

Macular zeaxanthins and lutein – a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease

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The retina is unique in the human body in containing three xanthophyll carotenoids; 3R,3'R-zeaxanthin, meso-zeaxanthin (MZ) and lutein. Humans consume 1 to 3 mg lutein per d and the lutein:zeaxanthin ratio in the diet is about 5:1. Xanthophyll pigments occur widely in vegetables and fruits but MZ is found in only a few foods such as the shrimp carapace and fish skin. In spite of the amounts of the different xanthophylls in the diet, zeaxanthin and MZ occur in approximately equal amounts in the eye, and their combined concentration can exceed that of lutein. In the present review the bioavailability of zeaxanthin and lutein is assessed using the plasma xanthophyll response to dietary intervention. A number of studies have used single and mixed sources of the pure xanthophylls to achieve steady-state plasma responses. Mostly these have been with lutein and zeaxanthin but two using MZ are also described. Responses following the intervention with the pure xanthophylls are compared with those following food intervention. Vegetables are the richest source of dietary lutein and several vegetable-feeding studies are discussed. Intervention studies with eggs, which are a good source of zeaxanthin, suggest that the xanthophyll carotenoids in egg yolk may be more bioavailable than those in other foods and are described separately. MZ has been a component of a xanthophyll supplement added to chicken feed in Mexico in the last 10 years. Egg consumption in Mexico is approximately one egg/person per d and the potential contribution of this food source of MZ to Mexican dietary intakes is described. Very limited information from human feeding studies of MZ-containing supplements suggests that MZ is less well absorbed than zeaxanthin. However, MZ is unusual in the diet and not reported in the plasma. Thus plasma responses may not reflect true absorption if it takes MZ longer to equilibrate with body tissues than the other xanthophylls and competition with zeaxanthin may lower the relative concentrations of MZ in plasma. Lastly, the effects of long-term feeding with both pure and food sources of the xanthophyll pigments on macular pigment optical density is compared and the importance of previous dietary intake on the effects of intervention is discussed.

Zeaxanthin: Meso-zeaxanthin: Lutein: Bioavailability: Macular disease

Introduction

Recent interest in zeaxanthin began with the discovery that it is a major carotenoid in the retinal pigment of the eye¹. The macula region of the eye has a particularly high concentration of pigment that is almost 1 mm within the macula and is visibly discernable as a yellow spot in the central retina². That is, the concentration of the macular pigment is three orders of magnitude above that in normal serum. Several workers have now shown that macular pigment is composed principally of three isometric

carotenoids, lutein, zeaxanthin and meso-zeaxanthin (MZ), present in the approximate proportions 2:1:1 in the retina^{1–4}. Landrum & Bone² found these pigments represented 72% of the total carotenoid content in the eye and other carotenoids are only present in very much smaller amounts. For example, Handelman and colleagues detected β -cryptoxanthin present but β -carotene, which is predominant in serum, was less than 1%⁵. These workers also noted that there were differences in the ratios of lutein and zeaxanthin in the central and peripheral retina, with

Abbreviations: AMD, age-related macular degeneration; GST, glutathione-S-transferase; MPOD, macular pigment optical density; MZ, meso-zeaxanthin; USDA, United States Department of Agriculture.

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zeaxanthin two times more concentrated than lutein in the fovea but lutein more dominant in the parafoveal region^{5,6}.

The stereochemical isomerism for both lutein and zeaxanthin poses interesting questions concerning potential functions of the different forms that are present. In fact macular lutein is composed solely of the most abundant natural stereoisomer ((3*R*,3'*R*,6'*R*)- β - ϵ -carotene-3,3'-diol) but zeaxanthin has been demonstrated to be primarily composed of two stereoisomers in the retina, the 3*R*,3'*R* form and the 3*R*,3'*S* (MZ) form (Fig. 1)^{2,4}. Evidence suggests that 3*R*,3'*R*-zeaxanthin is the major natural form and this is the only form found to exist in higher plants and most shellfish but all three optical isomers of zeaxanthin are found in the shrimp carapace, depot fat tissues in turtles and in the integument of twenty species of fish⁷. No MZ was detected in hens' egg yolk. As the MZ-containing foods are minor components of human diets, it is interesting to speculate why MZ should be a major form in macular pigment and what is the possible source of MZ in retinal tissue. Bone *et al.*⁴ and others^{8,9} suggested that dietary lutein may be the source of MZ in the eye and this has recently been confirmed in monkeys¹⁰. In the present paper it is proposed to (1) review the foods known to provide lutein and zeaxanthin, (2) relate the amounts of the xanthophyll pigments present in human diets with the concentrations found in human plasma, (3) examine the influence of different food and xanthophyll supplements on plasma concentrations and (4) examine plasma concentrations of xanthophyll carotenoids in studies of patients with macular disease and matched controls or where attempts have been made to relate plasma concentrations to macular pigment density.

There is as yet no direct evidence that either lutein or zeaxanthin will protect against macular disease either by prevention or treatment¹¹. However, the direct relationship between dosing with the different xanthophyll carotenoids and increases in macular pigment density¹²⁻¹⁴ may be evidence that dietary intake and plasma concentrations of xanthophyll carotenoids are inversely related to the risk of macular disease. Very recently a specific binding protein (GSTP1) for zeaxanthin has been isolated from human

retinas¹⁵. Dietary (3*R*,3'*R*)-zeaxanthin displayed high affinity for the protein with an apparent dissociation constant (K_d) of 0.33 μ M as also did (3*R*,3'*S*-*meso*)-zeaxanthin with only slightly less affinity (K_d 0.52 μ M) but (3*R*,3'*R*,6'*R*)-lutein displayed no affinity. The binding protein is a glutathione-*S*-transferase (GST). GST has been shown to exist in twelve different classes and within each class many sub-isoforms can exist. In man only one subclass of the Pi isoform is known to be expressed in human tissue, GSTP1. GSTP1 is widely expressed in human epithelial tissue and known to be in the retina but its cellular localisation was unknown until this report¹⁵. Using immunocytochemical techniques, the authors showed that high levels of GSTP1 are specifically localised in the parafoveal plexiform layers of the human macula and correlate well with the xanthophyll pigment distribution reported by Snodderly *et al.*¹⁶. The localisation of GSTP1 suggests that its primary role is to take up zeaxanthin from the blood. However, GSTP1 may possibly have other activities particularly since one of the isomers, (3*R*,3'*S*-*meso*)-zeaxanthin, is not normally present in blood. Another GST isoform, GSTA3-3, can catalyse a double-bond shift reaction in steroid biochemistry¹⁷. GSTP1 may be involved in analogous activity to convert (3*R*,3'*R*,6'*R*)-lutein to (3*R*,3'*S*-*meso*)-zeaxanthin as now shown to take place in monkeys¹⁰.

The identification and isolation of a specific binding protein for the two zeaxanthin diastereoisomers opens the possibility that activity-altering polymorphisms on the gene for GSTP1 may alter its binding properties, enzyme activity, interaction with intracellular mediators, etc, all of which may influence the risk of macular disease¹⁵. In such a situation, the availability of the non-dietary form of zeaxanthin, namely (3*R*,3'*S*-*meso*)-zeaxanthin, may be a timely addition to armoury of supplements to reduce the risk of macular disease.

