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ORIGINAL CONTRIBUTION

# Competitive inhibition of carotenoid transport and tissue concentrations by high dose supplements of lutein, zeaxanthin and beta-carotene

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**Abstract**
*Background* Carotenoids may interact differently in their absorption and transport in animals and humans. The simultaneous administration of large amounts of lutein, zeaxanthin and beta carotene would affect not only plasma values but also their concentrations in the retina and other tissues.
*Objective* In this study, we investigated the transport, distribution and interactions of lutein, zeaxanthin and beta-carotene in the plasma, retina and other tissues of chicks fed supplements rich in lutein, zeaxanthin or beta-carotene.
*Methods* Newly hatched male Leghorn chicks were randomly assigned to ten groups. One group provided baseline data (1-day-old group). The other groups were fed one of the following six diets for 14 or 28 days: high lutein diet; high zeaxanthin diet; three high beta-carotene supplemented diets and the control diet. Plasma and tissues including retina were analyzed for lutein and zeaxanthin and beta-carotene at baseline and at 14 and 28 days.
*Results* All tissues had increased concentrations of lutein after the high lutein diet and had increased concentrations of zeaxanthin after the high zeaxanthin diet. After 28 days, the retinal concentrations of lutein and zeaxanthin in the chicks supplemented with lutein (27.2 mg/kg diet) and zeaxanthin (15.3 mg/kg diet) increased 128 and 116%, respectively, compared to the retinas of chicks fed the control diet (lutein 5.2 mg/kg and zeaxanthin 1.7 mg/kg). Lutein was decreased in plasma and other non-retinal tissues when the diet was supplemented with zeaxanthin; likewise, zeaxanthin was decreased in plasma and non-retinal tissues after the lutein supplement. Zeaxanthin increased in the retina after the high lutein supplement, and retinal lutein was maintained after the high zeaxanthin supplement. The high beta-carotene supplement increased the beta-carotene content of plasma and liver very little, and beta-carotene was not found in any other tissue in the chick, including the retina. More importantly, beta-carotene decreased the concentrations of both lutein and zeaxanthin in the plasma and most tissues, including the retina.
*Conclusion* High dose dietary supplementation of a single carotenoid may alter the assimilation of other carotenoids. The retina appears to have the capacity to preserve accumulation of lutein and zeaxanthin, but this capacity is diminished when intake of beta-carotene is high.

**Keywords** Supplements · Carotenoid absorption and interactions · Retinal carotenoids

## Introduction

Carotenoids represent a group of widely distributed plant pigments that have strong antioxidant effects in humans and animals. It is hypothesized that lutein and zeaxanthin may protect against age-related macular degeneration (AMD) and atherosclerosis, and may decrease the propensity to cancer by enhancing immune function [19, 30, 37]. The results of several lines of evidence provide support for the hypothesis that lutein and zeaxanthin may help prevent AMD. The incidence of AMD is inversely related to the dietary intakes of lutein and zeaxanthin and also to their concentrations in the blood and retina [2, 6, 7, 14]. In patients with macular degeneration and in controls,

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a diet rich in fruits and vegetables more than doubled the plasma levels of lutein and zeaxanthin and beta-carotene [39]. Lutein supplementation increased macular pigment optical density eccentrically and may aid in prevention of AMD [15]. High levels of lutein and zeaxanthin in the diet correlate well with high amounts of these carotenoids in the retina. Light exposure is an important factor in age-related macular degeneration [19, 20, 26, 29, 32, 33]. Dietary zeaxanthin has been shown to be protective against light damage in quail [33]. The number of dying photoreceptors in light-damaged eyes correlated significantly and inversely with the zeaxanthin concentration in the contralateral retina [32, 33].

Beta-carotene serves as a vitamin A precursor in the intestine and may have a role as an antioxidant [26, 41–43]. In the past, it has been considered safe and has been used in a number of clinical trials to prevent cancers, but it was shown to increase the risk of lung cancer in smokers [3, 22, 23]. Currently, beta-carotene is a component of a vitamin mineral cocktail intended to prevent damage from age-related macular degeneration [10, 31].

The interactions of carotenoids and their absorbability by the gut has been previously reviewed [13, 43]. When beta-carotene and lutein were administered in equal doses to humans, beta-carotene depressed the plasma lutein values to 54–61% of control values, whereas the lutein dose had an inconsistent effect on plasma beta-carotene values [18]. In contrast, the results of other studies found no change in other carotenoids following beta-carotene supplementation and no change in other carotenoids following lutein or zeaxanthin supplementation [34, 35].

The purpose of the current study was to test the competitive effects of co-administration of beta-carotene, lutein and zeaxanthin on the assimilation and transport of these carotenoids. This was tested in the newly hatched chick given dietary supplements rich in lutein, zeaxanthin or beta-carotene. We especially contrasted the carotenoid content of the retina with other tissues. The results of this study may be relevant to the effects of various carotenoids being used to prevent disease in clinical trials.

