyll that is made available (i.e., delivered to the bloodstream) for its intended mode of action.

Four major events must take place for optimal absorption of xanthophylls (10). First, xanthophylls must be released from their food matrix. This process is not efficient as detailed below.

The second step of absorption is the transfer of xanthophylls to lipid micelles in the small intestines. This requires the presence of dietary fat in the small intestine, which stimulates the gallbladder to release bile acids (i.e., emulsifiers). Bile acids are synthesized by the liver and are composed of both polar and nonpolar ends, which allow binding of both
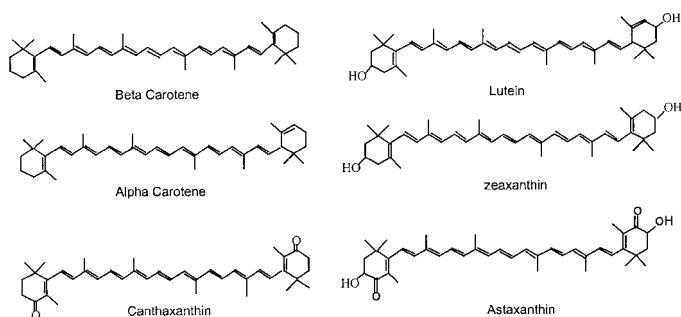
FIGURE 1 Chemical structures of the selected carotenoids.

lipophilic and hydrophilic molecules. Released xanthophylls then must be assimilated into the mixed lipid micelles in the lumen of the small intestine, most likely orienting themselves at the micelle surface. Polar compounds make up the exterior of the micelle, acting as a carrier for the xanthophylls to travel through the hydrophilic chyme in the intestine to the intestinal mucosal cell surface.

The third step is uptake by intestinal mucosal cells. It is thought that xanthophylls passively diffuse through the cell membrane and are released into the enterocyte. Some of the xanthophylls that are taken up by the mucosal cell are not absorbed because they are returned to the lumen of the intestine with the turnover of the mucosal cells, which have a half-life of ~3 d.

The final step in absorption is the transport of the xanthophylls or their metabolic products to the lymph system. In the Golgi of the enterocyte, xanthophylls are incorporated into chylomicrons. Due to their polarity, it is hypothesized that xanthophylls are surface oriented (4,5,10–12). The chylomicrons are eventually delivered to the blood stream and through the action of lipoprotein lipase, chylomicrons lose triglyceride content and shrink in size. It is postulated (10) that nontriglyceride components of the chylomicron, including surface molecules such as xanthophylls may be taken up by extra hepatic tissues or transferred to other blood lipoproteins. Eventually, the chylomicron remnant, including the remaining xanthophylls is taken up by the liver. Xanthophylls then can remain in the liver or be transported to the bloodstream by VLDL. They then are transferred to LDL and HDL with maturation of the lipoproteins. Tissues differentially take up carotenoids, with lutein and zeaxanthin specifically accumulating in the macula region of the eye.

Factors effecting bioavailability

Lutein, zeaxanthin and canthaxanthin are present predominantly in green leafy vegetables and in fruit. Carotenoids are particularly concentrated in chromoplasts or chloroplasts of plant foods and are noncovalently bound to protein or fiber, dissolved in oil or exist in crystalline form (12,13), making optimal absorption difficult to achieve (10). Some major factors limiting the availability of xanthophylls include physical disposition in food sources (food matrix), structure of the xanthophyll molecule, interaction of xanthophylls with other nutrients (mainly dietary fat) and malnutrition (2).

Food processing such as grinding, fermentation and/or mild heating usually improves bioavailability, most likely as a result of weakening the cell wall of plant tissues, dissociating the protein-oxycarotenoid complexes and/or dissolving the crystalline carotenoid complexes (5,10). Factors such as general malnutrition or intestinal parasites have been found to substantially reduce the efficiency of carotenoid absorption (14).

Human bioavailability studies

Effect of xanthophyll esterification, isomerization and dietary fat. How much fat is required to achieve maximal absorption of xanthophylls or xanthophyll esters? Could the more hydrophobic xanthophyll esters be distributed differently in the lipoproteins, affecting transfer among lipoproteins? These questions have been addressed only partially.