The aetiology of macular disease is still only poorly understood and both genetic and environmental factors may be involved. One environmental factor that may be especially important is exposure to sunlight and history of exposure to blue light has been associated with an increased risk of macular disease¹⁸. The retina is highly active metabolically and has a higher blood flow than other tissues¹⁹. High metabolic activity and the simultaneous presence of light and O₂ will generate reactive oxygen species which in the absence of suitable antioxidants can damage PUFA that are rich in the photoreceptor outer segments²⁰. The macula in particular may be intermittently exposed to high light intensity and the high concentration of carotenoids may exert a protective role against oxidative damage³. Carotenoids are potent quenchers of singlet oxygen²¹ and lipid radicals^{22,23}. Lutein and zeaxanthin both absorb blue light with a maximum wavelength of about 450 nm and their location in Henle's fibre layer, just in front of the photoreceptors, is appropriate to this filter action. The importance of carotenoids in protecting the eye has been shown in macaque monkeys raised on a carotenoid-free diet for 5 years²⁴, where there was a loss of macular pigment and more drusen and other indicators of photic damage were found. One of the first intervention studies which found evidence that zeaxanthin might protect the retina against

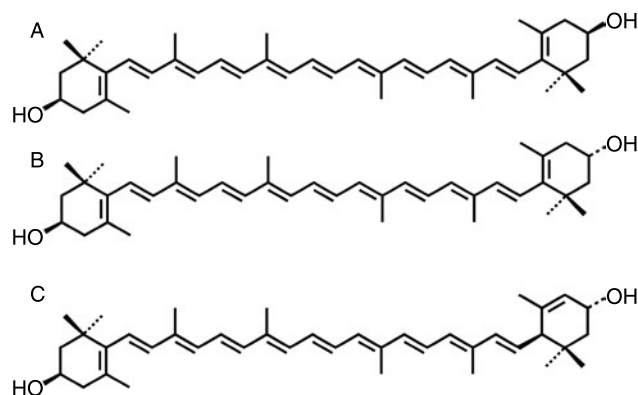


Fig. 1. The main xanthophyll carotenoids found in the macula. (A) Zeaxanthin (3*R*,3'*R*-dihydroxy- β , β -carotene); (B) *meso*-zeaxanthin (3*R*,3'*S*-dihydroxy- β , β -carotene); (C) lutein (3*R*,3'*R*,6'*R*-dihydroxy- β , ϵ -carotene).

light was reported by Dorey *et al.*²⁵. Quails were fed a carotenoid-free diet or with zeaxanthin (5 mg/kg) for 3 months. Birds were then exposed to intermittent white light for 28 h to induce photic damage to the retina. After 14 h in the dark, eyes were excised to determine zeaxanthin in the retina and to measure the number of apoptotic cells. The number of apoptotic rod and cone photoreceptor cells was significantly lower in the treated compared with the control birds. Furthermore those retinas containing more zeaxanthin, as assessed by HPLC, seemed better protected than those with less. Other xanthophyll-intervention studies to increase macular pigmentation are discussed later in the present review.

Dietary intake and bioavailability of xanthophylls

The predominant xanthophyll pigment in the diet is lutein and relatively high concentrations occur in dark green vegetables together with the carotenes, especially β -carotene. The intake of lutein (and zeaxanthin) in the USA is between 1 and 3 mg/d; white individuals tend to be nearer the bottom and blacks nearer the top of the intake spectrum^{26–28}. Analysis of survey data from US National Health Interviews between 1987 and 1992 suggests there was a significant decline in the consumption of dark green leafy vegetables that reduced lutein (and by proxy zeaxanthin) intakes particularly in white women²⁹. The content of zeaxanthin in the diet is usually much lower than either β -carotene or lutein and one recent estimate based on National Health and Nutrition Examination Survey (NHANES) data has suggested that the lutein:zeaxanthin ratio in the US diet is 5:1³⁰. Because of the low amounts of zeaxanthin in the diet, there are far fewer studies that have assessed its bioavailability than there are of lutein. However, as the structure of the two xanthophyll carotenoids is very similar, studies on lutein bioavailability may assist in understanding zeaxanthin bioavailability.

Bioavailability of lutein in vegetables

Several studies have examined the plasma responses in lutein and sometimes zeaxanthin concentrations to dietary and supplemental lutein (Table 1). The first experiment shown in the table³¹ illustrates plasma responses to lutein in a low- and high-vegetable diet, and to a third group receiving a low-vegetable diet and a lutein supplement. The authors reported that dietary lutein was absorbed 67% as efficiently as the supplemental lutein. Zeaxanthin was not assessed as intakes were not known but it should also be noted that plasma zeaxanthin concentrations increased more in the high vegetable group than the supplement group. The lutein supplement is reported as containing 1.4% zeaxanthin, i.e. approximately 126 μ g/d. In contrast, if the amount of zeaxanthin in vegetables is about 20% of the lutein, then there could be about 2 mg zeaxanthin/d in the high-vegetable diet.

In the second experiment shown in Table 1³², the authors tested the effects of the different processing methods on the bioavailability of lutein in spinach. However, none of the processing methods influenced relative bioavailability that ranged between 45 and 54% of the supplemental lutein in

the different groups. Thus bioavailability of lutein in spinach (50%) and in other green vegetables (67%) was of a similar magnitude in the two studies. However, the plasma response to the pure lutein supplement in the Castenmiller *et al.* study was two times higher than that reported by van het Hof *et al.*³¹ (0.116 compared with 0.053 μ mol/l per mg lutein, respectively), even though the supplemental lutein was from the same supplier and in both studies it was incorporated in the same carrier (a salad dressing). Thus it would appear that lutein absorption from a supplement taken with a vegetable-containing meal is impaired by the presence of the vegetables.

The study of Roodenburg *et al.*³³ showed the importance of dietary fat for lutein absorption. Although the authors found that 3 g fat was satisfactory for α - and β -carotene absorption (data not shown), there was a three-fold difference in lutein absorption between diets containing 3 and 36 g/meal. However, differences in fat intake are unlikely to explain any of the differences in the results in the other studies in Table 1 as all had a minimum of 30 g fat/meal but the plasma response to supplemental lutein in this experiment, even in the high-fat group, was relatively poor (0.048 μ mol/l per mg lutein) and comparable with the study by van het Hof *et al.*³¹. Also comparable with that study, volunteers received supplemental lutein together with 160 g vegetables. The vegetables contained almost no carotenoids (lutein < 0.02 mg/meal) but appeared to impair the plasma lutein response to the supplement.

The last study in Table 1 looked at possible interference in lutein absorption by lycopene in tomato purée or as the pure compound. Although chylomicron carotenoid responses³⁴ did suggest that supplements of lutein or lycopene decreased chylomicron lycopene or lutein respectively there was no evidence of interactions in the plasma carotenoid responses 3 weeks after dietary supplementation (Table 1). The relative plasma lutein responses to spinach (about 12 mg lutein/meal) alone or with tomato paste of supplemental lycopene were 0.075, 0.062 and 0.063 μ mol/l per mg respectively. In contrast the lutein response to supplemental lutein given with tomato paste (0.085 μ mol/l per mg) was 37% higher than that when the lutein was given as spinach with tomato paste (0.062 μ mol/l per mg). The higher response of supplemental lutein demonstrates the higher relative bioavailability of supplemental as opposed to food lutein but it was still not as high as when lutein was given without any vegetable food in the meal (0.116 μ mol/l per mg)³². These data therefore suggest that the presence of vegetables with or without carotenoids and including tomato paste depresses the plasma response to supplemental lutein.

In conclusion, although van het Hof *et al.*³¹ reported that the relative bioavailability of lutein from mixed vegetables was only 67% that of supplemental lutein, the response they obtained for supplemental lutein with vegetables (0.053 μ mol/l per mg) was low when compared with that obtained by Castenmiller *et al.*³² (0.116 μ mol/l per mg) who provided lutein as a supplement without food carotenoids. None of the responses in the other two Dutch papers^{31,33} achieved even half the response of Castenmiller *et al.*³² and even though those from the French study were somewhat higher, they too confirm that the response to supplemental lutein is depressed by food carotenoids.