## Materials and methods

The chicken was selected as the experimental animal for several reasons. One, unlike the rat, the chicken is a diurnal animal and has a cone-dominant retina, which is rich in lutein and zeaxanthin. Two, HDL, the major transport modality for lutein and zeaxanthin, is the predominant lipoprotein in the chicken. Third, because carotenoids are retained in the tissues of chickens, we selected newly hatched chicks as our model for the studies. Such chicks more immediately reflect metabolic processes. Further, on a weight basis growing chickens need more nutrients than adult chickens, so they are more sensitive to the interaction of carotenoids and their effects on absorption and distribution. And, as indicated subsequently, while beta-carotene is poorly absorbed in chicks, it does enter the intestinal mucosa where it functions as pro-vitamin A. Finally, we demonstrated previously the applicability of the chicken as a model for lutein and zeaxanthin metabolism [40].

### Dietary groups

Eighty-eight healthy 1-day-old male Leghorn chicks were randomly assigned to six dietary groups and fed for 14 or 28 days. Each group contained eight chickens (Tables 1, 2, 3). The composition of the six diets (control, the high lutein, high zeaxanthin and three beta-carotene diets) is given in Table 4. The control diet, obtained from Purina Mills Start & Grow Sunfresh, Purina Mills, LLC, St. Louis, MO, contained lutein, zeaxanthin and beta-carotene in the amounts of 5.2, 1.7 and 3.3 mg/kg diet, respectively, as analyzed in our laboratory. Lutein and zeaxanthin were obtained from DSM Nutritional Products Ltd and beta-carotene from Nature Made Nutritional Products. The beta-carotene was all-trans. Soy oil manufactured by ConAgra-Foods Inc was obtained from a local food market. The soy oil contained no carotenoids. All one-day-old chicks were obtained from the Featherland Farms in Oregon. The control diet (Purina Mills Start & Grow Sunfresh) for chicks was the same as the maternal diet. This was a complete diet in terms of all nutrients. All chicks were housed in the animal care facility of OHSU.

The chickens in the experimental groups were fed either the high lutein diet (27.2 mg/kg diet), the high zeaxanthin diet (15.3 mg/kg diet) or one of the three high beta-carotene diets (27, 58, or 227 mg/kg diet) for 14 or 28 days. The high lutein and zeaxanthin diet contained 3% fat (same as control diet). For the beta-carotene studies, the 14-day diet contained 3% fat (same as control diet). Twenty percent soy oil was added to the diet for the 28-day groups (total fat 23%) to ascertain any effects of dietary fat on the intestinal absorption of beta-carotene. All diets contained at least the basal amounts of lutein, zeaxanthin and beta-carotene (5.2, 1.7 and 3.3 mg/kg diet respectively). All supplements were incorporated into the Purina control diet.

The high lutein diet, contained lutein at a level of 27.2 mg/kg of chow, roughly five times greater than the lutein content of the control diet. The high zeaxanthin diet contained 15.3 mg zeaxanthin per kg of chow, nine times the amount in the control diet. Because zeaxanthin is less abundant than lutein in most foods and in the diet, we used a ratio similar to the usual diet of the chicks and humans.

**Table 1** The lutein content of the retina, plasma and other tissues in chicks fed the high lutein, high zeaxanthin and high beta-carotene or the control diet (mean ± SD)

| Tissue | Baseline 1 day ($n = 8$) | High lutein | | High zeaxanthin | | High beta-carotene | | Control diet 28 days ($n = 8$) |
|---|---|---|---|---|---|---|---|---|
| | | 14 days ($n = 8$) | 28 days ($n = 8$) | 14 days ($n = 8$) | 28 days ($n = 8$) | 14 days, ($n = 8$) 3% fat diet | 28 days, ($n = 8$) 23% fat diet | |
| Plasma (μg/dL) | 541 ± 126 | 1078 ± 359[a,c] | 1147.9 ± 289[a,c] | 300 ± 60[**a,c**] | 54.8 ± 8.9[**a,b,c**] | 285 ± 38[a,c] | 268 ± 70[a,c] | 544 ± 69 |
| Liver (μg/g) | 2.75 ± 1.46 | 7.64 ± 1.68[a,c] | 8.30 ± 2.52[a,c] | 1.53 ± 0.31[**a,c**] | 0.45 ± 0.15[**a,b,c**] | 2.5 ± 0.8[c] | 1.7 ± 0.4[a,b,c] | 3.57 ± 0.51 |
| Heart (μg/g | 2.23 ± 0.77 | 4.80 ± 1.35[a,c] | 3.85 ± 0.69[a,c] | 1.13 ± 0.35[**a,c**] | 0.12 ± 0.02[**a,b,c**] | 1.5 ± 0.4[c] | 0.9 ± 0.2[a,b,c] | 2.10 ± 0.66 |
| Adipose (μg/g) | 1.78 ± 0.41 | 3.82 ± 0.72[a,c] | 4.29 ± 1.54[a,c] | 0.90 ± 0.29[**a,c**] | 0.23 ± 0.08[**a,b,c**] | 1.1 ± 0.3[a,c] | 1.2 ± 0.4[a,c] | 1.53 ± 0.32 |
| Skin (μg/g) | 1.82 ± 0.56 | 7.50 ± 2.55[a,c] | 6.48 ± 1.55[a,c] | 1.03 ± 0.35[**a,c**] | 0.66 ± 0.11[**a,b,c**] | 1.6 ± 0.2 | 1.0 ± 0.3[a,b,c] | 1.90 ± 0.32 |
| Brain (μg/g) | 0.15 ± 0.09 | 0.23 ± 0.05[a,c] | 0.27 ± 0.07[a,c] | ND | ND | 0.15 ± 0.1 | 0.17 ± 0.1 | 0.12 ± 0.06 |
| Retina (μg/g) | 5.13 ± 1.06 | 11.40 ± 4.37[a,c] | 11.00 ± 3.26[a,c] | 4.56 ± 0.66 | 5.45 ± 1.34 | 5.9 ± 1.1 | 5.6 ± 1.5 | 4.82 ± 2.60 |