Using β-carotene as the representative carotenoid, Jayarajan et al. (15) concluded that at least 5 g of dietary fat in the same meal as β-carotene was necessary for optimal carotenoid absorption. A more recent study (16) tested the effects of dietary fat on the bioavailability of lutein diester, α-carotene, β-carotene and vitamin E in humans. The experimental design included two 7-d periods, with a 5-wk washout period between each test period. The control group received a placebo supplement and the experimental groups received supplements of vitamin E, lutein esters or a combined dose of α- and β-carotene supplements, which were ingested as part of either a high or low fat spread in a crossover design. The low fat meal contained 3 g of fat, whereas the high fat meal contained 36 g of fat. The increased plasma concentrations of vitamin E, α-carotene and β-carotene were not significantly changed by the amount of fat in the spread. However, the lutein diester supplement did show a significant enhancement in absorption (as measured by increase in serum carotenoid) with the higher dietary fat (207% increase with the high fat spread and 88% increase with the low fat spread). From these findings, it may be concluded that a limited amount of fat is required for optimal intestinal uptake of the hydrocarbon carotenes or vitamin E, whereas a greater amount of fat is required for the optimal deesterification of lutein esters and absorption of lutein. Thus, >3 g of fat were required for the sufficient solubilization of lutein diesters and/or secretion of esterases and lipases from the pancreas. The hydrolysis of lutein esters is mediated by these enzymes and is regulated by the presence of fat in the stomach and the duodenum (16). The diester form of lutein is more hydrophobic, making it more difficult to solubilize.

At levels found in foods, lutein mono- and diesters are poorly absorbed without deesterification. Therefore, lutein esters are not normally found in chylomicrons or in blood serum. However, Granando et al. (17) detected lutein esters in serum of subjects who received a supplement of 15 mg lutein/d as mixed esters for 4 mo. This dose level is 10 times the average U.S. intake of lutein (18) and likely exceeds the maximal deesterification activity of the enzymes in the gastrointestinal tract. Three weeks after completion of supplementation, the lutein diesters disappeared from the serum. Therefore, the presence of lutein esters in serum is reversible and is found only when total serum lutein concentrations are >1.05 μmol/L (17). More research is required to determine whether mono- or diester forms of lutein are taken up into specific tissues, e.g., the retina.

A recent study adopted the method used to radiolabel β-carotene with C^{13} via algae (19,20) and applied it to lutein (21). Four women ages 25–38 y were fed a strict diet of 14% of total energy from protein, 59% from carbohydrates and 27% from fat. Lutein was successfully radiolabeled with the stable isotope and fed as part of a low carotenoid meal. After the meal, blood was taken over the next 528 h. C^{13} Lutein was detected in plasma almost immediately and increased rapidly to its single plasma peak at 16 h (21). This innovative ap-

proach has the potential to be used to evaluate the differential absorption of free, mono- and diester lutein, as well as oxidative products, which are proposed to be present in serum (14).

The variable structures of xanthophylls could have an effect on their tissue uptake. An example, is astaxanthin whose predominant form in nature is all-*trans* astaxanthin but other isomers such as 9*cis*-, 13*cis*- and 15*cis*- isomers have also been detected. In one study, Osterlie et al. (22) examined the distribution of and the rate at which isomers of astaxanthin appeared in plasma. The plasma appearance and distribution of astaxanthin *trans/cis* and *R/S* isomers in plasma lipoproteins of three men was detected after ingestion of a single 100-mg dose. The astaxanthin dose was made up of 74% all *trans*-astaxanthin, 9% 9*cis*-astaxanthin, and 17% 13*cis*-astaxanthin. The astaxanthin levels in the plasma were measured for 72 h and the maximum peak was reached at ~7 h at 1.3 ± 0.1 mg/L. The plasma elimination half-life was found to be 21 ± 11 h. This study found that astaxanthin accumulates selectively in the VLDL-containing chylomicrons, whereas ~29 and 24% were distributed within LDL and HDL, respectively. The relative proportion of astaxanthin *cis*-isomers compared with all-*trans*-astaxanthin was increased apparently due to its selective absorption of *cis* isomers. However, the pharmacokinetics of astaxanthin isomers are similar to each other (22).