Table 1. Changes in plasma carotenoid concentrations in subjects in industrialised countries following consumption of vegetable foods with or without pure lutein

Subjects and study	Miscellaneous descriptors	Intervention		Baseline		Outcome change			
		Type‡	Lutein (mg/d)	Lutein (µmol/l)	Zeaxanthin (µmol/l)	Lutein		Zeaxanthin†	
						µmol/l	µmol/l per mg lutein	µmol/l	%
Fifty-four healthy Dutch adults enrolled for a 4-week dietary intervention study ³¹	Subjects received low- (<i>n</i> 22) or high- (<i>n</i> 22) vegetable diets or a low-vegetable (<i>n</i> 10) diet plus 6 mg β-carotene and 9 mg lutein. Fat about 30% of total energy intake §	Low-vegetable (130 g/d)	2.7	0.19	0.048	0.068	0.025	0.018	+38
		High-vegetable (490 g/d)	10.7	0.21	0.056	0.40*	0.037	0.13	+23
		Low-vegetable and supplemental lutein (130 g/d)	12	0.18	0.049	0.64*	0.053	0.022	+45
Seventy healthy Dutch men and women enrolled for a 3-week vegetable intervention study ³²	Allocated to control group (<i>n</i> 10) or one of four spinach groups (<i>n</i> 12) or pure supplements of β-carotene and lutein (<i>n</i> 12). Fat 110 g/d, i.e. about 33 g/meal§	Control	0.5	0.224	–	0.005	–	–	–
		Whole leaf	12.6	0.215	–	0.68*	0.054	–	–
		Leaves minced	11.2	0.266	–	0.691*	0.062	–	–
		Leaves – enzyme liquefied	11.3	0.187	–	0.735*	0.065	–	–
		As above plus fibre	10.9	0.197	–	0.691*	0.064	–	–
Four groups of fourteen or fifteen non-smoking Dutch adults age 18–70 years (twenty-three men and thirty-seven women) ³³	During two 7 d periods with a low-fat meal containing 160 g low-carotenoid vegetables, subjects received one of four supplements in high- or low-fat spread	Low-fat, 3.1 g/meal	8.0§	0.180	–	0.158	0.02	–	–
		High-fat, 36 g/meal	7.6§	0.176	–	0.365	0.048	–	–
		Lutein supplement¶	6.6	0.218	–	0.765*	0.116	–	–
Twenty healthy French women age 21–39 years (some taking oral contraceptives). Subjects split into two groups but low and high responders split equally ³⁴	Cross-over study with 3-week periods and 3-week washout. At the start and when checked during the study, the basic meal contained (means) about 1.0 mg lutein/d, about 5.45 mg β-carotene/d and 40 g fat/d	TP and spinach	12.35	0.29	0.125	0.76*	0.062	0.06	+48
		TP and lutein in oil††	12.42	0.44	0.125	1.05*	0.085	0.115	+93
		Spinach	11.93	0.50	0.125	0.85*	0.075	0.105	+85
		Spinach and lycopene in oil	11.93	0.63	0.175	0.75*	0.063	0.095	+80

TP, tomato puree.

* $P < 0.05$.

† Zeaxanthin in meals eaten was not quantified, so change in concentration of zeaxanthin recorded as a percentage of baseline plasma concentration.

‡ For experimental details and sources of zeaxanthin, see Table 3 for more information.

§ Vegex lutein esters mainly palmitate (0S30; Quest International Ireland Ltd, Dublin, Republic of Ireland).

|| Low-vegetable diet described as being comparable with the average vegetable intake of the Dutch population.

¶ Crystalline lutein from marigold flowers containing 5% zeaxanthin (FloraGLO; Kemin Industries, Inc., Des Moines, IA, USA).

†† All-*trans*-lutein purified from marigold flowers contained 1.4% all-*trans*-zeaxanthin (Kemin Industries, Inc.).

Bioavailability of lutein and zeaxanthin from eggs

Six studies have assessed plasma responses to egg lutein and three for zeaxanthin, to give some information on bioavailability (Table 2). In the study by Handelman *et al.*³⁵ the equivalent of 1.3 eggs was fed daily with two different fats (maize oil and beef tallow as two-thirds of the total dietary fat). Analyses of egg yolks by the authors indicated the amounts of lutein (0.38 mg) and zeaxanthin (0.28 mg) fed daily. After about 32 d the increases in plasma lutein and zeaxanthin in the recipients in the tallow- and maize oil-fed groups respectively were 0.094 and 0.134 ($\mu\text{mol/l}$ lutein) and 0.068 and 0.056 ($\mu\text{mol/l}$ zeaxanthin). There might have been a slightly better lutein response in the maize oil than the tallow group but the mean responses were calculated to assess the increases of the two carotenoids per mg dose. The average plasma responses for lutein (0.3 $\mu\text{mol/l}$ per mg) and zeaxanthin (0.221 $\mu\text{mol/l}$ per mg) were more than double any of the responses by lutein-in-oil groups in the studies described in Table 1. Unfortunately, there was no lutein-in-oil control group with which to compare the egg-lutein response. The latter is desirable because of the large variation in results both between and within laboratories. However, the apparently high bioavailability of the yolk xanthophylls is probably due to the small amount of xanthophyll supplement (0.66 mg/d) thoroughly solubilised in the high content of egg phospholipid. By comparison, the amounts of pure lutein given in the vegetable-feeding studies ranged from 6 to 12 mg/d.

The second study shown in Table 2 also lacked a pure lutein control group with which to assess relative bioavailability³⁶. The authors recruited two groups of subjects to which they fed one control or one lutein-enriched egg per d for 8 weeks. The mean lutein content of the control eggs was about half that of the eggs used by Handelman and colleagues and the amount of xanthophyll given per d was about one-third, and there was no change in plasma lutein concentration. However, in the group that received the lutein-enriched eggs, there was an increase in plasma lutein of 0.110 $\mu\text{mol/l}$ per mg egg lutein. Thus the response was similar to that produced by pure lutein in oil³² (see Table 1) but much lower than that reported by Handelman *et al.*³⁵. Of the possible reasons for the lower response, fat intake was not mentioned by Surai *et al.*³⁶ and in an 8-week study, there may have been changes in the intake or type of vegetables consumed during that period. Neither fat intake nor the background dietary intake was controlled.

The third study in Table 2 included four intervention groups³⁷. The supplements (powdered lutein, lutein esters, chopped spinach and lutein-enriched egg providing about 6 mg lutein/d) were incorporated into a frittata using egg white in those groups where egg was not the supplement. Supplements were fed for 10 d and there was 19–20 g oil with each meal. The results showed that egg lutein was three times more bioavailable than the pure lutein preparations; however, the lutein response in all groups was poor (compare with Table 1). Both pure lutein groups in the study of Chung *et al.*³⁷ showed similar responses (0.022 and 0.019 $\mu\text{mol/l}$ per mg) and although both spinach and egg lutein appeared more bioavailable than the pure lutein

preparations (0.032 and 0.067 $\mu\text{mol/l}$ per mg respectively) there were no significant differences in the responses by the different groups. The reason for the poorer overall responses may be a combination of the short intervention and the lower dietary fat than used in most studies. It has been shown that 17–18 d is necessary for both lutein and zeaxanthin to reach more than 90% of plateau plasma concentrations^{30,38}. In this study³⁷ egg lutein appeared more bioavailable than both spinach lutein and pure lutein but very little information was supplied on the pure lutein used.

Three more recent studies are also shown in Table 2^{39–41}. In all three studies there are plasma responses to egg lutein > 0.3 $\mu\text{mol/l}$ per mg, and only in one study where one of the lutein treatments provided 826 $\mu\text{g/d}$ for 12 weeks was a lower response of 0.136 $\mu\text{mol/l}$ per mg obtained⁴⁰. It could be argued that it might take longer for the plasma concentration to reach its plateau in response to large doses but Clark *et al.*³⁹ gave 600 μg egg lutein per d for only 3 weeks and the total response (lutein and zeaxanthin combined) was 0.367 $\mu\text{mol/l}$ per mg. So the reason for the relatively poor response to the high-xanthophyll-containing eggs used by Wenzel *et al.*⁴⁰ is not immediately apparent.

The last two studies in Table 2 also provided information on the plasma zeaxanthin responses to egg zeaxanthin intake. Two of the treatments were very similar: 94 $\mu\text{g/d}$ ⁴¹ and 114 μg zeaxanthin/d⁴⁰ but gave responses of 0.160 and 0.421 $\mu\text{mol/mg}$ respectively while the higher amount given by the latter workers (312 $\mu\text{g/d}$) gave a response of 0.151 $\mu\text{mol/l}$ per mg. There was some evidence from the lutein responses in the latter study⁴⁰ that higher doses may take longer to reach a steady state in the plasma but even the mean plasma concentration in response to the high dose (312 μg , 0.104 $\mu\text{mol/l}$) was lower than that achieved with the low dose (114 μg egg zeaxanthin/d, 0.149 $\mu\text{mol/l}$).