[a] Compared with 1 day old, $p < 0.01$

[b] Compared with 14 days old, $p < 0.01$

[c] Compared with 28 days, control diet, $p < 0.01$

*ND* not detectable

**Table 2** The zeaxanthin content of the retina, plasma and other tissues in chicks fed the high-lutein, high zeaxanthin, high beta-carotene or the control diet (mean ± SD)

| Tissue | Baseline 1 day ($n = 8$) | High lutein | | High zeaxanthin | | High beta-carotene | | Control diet 28 days ($n = 8$) |
|---|---|---|---|---|---|---|---|---|
| | | 14 days ($n = 8$) | 28 days ($n = 8$) | 14 days ($n = 8$) | 28 days ($n = 8$) | 14 days, ($n = 8$) 3% fat diet | 28 days, ($n = 8$) 23% fat diet | |
| Plasma (μg/dL) | 192 ± 46 | 53.4 ± 15.8[**a,c**] | 61.0 ± 10.9[**a,c**] | 340 ± 110[**a,c**] | 540 ± 91[**a,b,c**] | 68.7 ± 16.0[a,c] | 109.6 ± 34.6[a,b,c] | 229.5 ± 71.3 |
| Liver (μg/g) | 1.40 ± 0.74 | 0.80 ± 0.22[**a,c**] | 0.64 ± 0.21[**a,c**] | 3.10 ± 2.10[**a,c**] | 6.03 ± 1.34[**a,b,c**] | 1.1 ± 0.4[c] | 0.7 ± 0.2[a,b,c] | 1.86 ± 0.43 |
| Heart (μg/g | 0.45 ± 0.21 | 0.43 ± 0.11[**c**] | 0.31 ± 0.10[**a,b,c**] | 1.90 ± 0.40[**a,c**] | 2.57 ± 0.31[**a,b,c**] | 0.6 ± 0.2 | 0.5 ± 0.1 | 0.60 ± 0.18 |
| Adipose (μg/g) | 0.57 ± 0.25 | 0.54 ± 0.53 | 0.40 ± 0.16 | 1.20 ± 0.40[**a,c**] | 1.81 ± 0.64[**a,b,c**] | 0.7 ± 0.2[c] | 0.8 ± 0.2[a,c] | 0.46 ± 0.16 |
| Skin (μg/g) | 0.66 ± 0.35 | 0.89 ± 0.28 | 0.55 ± 0.12[**b,c**] | 4.80 ± 0.90[**a,c**] | 6.23 ± 1.07[**a,b,c**] | 0.7 ± 0.2 | 0.7 ± 0.2[c] | 0.97 ± 0.39 |
| Brain (μg/g) | 0.06 ± 0.05 | 0.08 ± 0.03[**c**] | 0.08 ± 0.02[**c**] | 0.08 ± 0.03[**c**] | 0.11 ± 0.04[**a**] | 0.15 ± 0.1 | 0.16 ± 0.1[a] | 0.13 ± 0.03 |
| Retina (μg/g) | 5.02 ± 1.28 | 7.49 ± 2.95[a] | 6.76 ± 2.58[**a**] | 11.30 ± 2.40[**a,c**] | 13.65 ± 4.36[a,b,c] | 4.1 ± 1.1[c] | 4.0 ± 1.5[c] | 6.32 ± 2.80 |

[a] Compared with 1 day old, $p < 0.01$

[b] Compared with 14 days old, $p < 0.01$

[c] Compared with 28 days, control diet, $p < 0.01$

The amount of beta-carotene used was 8, 17 and 68 times greater than the control diet.

The results from 1-day-old newly hatched chickens were considered baseline data. The 1-day-old chicks were unfed. We compared the 1-day results with the 14 and 28-day results.