Effects of other food components on bioavailability. It is important to determine the effects of other food components on the bioavailability of xanthophylls. For instance, the bioavailability of both lutein and canthaxanthin can be reduced significantly when they are consumed with some forms of dietary fiber (23,24). Another food component that has been shown to reduce bioavailability of carotenoids is the fat substitute, sucrose polyester (SPE). A significant reduction of lutein and zeaxanthin was found in human plasma with 3g/d consumption of SPE; however, SPE does have a greater effect on carotenes than xanthophylls. SPE affects carotenoid absorption when consumed in the same meal as the carotenoids (25).

Interaction among carotenoids

When a diet is high in carotenoid-rich foods, it will usually be high in several carotenoids; thus, it is important to determine whether there are interactive effects among carotenoids. In addition, the use of high dose, single-carotenoid supplements in clinical trials or for self-medication increases the risk of negative interactions. The question of whether oxygenated xanthophylls are absorbed more rapidly than carotenes was addressed in a pharmacokinetics study of β-carotene and canthaxanthin. Two subjects ingested either a 25-mg dose of canthaxanthin, a 25-mg dose of β-carotene or a combined dose of canthaxanthin and β-carotene, which contained 25 mg of each over three 5-d study periods. The order of carotenoid treatments varied among the subjects, i.e., either canthaxanthin or a combined dose of β-carotene and canthaxanthin were administered for the first and second study periods. After a 33-wk washout period the subjects ingested an individual β-carotene dose. It was concluded that canthaxanthin did not inhibit the appearance of β-carotene in serum. However, this combined dose did reduce the bioavailability of canthaxanthin. β-Carotene reduced the serum canthaxanthin concentration by ~39%; even after 72 h, a significant reduction of 34% was observed (26). Therefore, when high doses of β-carotene and canthaxanthin are ingested together, one should expect a reduction of canthaxanthin absorption.

Another dietary supplement study compared the plasma appearance of both β-carotene and canthaxanthin in nine normolipidemic premenopausal women (27). Each subject ingested individual doses of 25 mg canthaxanthin or β-carotene, as well as a combined dose of 25 mg each. Plasma β-carotene had a small plasma peak appearance at 5 h, most likely the chylomicron peak, and then a sustained serum concentration peak from 24 to 48 h. The appearance of canthaxanthin in the plasma was monophasic, with a rapid increase at 12 h and a steady decrease at 24 h. The combined dose of β-carotene and canthaxanthin reduced the level of canthaxanthin in the VLDL subfraction ($P < 0.05$) but did not significantly reduce its appearance in LDL. There was an insignificant change in the appearance of β-carotene in both plasma and plasma lipoproteins (27). Therefore, both studies demonstrated a reduction of canthaxanthin absorption when consumed concurrently with β-carotene.

The relative bioavailability of carotenes compared with the oxycarotenoids was studied from a natural carotenoid supplement Betatene, which is derived from *Dunaliella salina* (0.5% lutein, 0.75% zeaxanthin, 3.6% α-carotene, 70.3% all *trans* β-carotene, 22.7% *cis* isomers and 2.1% unidentified carotenoids). A single dose of 5.6 μmol total carotenoids/kg body wt was given to eight healthy subjects (5 men and 3 women) with 500 mL milk (3.5% fat). Both lutein and zeaxanthin were taken up from the intestinal lumen into chylomicrons more efficiently than β-carotene and α-carotene from the same supplement. The content of lutein and zeaxanthin in the chylomicron were 14- and 4-fold greater, respectively, relative to Betatene composition, whereas relative composition of β-carotene in the chylomicron was substantially lower than Betatene (28). Although some of the α- and β-carotene may have been converted to vitamin A in the enterocyte, this study suggests that lutein's relative bioavailability is greater than that of β-carotene. Lutein may be more easily incorporated into the micelle because it is more polar than β- and α-carotene and as such may be incorporated into the polar exterior of the micelle. In the same regard, the membranes of enterocytes may take up lutein more readily, which will increase bioavailability (10,28).