In conclusion, four out of the six studies indicated that that egg lutein is three to four times more bioavailable than pure lutein^{35,39–41}. There were slightly poorer responses in those studies where relatively high intakes of lutein were used^{36,40} or where the period of supplementation was shorter than the 3 weeks probably needed to reach equilibrium³⁷. In the case of egg zeaxanthin, all responses were > 0.150 $\mu\text{mol/l}$ per mg with one particularly high response of 0.42 $\mu\text{mol/l}$ per mg. Some of the variability in the plasma response to egg lutein and zeaxanthin may be due to the very large variability in the content of the xanthophyll pigments in eggs where a 10-fold range was reported for lutein and a 4–5-fold range in the case of zeaxanthin⁴⁰. Unfortunately, apart from the one study which was only 10 d, none of the egg studies included a pure lutein or zeaxanthin control but the frequency of high plasma responses to egg xanthophyll supplements is a clear indication of the high bioavailability of xanthophyll pigments in egg yolk.

Bioavailability of pure xanthophyll supplements

Pharmacokinetics of lutein and zeaxanthin

Two recent studies by Hartmann *et al.*³⁰ and Thurmann *et al.*³⁸ suggest that the pharmacokinetics of lutein and zeaxanthin are similar (Table 3; Fig. 2). In these two

Table 2. Changes of plasma xanthophyll concentrations after the consumption of eggs

Subjects and study	Experimental details	Intervention		Baseline (μmol/l)		Change – post-treatment†			
		Type	Amount	Lutein	Zeaxanthin	Lutein		Zeaxanthin	
						μmol/l	μmol/l per mg lutein	μmol/l	μmol/l per mg Zeaxanthin
Eleven American, non-smoking, moderately hypercholesterolaemic men (<i>n</i> 6) and women (<i>n</i> 5) ³⁵	Two diets containing 29–33% energy as fat (mainly beef fat or maize oil (20%)) for 4-5 weeks with 2-week washout	1.3 eggs with 20% of energy as beef fat‡	380 μg lutein and 280 μg zeaxanthin (plus 4.7 μg β-carotene + 344 mg cholesterol)/d	0.333	0.048	0.094*	0.247	0.068**	0.242
		1.3 eggs with 20% of energy as maize oil‡		0.269	0.049	0.134*	0.352	0.056**	0.2
Twenty Scottish men and twenty women. Smoking habits not described ³⁶	Stratified by age and sex, then randomly assigned to receive one egg/d for 8 weeks. Fat intake with egg uncontrolled	Control	0.12 mg lutein/egg	0.21	–	0	–	–	–
		Eggs enriched with Se, lutein and DHA§	1.91 mg lutein/egg	0.24	–	0.21***	0.110	–	–
Ten healthy American men (age 26–75 years) completed four treatments in random order. Smoking not permitted during course of study ³⁷	All doses consumed with test meal of frittata containing 19–20 g fat for 10 d. Interventions separated by 2 weeks on low-carotenoid diet	Lutein	6 mg/d	0.158	–	0.13***	0.022	–	–
		Lutein ester¶	5.5 mg lutein/d	0.131	–	0.107***	0.019	–	–
		Spinach	6 mg lutein/d	0.136	–	0.19***	0.032	–	–
		Lutein-enriched eggs††	6 mg/d	0.125	–	0.404***	0.067	–	–
Twenty hyper and twenty hypo responders to dietary cholesterol. Twenty men and twenty women (premenopausal) equally distributed in the groups ³⁹	Cross-over study, subjects consumed one egg or placebo for 30 d, 3-week washout, second dietary period	Egg. Placebo was egg substitute that contained 568 μg β-carotene	600 μg lutein and zeaxanthin combined	Placebo Male 0.375 Female 0.505	Placebo Male 0.08 Female 0.13	Egg Male 0.63 Female 0.75	0.367‡‡	Egg Male 0.095 Female 0.155	
Seven men and twenty-six women age > 60 years ⁴¹	Cross-over study, one egg/d or egg substitute for 5 weeks with 4-week washout	Egg or egg substitute	Lutein 143 μg/d; zeaxanthin 94 μg/d	0.164	0.042	0.210	0.322	0.057	0.160
Twenty-four women, age 24–59 years recruited for intervention changes shown at 12 weeks ⁴⁰	Three groups: pill (sugar), or eggs with low and high xanthophyll content	1. Pill	Zero	0.505	0.096	0.457	–	0.080	–
		2. Six eggs/week: lutein 331 μg/yolk; zeaxanthin 133 μg/yolk	Lutein 284 μg/d; zeaxanthin 114 μg/d	0.389	0.101	0.477	0.310	0.149	0.412
		3. Six eggs/week: lutein 964 μg/yolk; zeaxanthin 364 μg/yolk	Lutein 826 μg/d; zeaxanthin 312 μg/d	0.428	0.057	0.540	0.136	0.104	0.151

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Final minus baseline concentrations expressed as μmol/l and as μmol/l per mg daily intake of respective carotenoids.

‡ Commercial chicken eggs purchased in autumn 1997 in the Boston metropolitan area. Analyses done on eggs classified as large, except one which was extra large.

§ Chickens were fed marigold extract, and xanthophyll esters in egg were > 85% lutein and zeaxanthin was the other main component.

|| Only described as vitamin powder.

¶ Obtained from Cognis, Nutrition and Health.

†† Provided by Kemin Industries Inc. (Des Moines, IA, USA).

‡‡ Responses calculated from difference between egg and placebo arms of the trial after lutein and zeaxanthin combined and averages of males and females, hyper- and hypocholesterol groups calculated.

Table 3. Long-term supplementation studies to measure plasma responses and kinetics of lutein, zeaxanthin and meso-zeaxanthin uptake and elimination

Subjects and study	Experimental details	Xanthophyll dose	Baseline blood measurements ($\mu\text{mol/l}$)			Changes – post-supplementation					
			Lutein	Zeaxanthin	Meso-zeaxanthin	Lutein		Zeaxanthin		Meso-zeaxanthin	
						$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg	$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg	$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg
Three male non-smokers ⁴⁴	Marigold extract prepared in olive oil (10 mg/4 ml)	10 mg/d for 21 d	0.200	–	–	0.80	0.08	–	–	–	–
	Zeaxanthin isolated from guji (a Chinese fruit) and extract prepared in olive oil (10 mg/4 ml)	10 mg/d for 21 d	–	0.03	–	–	–	0.12	0.012	–	–
One subject, age 53 years, male ^{11,12}	Source marigolds. Taken with food and no control of fat intake	30 mg lutein in 2 ml rapeseed oil for 140 d	0.150	–	–	1.59	0.053	–	–	–	–
One subject, age > 42 years ^{11,12}			0.165	–	–	2.215	0.074	–	–	–	–
One subject age 53 years, male ¹²	Source flavobacteria. Taken with food and no control of fat intake	30 mg zeaxanthin for 120 d	–	0.097	–	–	–	0.463	0.015	–	–
One subject, age 18 years ¹²		30 mg zeaxanthin for 60 d	–	0.086	–	–	–	0.394	0.013	–	–
Seventeen female and four male subjects age, 19–59 years ¹²	Source marigolds. Taken with food and no control of fat intake	2.4 mg lutein (as esters) for 180 d	0.245	–	–	0.239	0.099	–	–	–	–
Two female subjects, age > 18 years ¹²	Lutein esters from marigold. Taken with food and no control of fat intake	5 mg lutein for 120 d	–	–	–	0.74*	0.098†	–	–	–	–
Eight females and four males, age 19–60 years ¹²		20 mg for 120 d	–	–	–	1.14*	0.178†	–	–	–	–
Twenty healthy adults, ten (five male and five female) allocated to each treatment ^{30,‡}	Zeaxanthin finely dispersed in maize starch as a beadlet formulation. Taken in the morning, fat intake not specified. Post-supplementation concentration on day 42	1 mg (1.76 μmol) zeaxanthin hard gel capsules	–	0.051	–	–	–	0.17	0.17	–	–
		10 mg (1.76 μmol) zeaxanthin hard gel capsules	–	0.045	–	–	–	1.01	0.101	–	–
Nineteen healthy adults, eight (four male and four female) allocated to each lutein treatment, three controls ^{38,‡}	Lutein from marigolds, formulated in beadlets, hard gelatin capsules contained 4.1 mg <i>all-E</i> -lutein and 3.4 mg <i>all-E</i> -zeaxanthin. Capsules taken daily with 150 ml water and light breakfast for 42 d	One capsule containing 4.1 mg lutein and 0.34 mg zeaxanthin	0.140	0.052	–	0.425	0.104	0.033	0.097	–	–
		Five capsules containing 20.5 mg lutein and 1.7 mg zeaxanthin	0.148	0.035	–	1.320	0.064	0.113	0.066	–	–