## Procedures

Chicks from each group were euthanized on day 1, 14, or 28. The 1-day-old chicks were euthanized soon after hatching.

**Table 3** Beta-carotene content of the retina, plasma and other tissues in chicks fed different amounts of beta-carotene and dietary fat (mean ± SD)

| Tissue | 1 day baseline | 14 days, dietary fat 3% | | 28 days, dietary fat 23% | | | 28 days control diet ($n = 8$) |
|---|---|---|---|---|---|---|---|
| | | Beta-carotene | | Beta-carotene | | | |
| | | 27[b] ($n = 8$) | 58[b] ($n = 8$) | 27[b] ($n = 8$) | 58[b] ($n = 8$) | 227[b] ($n = 8$) | |
| Plasma (μg/dL) | ND | 17.9 ± 5.4 | 18.5 ± 4.4 | 14.9 ± 4.8 | 22.0 ± 9.1[a] | 43.9 ± 11.8[a] | ND |
| Liver (μg/g) | ND | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.2 | 0.5 ± 0.2 | ND |
| Heart (μg/g | ND | ND | ND | ND | ND | ND | ND |
| Adipose (μg/g) | ND | ND | ND | ND | ND | ND | ND |
| Skin (μg/g) | ND | ND | ND | ND | ND | ND | ND |
| Brain (μg/g) | ND | ND | ND | ND | ND | ND | ND |
| Retina (μg/g) | ND | ND | ND | ND | ND | ND | ND |

[a] Compared with 14 days old, $p < 0.01$

[b] mg/kg diet

*ND* not detectable

**Table 4** Composition of the chicken diets (mg/kg)

| Nutrient | Control diet[a] | High lutein supplement | High zeaxanthin supplement | High beta-carotene supplement[b] |
|---|---|---|---|---|
| Lutein | 5.2 | 27.2 | 5.2 | 5.2 |
| Zeaxanthin | 1.7 | 1.7 | 15.3 | 1.7 |
| Beta-carotene | 3.3 | 3.3 | 3.3 | 27 or 58 or 227[c] |

[a] From Purina Mills, LLC, St. Louis, P.O. Box 66812, MO, USA. This diet contains 18%, protein, 3% fat and other complete nutrients

[b] 20% fresh soy oil was added to 28-day supplements

[c] Three different amounts of beta carotene

The eyes were removed and the retinas dissected out and immediately frozen at −80 °C. The brain, heart, liver, and adipose tissue also were harvested and frozen. All tissues were light protected and were washed with saline and stored at −80 °C until analysis.

The chickens were anaesthetized by using 60 mg/kg BW ketamine + 10 mg/kg BW xylazine, IM. Then the chickens were euthanized by lethal dose of potassium chloride. Dosage was 1–2 mmol/kg BW, and injected intracardially.

## Plasma and tissue analyses for lutein and zeaxanthin

A 200 μL aliquot of plasma was diluted with 0.5 mL of 0.9% saline solution. 60 μL BHT (10 mg/mL stock solution) was added to the samples as an antioxidant. Echinenone, in ethanol, was added as an internal standard. The mixture was extracted twice with 2 mL $CHCl_3$: $CH_3OH$ (2:1, by vol) and 3 mL hexane. The extract was evaporated to dryness under nitrogen. The residue from plasma was re-dissolved in 150 μL ethanol. A 50 μL portion was used for HPLC analysis.

To facilitate the extraction of lutein and zeaxanthin from solid tissues, 30 mg of tissue was added to a mixture of 100 μL 12% pyrogallol in ethanol, 200 μL echinenone (43 μg/dL) as an internal standard, 200 μL 30% KOH, 60 μL BHT (10 mg/mL) and 1 mL ethanol. The samples were then saponified at 37 °C for 2 h. The mixture was extracted twice with 3 mL ether: hexane (2:1, by vol). The extract was evaporated to dryness under nitrogen. The residue was re-dissolved in 150 μL ethanol, and a 50 μL sample was used for HPLC analysis [16, 28].

In a previous study [40] and the present study, the chick retina contained both *trans*- and *cis*-zeaxanthins, but *cis*-zeaxanthin was not found in any other tissues.

## HPLC analysis

The HPLC system consisted of a series 410 LC pump, an UV/Vis detector plus an autosampler, and a C30 carotenoid column (3 μm, 4.6 × 150 mm). The HPLC mobile phase was methanol:tert-butyl-methyl-ether:water (83:15:2, by vol, solvent A) and methanol:tert-butyl-methyl-ether:water (8:90:2, by vol, solvent B). The gradient procedure, at a