Kostic et al. (29) investigated the interaction between β-carotene and lutein during intestinal absorption, metabolism and serum clearance. Four men and four women were each assigned to one of three groups. Group one was administered a combined dose, then a single dose of lutein and finally a single dose of β-carotene. Group 2 received β-carotene, the combined dose and then a single dose of lutein, whereas group 3 ingested doses in the following order: lutein, β-carotene and a combined dose. Each phase lasted 5 wk plus 10-d washout periods. Each subject ingested 0.5 μmol/kg body wt of either β-carotene or lutein in oil (0.16 and 0.13 mL oil/kg body wt, respectively) or both carotenoids in a combined dose in 0.24 mL oil/kg body wt. The mean serum appearance of lutein was monophasic and had a peak at 16 h; in contrast, the serum appearance of β-carotene peaked at 6 h and again at 32 h. As found for canthaxanthin, the consumption of the combined dose of β-carotene and lutein reduced the absorption of lutein. However, it was also shown that lutein influenced β-carotene absorption depending on the area under the plasma appearance curve (AUC) values of individuals receiving β-carotene alone. Lutein enhanced absorption of β-carotene when the AUC values of individually dosed β-carotene was <13 (μmol × h)/L. Lutein reduced absorption of β-carotene when subject's AUC values of individual β-carotene doses were >25 (μmol × h)/L (29). Thus it is clear that carotenoids can interact with each other during intestinal absorption, metabolism and serum clearance, and individual responses may vary markedly. The ability of an individual to convert β-carotene

to vitamin A when consuming lutein supplements warrants further study.

Food processing also affects carotenoid bioavailability. Castenmiller et al. (30) studied the effects of food processing on the relative bioavailability of β-carotene and lutein from spinach compared with supplemental sources in humans. In this study, spinach was consumed in the minced, whole-leaf, liquefied (enzymatically digested) or liquefied with added fiber form. The supplemental standards were β-carotene (vegetable oil with microcrystalline β-carotene) or lutein plus zeaxanthin (marigold source) primarily in diester form. Carotenoid supplements were added to the control diet. The percentage of relative bioavailability of β-carotene from spinach ranged from 5.1–9%, whereas for lutein it ranged from 45 to 55%. In both cases, the whole leaf proved to have the lowest percentage of bioavailability of lutein or β-carotene; the liquefied form had the highest (30). It can be concluded from this study that spinach xanthophylls are more bioavailable than β-carotene and that food processing improves the relative bioavailability of β-carotene more than it does lutein and zeaxanthin.

In conclusion, the xanthophylls, lutein and zeaxanthin have specific distribution patterns in human tissue especially in the retina of the eye. The presence of these xanthophylls is thought to provide protection from macular degeneration. As this review has shown, the complexity of the bioavailability of these compounds is far from fully understood. Environmental factors, food processing, food matrix, structural differences and the interaction among other food components all have an effect on their efficiency of uptake and absorption.

From the limited human studies, lutein appears to be more bioavailable from food sources than does β-carotene. The disruption of the food matrix seems to improve β-carotene's bioavailability more than that of lutein. There is no evidence that a negative interaction between carotenoids occurs when foods are ingesting. However, interactions do occur between xanthophylls and carotenes when supplements are consumed. Several studies found that when they were consumed simultaneously, β-carotene reduced lutein bioavailability. With the broad consumption of lutein supplements from marigold flowers, some of which are high in lutein diesters, the question of lutein diester bioavailability arises. More dietary fat seems to be required for efficient absorption of lutein from lutein diester sources.

The current research on xanthophyll bioavailability is limited and inconsistent. Knowledge is lacking on the bioavailability of xanthophylls other than lutein. More work should be carried out to compare the bioavailability of free xanthophylls to mono- and diester forms and to carotenes. More research should be performed using similar levels of xanthophylls from foods and supplements. It is also important to understand more fully the effect of food processing techniques on the bioavailability of xanthophylls. More complete work on the kinetics of absorption, transport, and turnover and tissue uptake of xanthophylls, especially in the eye, is greatly needed.

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