Table 3. Continued

Subjects and study	Experimental details	Xanthophyll dose	Baseline blood measurements ($\mu\text{mol/l}$)			Changes – post-supplementation					
			Lutein	Zeaxanthin	Meso-zeaxanthin	Lutein		Zeaxanthin		Meso-zeaxanthin	
						$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg	$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg	$\mu\text{mol/l}$	$\mu\text{mol/l}$ per mg
Eight male and two female American subjects (ages 21–58 years) ⁴⁵ §	One capsule per d with meal for 180 d	Gelatin capsules containing 14.9 mg meso-zeaxanthin,	0.305	0.097	–	0.075	0.014	0.167	0.119	–	–
One male subject ⁴⁵ §	One capsule per d with meal for 42d	5.5 mg lutein and 1.4 mg zeaxanthin	0.229	0.044	0	0.01	0.002	0.043	0.031	0.044	0.003
One male subject ⁴⁵ §	One capsule per d with meal for 42 d		0.148	0.021	0	0.306	0.056	0.133	0.095	0.145	0.010
Nineteen healthy European subjects (ten men and nine women age 21–46 years) ⁴⁶ §	One capsule per d with meals for 21 d	Gelatin capsules containing 8 mg meso-zeaxanthin, 10.8 mg lutein and 1.2 mg zeaxanthin	0.275	0.054	0	0.882	0.082	0.160	0.133	0.209	0.026
100 subjects with AMD and eight healthy subjects (sixty-two men and forty-six women) ⁴⁷	One capsule Ocuvite Lutein™ daily for 6 months (manufactured by Bausch & Lomb, Berlin)	12 mg lutein and 1 mg zeaxanthin both as esters; 120 mg vitamin C, 17.6 mg vitamin E, 10 mg Zn and 40 μg Se	0.278	0.142	–	0.795	0.066	0.010	0.010	–	–

* Final concentration shown, as baseline values not given.

† Serum concentration change/mg calculated by assuming baseline lutein to be 0.25 $\mu\text{mol/l}$.

‡ Source of xanthophyll was Roche Chemicals Ltd.

§ Non-esterified mixture of meso-zeaxanthin, lutein and zeaxanthin obtained from Industrial Organica SA, Monterrey, Mexico.

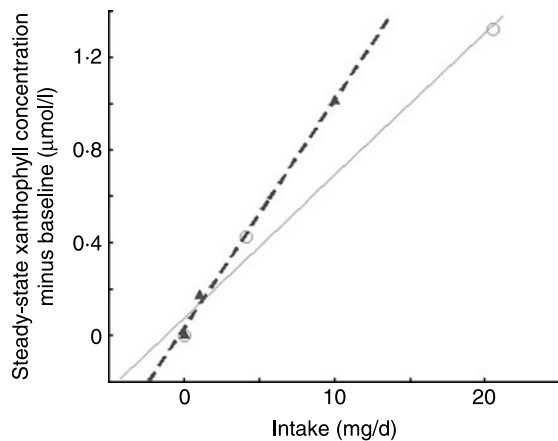


Fig. 2. Steady state plasma concentrations of zeaxanthin (▲) and lutein (○). Amounts shown were fed for 42 d. Regression lines for the separate carotenoids indicate plasma responses of 0.10 and 0.075 μmol per mg daily for zeaxanthin³⁰ and lutein³⁸ intakes respectively.

studies^{30,38}, supplements of zeaxanthin (1 and 10 mg) and lutein (4 and 20.5 mg) were taken daily in a hard-gelatin capsule by small groups of men and women for 42 d. More than 90% of steady-state concentrations were achieved around day 17–18 for both xanthophylls while the half-life for disposal of xanthophylls after the supplementation appears to be rapid initially (within the period of observation) but slowed down considerably when concentrations approached baseline. Hence disposal half-lives of lutein and zeaxanthin were estimated at 5–7 d and 10 d respectively. The small difference between these measurements are probably related to the periods during which the falling concentrations of the respective xanthophylls were measured, namely 38 d (lutein) and 48 d (zeaxanthin)³⁸. Others using depletion studies to estimate terminal half-lives of lutein have reported 33–61 d⁴² and 76 d⁴³. What is especially interesting, however, in these studies was that the plasma responses at plateau of zeaxanthin and lutein lay on very similar concentration response lines (Fig. 2), possibly suggesting that the formulations used by these workers was of a similar bioavailability.

Bioavailability of zeaxanthin

Other studies on the bioavailability of zeaxanthin are also shown in Table 3. Khachik *et al.*⁴⁴ reported separate supplementation studies with lutein and zeaxanthin isolated from marigold flowers and Chinese berries (*Fructus lycii*) respectively. The extracted carotenoids were prepared as suspensions in olive oil (10 mg per 4 ml) and were given in separate experiments to the same three subjects for 21 d. In the case of lutein, plasma concentrations rapidly increased from about 0.200 to between 0.8 and 1.0 $\mu\text{mol/l}$ (5–6-fold) and approached plateau concentrations around day 21. The lutein results are comparable with those shown in Fig. 2. In the case of zeaxanthin, even though the same dose was given, the increase was far less than for lutein. Starting plasma concentrations of zeaxanthin were about 0.03 $\mu\text{mol/l}$ and only increased to about 0.12 $\mu\text{mol/l}$ (far less than the

response in Fig. 2). These data suggest that the efficiency of zeaxanthin absorption from an oily suspension is only about 10% that of lutein.

More recently, two studies have been done in which the plasma response has been obtained to a combined supplement of non-esterified lutein, zeaxanthin and MZ suspended in soyabean oil in gelatin capsules. The results for lutein and zeaxanthin are discussed first and those for MZ later. In both cases the source of the material was the same (Industrial Orgánica SA, Monterrey, Mexico) but the composition differed. In the first study, two male subjects took the supplement which provided a mixture of lutein, zeaxanthin and MZ (5.5:1.4:14.9 mg/d or 25:6:68% respectively) daily with a meal for 6 weeks⁴⁵. In the case of both plasma lutein and zeaxanthin concentrations, there were very large differences in the response to the two xanthophylls by the two subjects. In one subject the plasma responses were similar to those seen in Fig. 2 while in the other subject, responses to both carotenoids were very poor. In the second study there were nineteen volunteers who took capsules containing 20 mg lutein, zeaxanthin and MZ in the proportions 54:6:40 for 21 d⁴⁶. The average plasma responses for lutein (0.882 $\mu\text{mol/l}$) and zeaxanthin (0.16 $\mu\text{mol/l}$) were reasonably close to those shown in Fig. 2.

In the last study shown in Table 3, most of the subjects (n 100) exhibited features of age-related macular degeneration (AMD). In addition many had hypertension (n 63), diabetes (n 11) or coronary vascular disease (n 20); there were thirty subjects currently taking lipid-lowering drugs, five smokers and three had recently had a stroke. In spite of all these indications of chronic disease, the response to 12 mg lutein/d (0.795 $\mu\text{mol/l}$) almost exactly corresponds with that shown in Fig. 2. In contrast there was almost no response to the 1 mg zeaxanthin/d (0.010 $\mu\text{mol/l}$). Thus there is similarity in the plasma zeaxanthin responses from the Roche Chemicals and Industrial Organica preparations except in one of the two subjects reported by Bone *et al.*⁴⁵; however, from the material prepared from guji⁴⁴, flavobacteria^{12,13} and by Bausch & Lomb⁴⁷ there were very low plasma responses (Fig. 3).