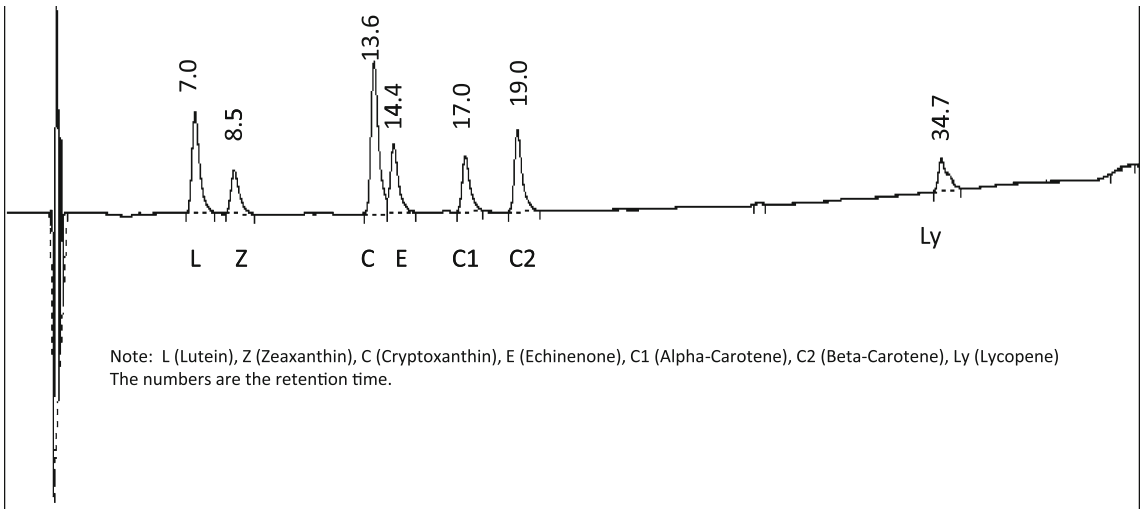


**Fig. 1** The representative HPLC chromatogram

flow rate of 1 mL/min at 16 °C, began at 100% solvent A before dropping to 93% solvent A and 7% solvent B over a 1-min linear gradient. This was followed by a 3-min hold at 93% solvent A, followed by a 17-min linear gradient to 45% solvent A and a 1-min hold at 45% solvent A, an 11-min linear gradient to 95% solvent B, a 4-min hold at 95% solvent B, and finally a 2-min gradient back to 100% solvent A.

Lutein, zeaxanthin and beta-carotene were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. In current HPLC analysis of carotenoids, lutein and zeaxanthin elutes are different. The difference of retention times between lutein and zeaxanthin usually is 1–2 min (Fig. 1). The values were corrected for extraction and handling losses by monitoring the recovery of the internal standard. Recovery rate for carotenoid extraction is 90–99%. The values for retinal zeaxanthin include total *trans* and *cis* zeaxanthin.

The detection limit of the HPLC (μg/dL): lutein 5.85; zeaxanthin 1.55; cryptoxanthin 6.35; echinenone 3.1; a-carotene 2.3; b-carotene 3.2; lycopene 3.65. Linear range (μg/dL): lutein: 5.85–187.2; zeaxanthin 1.55–49.6; cryptoxanthin 6.35–203.2; echinenone 3.1–99.2; a-carotene 2.3–73.6; beta-carotene 3.2–102.4; lycopene 3.65–116.8.

### Statistical analyses

Means and SDs were calculated to characterize the lutein and zeaxanthin content of plasma and tissues. Differences between tissues were detected by use of the appropriate one-way ANOVA statistic using the Bon-Ferroni inequality to control the overall α-level [5]. Data were analyzed using SPSS for WINDOWS (release 10.0.1, 27, Oct, 27, 1999; SPSS Inc, Chicago).

### Institutional review

This study protocol was approved by the "Institutional Animal Care Use Committee" of Oregon Health and Science University, which adheres to the ARVO animal statement.

## Results

### Food intake and weights of the chicks

Food intake increased greatly from day 1 to day 28, since these newly hatched chicks grew rapidly, but food intake at each age was comparable for the six diets. They weighed 39.0 ± 2.6 g at hatching and 295.8 ± 22.3 g at 28 days. The food intake and weights of all groups were similar. At 28 days, the average daily intake of lutein was 119.1 ± 9.1 μg and for zeaxanthin 38.9 ± 0.1 μg for chicks fed the control diet. Daily intake of beta-carotene for 28-day-old chicks:

| Levels of beta-carotene in diet (mg/kg) | Daily intake of beta-carotene (μg) |
|---|---|
| 27 | 618.3 ± 48.7 |
| 58 | 1328.2 ± 104.5 |
| 227 | 5198.3 ± 409.1 |

All chicks were healthy until termination.

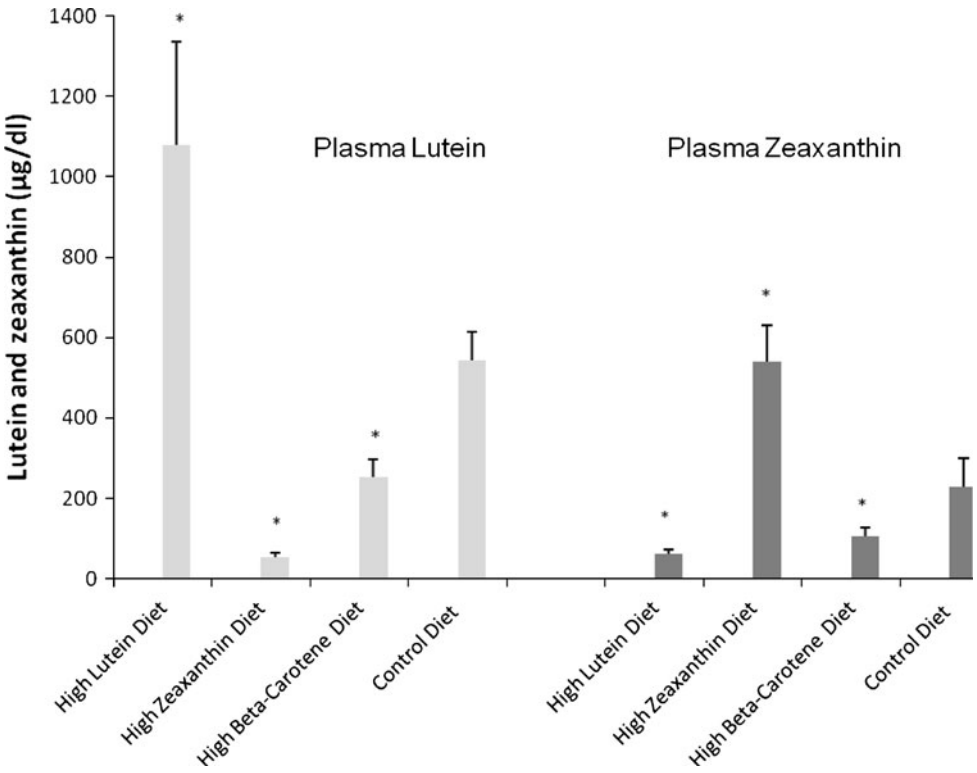


**Fig. 2** The effects of supplements of lutein, zeaxanthin and beta-carotene upon the plasma content of lutein and zeaxanthin after 28 days. *Asterisks* indicate comparison to the control diet, $P < 0.01$

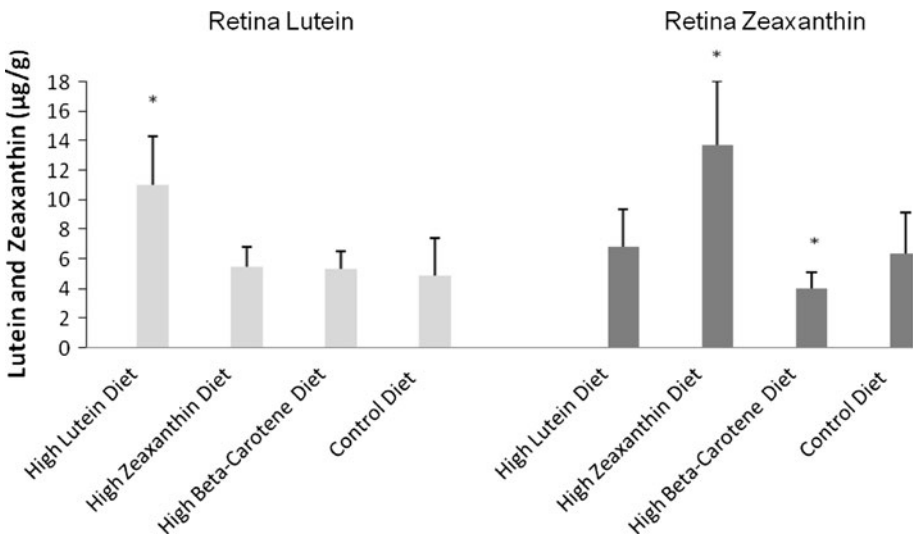


**Fig. 3** The effects of supplements of lutein, zeaxanthin and beta-carotene upon the retina content of lutein and zeaxanthin after 28 days. *Asterisks* indicate comparison to the control diet, $P < 0.01$

## Effects of the high lutein supplement

The high lutein supplement significantly increased the plasma and tissue content of lutein (Table 1; Figs. 2, 3). By day 14, the plasma and all tissues seemed replete with lutein, which did not increase subsequently. In particular, the lutein content of the retina increased from 5.13 to 11.4 µg/g ($P < 0.01$) at day 14 and remained at that level at 28 days (Table 1; Fig. 3). The zeaxanthin concentration in the plasma and most tissues decreased during the high lutein diet, despite consumption of the same amount of zeaxanthin as control chicks. The retina, however, increased its zeaxanthin content (from 5.02 to 7.49 µg/g at 14 days, a 49% increase, $P < 0.01$) (Table 2; Fig. 3). Thus, the high lutein supplement did not suppress the continued uptake of zeaxanthin by the retina as will be discussed later.