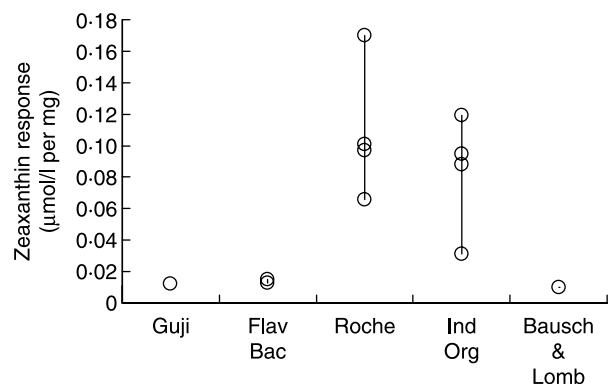


Fig. 3. Zeaxanthin steady-state plasma responses from different preparations. All preparations were fed for a minimum of 21 d. For experimental details, sources of zeaxanthin and source of the data shown in the figure, see Table 3. Flav Bac, flavobacteria; Roche, Roche Chemicals Ltd; Ind Org, Industria Organica SA.

All the studies shown in Table 3 fed the xanthophyll supplements for a minimum of 21 d, so the time of supplementation should have been adequate to achieve steady-state concentrations of both lutein³⁸ and zeaxanthin³⁰. Two studies fed the pigments for the minimum time of 21 d^{44,46}, while all the other studies were much longer. There were other differences in the sources of manufacture and in the oils used to suspend the xanthophylls that might influence the bioavailability of zeaxanthin, and the amount of fat taken with the xanthophylls was not always specified. Results suggest the plasma response to lutein is strongly related to the intake of dietary fat³³ and it is possible that different fats may also influence bioavailability³⁵. The number of subjects in two of the studies on zeaxanthin was very small. Furthermore, the dietary habits or history of these subjects was not described. There is evidence suggesting that a high intake of vitamin A-containing foods strongly depresses the absorption of stable-isotope-labelled β -carotene⁴⁸, and in dietary intervention studies to increase macular pigment optical density (MPOD), the non-responders to a lutein supplement were those whose previous intake of lutein and zeaxanthin was four times higher than that of those who later responded¹⁴. Thus previous carotenoid intake can markedly affect response to a carotenoid supplement, and the variable responses to pure lutein described in Table 1 indicate that there is much that is still not known about the absorption of lutein.

Bioavailability of the stereoisomers of zeaxanthin

There is almost no work reported in the literature on the comparative bioavailability of the three stereoisomers of zeaxanthin. In chickens, 3*R*,3'*R*-zeaxanthin is slightly better absorbed than is the 3*S*,3'*S*-diastereoisomer, but the absorption of the meso (3*R*,3'*S*) form is only 40% of that of the chiral forms. However, when a racemic mixture of all three isomers was fed, MZ was better absorbed⁴⁹. Bone *et al.*⁴⁵ reported the serum concentrations of natural (3*R*,3'*R*) zeaxanthin and MZ in two human subjects who were given a mixed suspension of lutein, zeaxanthin and MZ in soyabean oil in gelatin capsules (5.5:1.4:14.9 mg/d respectively; Industrial Orgánica SA, Monterrey, Mexico) that was taken daily with a meal for 6 weeks. The serum concentrations of zeaxanthin and MZ in one subject increased over this period by 0.043 and 0.044 $\mu\text{mol/l}$ respectively, and in the second by 0.133 and 0.145 $\mu\text{mol/l}$, i.e. the increase in the serum concentrations were approximately equimolar in both subjects for the two isomers of zeaxanthin. As the relative amount of the 3*R*,3'*S* (*meso*) was ten times greater than that of the 3*R*,3'*R* form of zeaxanthin in the supplement, this suggests that the plasma uptake of MZ in man is only 10% of the natural form of zeaxanthin in the diet.

In the second of the two studies using the mixed supplement, the uptake of MZ into the plasma was slightly greater⁴⁶. The change in the plasma MZ concentration after 21 d was 0.026 $\mu\text{mol/l}$ per mg whereas uptake in the above study was 0.003 and 0.01 $\mu\text{mol/l}$ per mg for the two volunteers⁴⁵. These results for MZ should be interpreted cautiously. The apparently lower response to MZ than

zeaxanthin may be because MZ is not a normal dietary component and is not normally found in serum. The serum carotenoids are also found throughout the body in fat deposits, thus any new carotenoid entering the body is likely to be distributed throughout the body before noticeable increases in plasma concentrations occur. It is also possible that MZ is selectively taken up by the optical tissues for incorporation into the macula although it is unlikely that this would have a major impact on plasma concentrations of MZ. The average amount of MZ per human donor eye taken within 24 h of death was reported to be 7.7 ng⁸ while even in the supplemented subject with the lower plasma concentration of MZ (0.044 $\mu\text{mol/l}$ ⁴⁵), the amount of MZ in the plasma was about 120×10^3 ng assuming 5 litres to be the blood volume. Thus the relatively lower concentrations of plasma MZ than zeaxanthin following receipt of the mixed supplement may be due to poor bioavailability of MZ and/or a greater uptake of MZ than zeaxanthin into fatty tissues in the body. A resolution to these uncertainties will only be obtained when some stable-isotope-labelled MZ is available and its fate determined following supplementation.

Zeaxanthin in foods

As previously indicated⁷ very few foods contain MZ. Fish skin is the most likely dietary source but there is no information in food tables⁷. Zeaxanthin is also a minor dietary component and the amount in the diet will depend very much on the specific foods consumed. United States Department of Agriculture (USDA) Nutrition Coordinating Center food composition tables⁵⁰ provide zeaxanthin concentrations in twenty-two commonly-eaten foods and indicate that yellow maize (canned) is by far the richest vegetable source of zeaxanthin in the diet (528 $\mu\text{g}/100$ g); the concentration even exceeding lutein (356 $\mu\text{g}/100$ g). However, the composition of maize can be variable. The lutein and zeaxanthin contents of the maize used by Hammond *et al.*¹⁴ were 270 and 200 $\mu\text{g}/100$ g respectively. Thus the zeaxanthin content was only half that reported by the USDA. Vegetables and fruits in the USDA report contain on average only one-quarter of the zeaxanthin in maize (125 and 129 $\mu\text{g}/100$ g respectively) but differed considerably in their content of lutein (3904 and 143 $\mu\text{g}/100$ g respectively). Thus the lutein:zeaxanthin ratio in vegetables (36:1) and fruits (3.5:1) differs considerably and fruit consumption rather than vegetables is likely to have a larger impact on zeaxanthin consumption in humans. It is reported that the lutein:zeaxanthin ratio in the diet in the USA is 5:1 based on the USDA data³⁰ while in Europe reports indicate 5.5:1 in fresh fruit and vegetables consumed in a large Spanish survey⁵¹, 15:1 in the diet of young, type 1 diabetics and 8:1 in that of age- and sex-matched controls⁵².

Populations consuming yellow maize as a staple food are likely to have the highest consumption of zeaxanthin. Plasma concentrations of lutein and zeaxanthin in Northern Chinese adults where the staple cereal was yellow maize were 1.0 (SD 0.4) and 0.13 (SD 0.06) $\mu\text{mol/l}$ respectively⁵³. Surprisingly, although the plasma lutein values in the Chinese were considerably higher than those found in European or American samples⁵⁴, the zeaxanthin concentrations were not very different from mean values reported

in Europe or America (0.07–0.09 $\mu\text{mol/l}$)^{27,52} but this may be attributable to the method of analysis used¹ and not to green vegetables diluting out the zeaxanthin. (It should be noted that the Chinese samples were measured using a liquid chromatography method that did not separate lutein and zeaxanthin⁵⁵. Using this method zeaxanthin is normally concealed within the lutein peak; however, in the Chinese samples zeaxanthin was much more obvious and appeared as a smaller peak or shoulder on the side of the lutein peak. It was integrated by the peak-skimming method and that may have underestimated the size of the zeaxanthin contribution.) The blood was taken in the spring when the main vegetable being consumed was Chinese cabbage and the main source of lutein was green leaves from garlic sprouts.

Sommerburg *et al.*⁵⁶ measured zeaxanthin and other carotenoids in thirty-six commonly consumed fruits and vegetables but do not report concentrations, only ratios of lutein and zeaxanthin. Several reports agree that the fruits providing the largest amounts of zeaxanthin are orange peppers, oranges (both fruit and juice), honeydew melon, mango and peaches^{44,56} and there are particularly large amounts in persimmons (488 $\mu\text{g}/100\text{g}$)⁵⁰.