### Effects of the high zeaxanthin supplement

The high zeaxanthin diet significantly increased the plasma and tissue content of zeaxanthin (Table 2; Figs. 2, 3). By day 28 of the high-zeaxanthin diet, the zeaxanthin concentration in plasma increased from 192 μg/dL (day 1) to 540 μg/dL (28 days). In other tissues, the level increased from 1.40 to 6.03 μg/g in the liver, from 0.45 to 2.57 μg/g in the heart, from 0.57 to 1.81 μg/g in the adipose tissue, and from 0.06 to 0.11 μg/g in the brain. In particular, the content of zeaxanthin in the retina increased more than twofold from 5.02 to 13.65 μg/g (Fig. 3). After 28 days of the high zeaxanthin supplement, the ratio of lutein to zeaxanthin in the retina decreased to 0.4 in comparison to a ratio of 0.8 in the control chicks, emphasizing the retinal preference for zeaxanthin.

The high zeaxanthin supplement resulted in a profound decrease in the lutein content of the plasma and tissues despite the fact that this diet still contained considerable lutein (5.2 mg/kg of diet) (Table 1; Fig. 2). The plasma lutein concentration declined from 541 to 54.8 mg/dL at 28 days. Lutein in the liver, heart, skin and adipose tissue almost disappeared. No lutein was found in the brain. Heart lutein at 28 days was only 5% of the 1-day value. However, retinal lutein remained unchanged, 5.13 versus 5.45 ($P > 0.05$). These effects were much greater than the effects of the high lutein diet on the zeaxanthin content of plasma and tissues. For example, the high lutein diet suppressed heart zeaxanthin by 69% at day 28.

### Effects of the high beta-carotene supplement

The plasma and tissues, including the retina, of the 1-day-old chick contained no beta-carotene. The medium dose beta-carotene diet (58 mg/kg diet) increased the concentration of beta-carotene in the plasma from undetectable on day 1 to 18.5 μg/dL at 14 days and to 22.0 μg/dL at 28 days. The plasma level of beta-carotene increased from undetectable on day 1 to 43.9 μg/dL at 28 days during the high dose 227 mg/kg diet (Table 4). Beta-carotene could be detected in the liver, but in no other tissues. The liver beta-carotene content was maximized by the lowest dietary content of beta-carotene (27 mg/kg) compared to the control diet (Table 4).

Increasing the fat content of the diet from 3 to 23% by weight for 28 days did not raise the beta-carotene concentrations in the plasma and liver. Concentrations of beta-carotene in the plasma of chicks fed 3% fat and either 27 or 58 μg/g beta-carotene were 17.9 and 18.5 μg/dL at 14 days, while that in the plasma of chicks fed 23% fat and either 27 or 58 μg/g of beta-carotene were 14.9 and 22.0 μg/dL ($P > 0.05$).

The main effect of the high beta-carotene supplement was to decrease the lutein and zeaxanthin concentrations in plasma and tissues compared to chicks fed the control diet (Tables 1, 2; Figs. 2, 3). The high beta-carotene and control diets contained the same amounts of lutein (5.2 mg/kg) and zeaxanthin (1.7 mg/kg). These results suggested that high beta-carotene intake suppressed the concentrations of lutein and zeaxanthin in the plasma and most tissues. During high beta-carotene feeding supplements, the retina failed to increase its content of zeaxanthin from day 1 to 28 days. Retinal lutein levels increased only 10% compared to baseline. The three different doses of beta-carotene (27, 58 and 227 mg/kg) had similar effects on the lutein and zeaxanthin content of plasma and tissues.

The plasma values (Fig. 2) of these carotenoids probably reflected the amounts absorbed by the intestinal tract. After the high lutein supplement, the plasma level of zeaxanthin declined strikingly in 28-day-old chicks to one-third of 1-day values. After the high zeaxanthin supplement, the plasma lutein was only about 10% of the baseline and 28-day control values; i.e., 54.8 μg/dL versus 541 (1 day) or 544 μg/dL (28-day chicks). Lutein was also lower in adipose tissue (1.53 vs. 0.23 μg/g). However, the high dose of beta-carotene suppressed levels of lutein and zeaxanthin in the chicks less than high doses of either lutein or zeaxanthin.

### Composite effects upon the retina

As already suggested, the changes in the retinal xanthophyls after feeding supplements high in lutein, zeaxanthin or beta-carotene provided the most important information because the retina is the organ where the activity of these antioxidant and light-quenching compounds may be most important. The beta-carotene supplement decreased zeaxanthin concentrations in the retina, from 6.32 to 4.10 μg/g ($P < 0.01$, Table 2; Fig. 3). All of the high beta-carotene diets reduced the retinal zeaxanthin about 30% versus the control retina at 28 days of age. The high lutein supplement almost doubled the content of lutein in the retina to a maximal level at 14 days, but did not suppress the retinal zeaxanthin content. The high zeaxanthin diet increased retinal zeaxanthin levels by twofold, but retinal lutein levels were unchanged.