Meso-zeaxanthin content of chicken eggs in Mexico

One of the foods identified by Sommerburg *et al.*⁵⁶ and the USDA⁵⁰ as being relatively rich in zeaxanthin was chicken eggs. This is because in many parts of the world, including the USA, the poultry industry uses xanthophylls for broiler and egg pigmentation. However, this is not an unnatural situation since when chickens are allowed to forage naturally, they will acquire lutein and zeaxanthin from grasses and other vegetation, to deposit in the yolk⁴⁹. In young birds the carotenoids are deposited in the flesh but with sexual maturity, the carotenoids are mobilised from the tissues and transferred to the reproductive organs and the eggs. In laying hens most of the absorbed carotenoids are transferred to the eggs⁴⁹.

Xanthophyll carotenoids in chicken eggs also contain a relatively high proportion of zeaxanthin where lutein:zeaxanthin ratios of about 1.4:1 have been reported^{35,50,56}. However, there is a wide variation in xanthophyll pigments in eggs^{35,36,40,50,57,58}, with values ranging from 1.4⁵⁰ to 41.5⁴⁰ $\mu\text{g}/\text{g}$ egg yolk for zeaxanthin and 1.9⁵⁰ to 129⁵⁷ $\mu\text{g}/\text{g}$ egg yolk for lutein assuming a yolk size of 17 g. The relatively high zeaxanthin:lutein ratio in eggs suggests that the chicken specifically concentrates zeaxanthin in the egg, as the marigold extract fed currently to most laying hens generally contains < 10% zeaxanthin as would any grasses consumed in the wild. A maize feed would contribute a higher proportion of zeaxanthin than marigold extract or wild grasses, but the extent to which selective uptake of zeaxanthin occurs requires more information on the absolute amounts of zeaxanthin in the chicken feed.

The amount of pigment used in the feed of laying hens is determined by the density of the yellow or yellow-orange colour of the yolk that is preferred by the consumer. In the USA, the colour approximates 7–8 as measured by the Roche fan⁵⁹ whereas in Mexico consumers prefer a deeper colour, 11–13. Over the last 10 years, Industrial Organica

SA (Monterrey, Mexico) has supplied pigment to 25–28% of the combined broiler and layer pigment market in Mexico (personal communication, José Torres, Industrial Organica SA 2006). The main pigment used for layers in Mexico is Yemix that comprises 70% xanthophyll concentrate (of which a half is MZ) and 30% capsanthin, although more recently the capsanthin has been replaced by lutein and some canthaxanthin. We recently measured the xanthophyll pigments in a lyophilised sample of Mexican chicken egg yolks (Table 4) obtained from birds that had been given feed containing 13–14 parts per million Yemix® of the newer composition. The analysis indicated that the xanthophyll concentration in the yolk was about 10 $\mu\text{g}/\text{g}$, containing approximately 10% MZ. The lutein:total zeaxanthin ratio was 1.7.

Mexico is the third largest consumer of eggs in the world. The annual per capita consumption of eggs increased from 13.1 kg in 1995 to 18.3 kg in 2002 and most of the eggs consumed in Mexico are supplied by the domestic market⁶⁰. Assuming an egg weighs 60 g⁵⁷, egg consumption in Mexico is approximately one egg/person per d. That is, in 2002, 25–28% of the Mexican population (26–29 million Mexicans) would have received about 170 μg (if the egg yolk is 17 g) xanthophyll pigments per d from eggs, of which about 34 μg was 3R,3'R-zeaxanthin, of which 35% was MZ. In the USA, eggs probably contribute little to the zeaxanthin intake of most Americans⁶¹ as light-coloured yolks are preferred in the USA. Mexican Americans living in the USA are reported to consume 1.1–1.2 mg lutein/d across the ages 40–80 years²⁸. If the average lutein:zeaxanthin ratio in food in the USA is 5:1³⁰, the average intake of zeaxanthin is about 200 $\mu\text{g}/\text{d}$. Thus about 28 million Mexicans may have consumed about an additional 34 μg mixed zeaxanthin isomers compared with other Americans. While numerically the additional amount of xanthophyll pigment may be small, the three to four times better bioavailability of egg compared with vegetable

Table 4. Xanthophyll composition of yolk lyophilisate from chickens fed Yemix®*

Xanthophyll†	% of total area	$\mu\text{g}/\text{yolk}$	ppm (yolk)
Lutein	34	57.74	3.4
3R,3'R-Zeaxanthin	12.8	21.74	1.3
Meso-zeaxanthin	7.2	12.22	0.7
Canthaxanthin	16	27.17	1.6
β -Cryptoxanthin	10	16.98	1
Unknown	20	33.96	2
Total	100	169.8	10

ppm, Parts per million.

* Yemix® (Industrial Orgánica SA, Monterrey, Mexico) contains 60–65% mixed zeaxanthin isomers (mainly meso-zeaxanthin), 25–30% lutein and a small amount of canthaxanthin and was fed to chickens at 13–14 ppm.

† The method used was the standard operating procedure employed in Industrial Orgánica SA (PEO-ACC-005, revision 01, 2 May 2005). In brief, samples of lyophilisate (about 2 g) were sonicated briefly in 30 ml of solvent mixture (hexane–acetone–ethyl alcohol–toluene, 100:70:60:70, by vol.) to suspend the sample and 2 ml 40% methanolic KOH was added in a 100 ml volumetric flask. The solution was mixed, left at 4°C overnight, heated at 56°C for 20 min, cooled to room temperature and the contents made up to 100 ml by the addition of 30 ml hexane and 10% aqueous anhydrous sodium sulfate. After vigorous mixing, the flask was again stood in darkness at 4°C overnight (about 20 h) to allow the supernatant fraction solution to clarify. Absorbance was then measured at 474 nm against a hexane blank. The composition of the carotenoids was then measured as described elsewhere⁴⁵.

xanthophylls may be equivalent to doubling the dietary intake of xanthophyll pigments, so reducing the potential risk of macular disease, but currently there is no evidence on the comparative risks of Mexicans to macular disease in the two countries available or of measurements of blood xanthophyll concentrations in Mexico. However, in a recent study, workers gave six eggs/week for 12 weeks to adult females (Table 2) and reported a significant increase in MPOD⁴⁰. The xanthophyll content of the eggs in this study was double that found in the Mexican egg lyophilisate but the study shows the potential advantage of a daily intake of eggs.

Serum zeaxanthin and lutein concentrations, macular pigment optical density and age-related macular disease

The very high concentrations of lutein and the two stereoisomers of zeaxanthin in the macula has stimulated much speculation as to the function of these compounds^{1,3,4}. It is generally believed that the macular pigments by their presence in the photoreceptor axons¹⁶ may filter out blue light (about 400–500 nm) and shield posterior tissues such as the photoreceptor outer segment from the photo-oxidative damage caused by blue light⁶². The retina is vulnerable to oxidative damage for several reasons: constant exposure to light and high levels of oxygen, the lipid bilayer of the photoreceptor outer segments contain high concentrations of PUFA, and the retina and the retinal pigment epithelium have an abundance of photosensitisers⁶. The antioxidant activity and free-radical-scavenging properties of macular pigment may limit photo-oxidative damage caused by blue light³ and the higher concentration of the zeaxanthin stereoisomers than lutein in the macula may also relate to the more effective quenching activity of zeaxanthin than lutein for singlet oxygen⁶³. It follows from these factors that maculae with high pigment densities should provide more protection from oxidative damage than that of maculae with less pigment, and that those individuals with poor pigment densities will be more at risk of damage. The risk of AMD may be a consequence of poor macular pigmentation and as the source of these pigments is from the diet, there is considerable interest in understanding the relationships between dietary intake and serum concentrations of the macular pigments and the xanthophyll content of the macula that is measured as MPOD⁶⁴.