## Discussion

There were several key findings from these studies. First, the retinal content of lutein and zeaxanthin increased greatly when either of these carotenoids was augmented in the diet. These results suggested the presence of specific pathways for transferring lutein and zeaxanthin from blood

into the retina. Berstein and colleagues pioneered this view and have proposed special binding proteins [4, 21]. The data from the WHAM chicken with a mutation of the ABCA1 transporter supports the crucial role of HDL and the ABCA1 transporter in this transport pathway [11]. Specific transport and binding proteins in the retina may explain the influx of lutein and zeaxanthin from the plasma across the blood–retina barrier and subsequent uptake by the retina. Bernstein et al. [1] reported that tubulin is major soluble carotenoid-binding protein in bovine and human retinal tissues. Lutein and zeaxanthin were found to copurify with tubulin. Their recent study indicates that the Pi isoform of human glutathione S-transferase (GSTP1) is also a specific xanthophyll-binding protein (XBP) in the human macula that interacts with meso-zeaxanthin and dietary zeaxanthin [4]. Lutein and zeaxanthin are present in the cones of the chick retina as esters in oil droplets [8, 9]; this feature may also stabilize their content in the retina. With lutein and zeaxanthin present in oil droplets, this feature may prevent their mobilization when the plasma level declines to almost zero. Other than for storage of carotenoids, the exact function of these oil droplets remains for future elucidation. We hypothesize that they may protect the retina against light damage.

Second, the dietary intake of large amounts of any one of the three carotenoids depressed the concentrations of the other carotenoids in plasma and most tissues. The high lutein supplement decreased levels of zeaxanthin; the high zeaxanthin supplement decreased lutein levels; and the high beta-carotene supplement decreased levels of both lutein and zeaxanthin. In the face of these dietary perturbations, the retinal content of lutein and zeaxanthin varied considerably. The preference of the retina for zeaxanthin over lutein was well illustrated. After the high zeaxanthin diet, the ratio of lutein to zeaxanthin in the retina declined from 0.8 to 0.4. The source of the increased retinal zeaxanthin at day 14 during the high lutein diet must have been zeaxanthin from the yolk sac [40] and from the diet, with subsequent preferential uptake and binding of zeaxanthin in the retina. This preference of the retina for zeaxanthin has been noted in other studies [16, 17, 27, 33, 36]. As indicated, the high zeaxanthin diet depleted the lutein content of the tissues more than the high lutein diet suppressed the zeaxanthin content, again suggesting a higher preference for zeaxanthin. Lutein and zeaxanthin were retained in the retina, in contrast to their decreases in the plasma and other tissues, when dietary sources of both were completely absent over a 28-day period [40]. Despite the retinal preference for zeaxanthin, the high beta-carotene diet depressed the retinal level of zeaxanthin. However, these effects have been shown only in chickens and have not yet been shown in humans who have predominantly LDL rather than in chickens with predominantly HDL.

In contrast to the retina, brain concentrations of both lutein and zeaxanthin were minimal or undetectable even after feeding high amounts of either in the diet. This indicated that the blood–brain barrier blocked entry of these carotenoids into the brain, whereas, the specific transport pathways enriched the retina with lutein and zeaxanthin [1].

A third finding from our study is that the content of beta-carotene in the chicken body was very low or absent. There was no beta-carotene in chicken eggs, the newly hatched chicks or in 28-day-old chicks, despite its presence in the diet (Table 4) [40]. Chicken bile is rich in lutein and zeaxanthin but contains no beta-carotene [38], which suggests that chickens may poorly absorb beta-carotene. The beta-carotene was not transported into any tissues other than the plasma and liver. There was no beta-carotene in the retina. Beta-carotene seems to be predominantly excluded from the physiology of the chick except for providing the substrate for the synthesis of vitamin A in the intestine [25]. In humans, beta-carotene is well absorbed into the body. A large dietary intake produces a yellowing of the skin as is well known. Whether the transport lipoprotein for beta-carotene causes a different metabolism in chicks and humans may be important to determine.

The results of human trials showing adverse effects of beta-carotene supplementation have raised questions about the effects of beta-carotene beyond its all-important role as a precursor for vitamin A synthesis. The report from the National Academy of Sciences casts some doubts about other presumed benefits of beta-carotene, such as those attributed to antioxidant properties [12]. In several trials, high-dose beta-carotene supplements have been associated with increased lung cancer in smokers and asbestos exposed workers [3, 22, 23]. The mechanism of this relationship has not been defined as yet, but could be related to suppressed levels of other carotenoids and/or eccentric cleavage products of beta-carotene [24]. In a report from the Food and Nutrition Board of the National Academy of Sciences, it is stated that, based upon the evidence, the use of beta-carotene supplements to prevent chronic diseases is not advisable. No benefit has been seen and harm has resulted in certain population sub-groups [12]. However, the ingestion of beta-carotene as a vitamin A precursor is important, but the amount needed for this purpose is small, only 6 mg/day.

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