The Eye Disease Case–Control Study Group was among the first groups to show an inverse correlation between the risk of AMD and the combined concentrations of serum lutein and zeaxanthin in man^{65,66}. The authors also found that a higher frequency of intake of spinach or collard greens was also associated with a substantially lower risk of AMD. The Eye Disease Case–Control Study Group evaluated the hypothesis that higher serum levels of micronutrients with antioxidant capabilities may be associated with decreased risk of neovascular AMD. The study compared serum levels of carotenoids, vitamins C and E and Se in approximately 421 cases and 615 control individuals with other eye diseases recruited in the same geographic areas in five centres. Micronutrients were classified by blood concentrations into three groups (low, medium and high). There were no individually significant effects for vitamin C,

vitamin E or Se, but for individuals with carotenoid levels in the medium and high groups (except lycopene) the risk of AMD was statistically significantly lower⁶⁵. For individuals with lutein or zeaxanthin, β -carotene, α -carotene and cryptoxanthin concentrations in the high percentile groups, OR of neovascular AMD ranged from 0.3 to 0.5 compared with those in the low percentile group.

More recently, another study was undertaken to investigate MPOD with respect to risk factors for AMD and dietary and serum concentrations of lutein and zeaxanthin in 828 healthy Caucasian Irish subjects aged 20–60 years^{64,67}. The authors found a statistically significant age-related decline in MPOD, that current and past smokers had lower MPOD than never smokers, and subjects with a confirmed family history of AMD had lower MPOD than those with no known history⁶⁷. In addition, the relationships between MPOD, serum concentrations of lutein and zeaxanthin, and dietary intake of lutein and zeaxanthin were positive and statistically significant when analysed for the entire study group (r 0.136–0.303; $P < 0.01$ for all). However, in sub-group studies, for subjects with a clinically confirmed family history of AMD, subjects aged more than 53 years, and subjects with a BMI > 27 kg/m², there were positive and significant relationships between MPOD and serum concentrations of lutein but not with zeaxanthin. In current heavy cigarette smokers, there were no significant relationships between MPOD and serum concentrations of either lutein or zeaxanthin. Thus in those subjects at increased risk for AMD, the authors suggested the results indicated impaired ability to accumulate circulating concentrations of zeaxanthin and in current heavy smokers this extended to lutein as well⁶⁴.

The fact that the Eye Disease Case–Control Study found the risk of macular disease to be inversely related to all the serum carotenoid concentrations except lycopene and not just lutein and zeaxanthin is understandable because high intakes of vegetables and fruits will tend to raise plasma concentrations of all the carotenoids. Lycopene, however, is different to the other carotenoids since there is really only one dietary source, that is tomatoes, and these fruits are used to make ketchup that is often consumed in large amounts and in separate meals from the other carotenoids. We also found plasma lycopene concentrations differed from the other carotenoids in not being lower in smokers than non-smokers in survey data from UK adults⁶⁸. The carotenoids believed to be of greatest importance for macular pigmentation, however, are lutein and zeaxanthin, as the others are there in only small amounts⁵.

Various workers have shown that MPOD is positively correlated with both dietary intakes^{19,27,64} and serum concentrations^{19,26,64,69,70} of lutein and zeaxanthin. These relationships suggest that dietary intervention alone should influence MPOD. A number of workers have shown that by using relatively pure supplements of lutein and more recently zeaxanthin^{12,13,71} it is possible to increase MPOD but food has also been used successfully^{14,40}. Hammond *et al.*¹⁴ fed 60 g spinach (10.8 mg lutein and 0.3 mg zeaxanthin) to eleven adult subjects. Ten of the subjects also added 150 g maize (0.4 mg lutein, 0.3 mg zeaxanthin) and two other subjects were given only maize. The intervention lasted 15 weeks and the subjects ate the supplement with a

meal or with a fat source. Eight subjects responded fully with significant increases in both serum lutein concentrations (33%) and MPOD (19%). The increase in both measurements was rapid (4 weeks) and MPOD was still elevated up to 9 months after treatment was discontinued. Two non-responders showed an increase in serum lutein concentrations but no change in MPOD and one non-responder had no change in either measurement. However, it emerged in the discussion of these results that the dietary intake of lutein and zeaxanthin of the non-responders before the study was four times higher than that of those who later responded and the concentrations of serum lutein and zeaxanthin were 17 and 52% higher at baseline respectively. As the baseline intake of the responders was approximately 2.3 mg xanthophyll carotenoids, the baseline intake of the non-responders will have been 8–9 mg of xanthophyll carotenoids, suggesting that the lack of response was due to the already high intake of xanthophyll carotenoids.

In another study using food, MPOD increased significantly in female adults who consumed six eggs/week for 12 weeks⁴⁰. Twenty-four subjects were allocated to three groups; egg I (lutein 167, zeaxanthin 166 µg/d), egg II (lutein 519, zeaxanthin 308 µg/d) or a pill containing sugar. There were significant increases in serum zeaxanthin in both egg groups but lutein only increased in response to the egg I treatment (Table 2). Three subjects were dropped from the egg groups because of concern over their cholesterol. Of the remainder, MPOD increased in eight and failed to increase in five subjects. A possible reason for non-response may have been the significantly higher MPOD at baseline in the egg II group since the authors reported an inverse relationship between the change in MPOD and baseline MPOD.

Three important factors emerge from these studies: baseline vegetable intake and baseline MPOD are determinants of the potential response from a xanthophyll supplement and that the xanthophylls in eggs are much more effective in increasing MPOD than those in vegetables. The increase in plasma zeaxanthin concentrations in the egg study in both egg-treatment groups may be causally related to the increase in MPOD but alternatively the increase in the plasma zeaxanthin concentration may simply be a consequence of the lower baseline plasma zeaxanthin concentration than lutein.

Dietary zeaxanthin concentrations may have a greater influence on MPOD than we currently realise. Zeaxanthin is after all the major carotenoid in the macula¹ and low serum zeaxanthin concentrations inversely correlated with the risk of AMD in a recent study in Sheffield⁷². A xanthophyll-rich diet, like spinach in the study of Hammond *et al.*¹⁴, also supplies zeaxanthin, and may fail to increase MPOD if plasma or tissue concentrations of zeaxanthin are already high in someone consuming an above average intake of vegetables. Very little is still known of the factors determining the conversion of lutein to MZ, and zeaxanthin may bind preferentially to the GSTP1-binding protein¹⁵ and suppress the formation of or prevent uptake of newly formed MZ. That is, a negative-feedback mechanism may prevent excessive pigmentation of the retina. In this respect, Hammond and colleagues reported that two of the non-responders showed a slight decrease (11%; NS) in MPOD¹⁴.

It may also be important to note that in the recent large Irish study, the authors noted that in subjects with a higher risk of AMD, there was a lack of correlation between MPOD and serum zeaxanthin concentrations which the authors suggested may indicate an impaired ability to accumulate circulating zeaxanthin⁶⁴.

The recent study where serum zeaxanthin concentrations were linked with a higher risk of AMD also found birth weight was positively associated with a risk of AMD. Birth records of 660 men and women born between 1922 and 1930 were examined and 392 of the subjects were contacted to do an ophthalmic examination. Of the subjects, 20.5% (forty-five men, thirty-three women) had signs of mostly early (17%) or late (3.7%) AMD. Subjects with AMD were significantly older than those without but there was no sex difference. After controlling for age, sex, serum zeaxanthin concentration, smoking and beer drinking etc, the surprising finding was that birth weight was positively related to the risk of AMD⁷³. However, the workers also measured serum concentrations of lutein and zeaxanthin and found that the risk of early or late AMD was significantly higher in those with the lower concentrations of serum zeaxanthin. A slightly lower risk of AMD was obtained when lutein was examined separately or if lutein and zeaxanthin were combined, but neither of the latter results were significant (Table 5)⁷². The writer is not aware of any association between birth weights and serum xanthophyll concentrations in adults.

Several studies have shown that smokers have a higher risk of AMD than non-smokers^{74,75} and lower MPOD than non-smokers⁶⁷. In The Rotterdam Study, in subjects under 85 years, current smokers had a 6.6-fold higher risk of neovascular AMD and adjusting the results for atherosclerosis did not change the association⁷⁴. In the Beaver Dam Study it was reported that men (but not women) who smoked greater amounts of cigarettes were more likely to develop early age-related maculopathy than men who smoked less⁷⁵. The conclusion in both these studies was that there was a dose–response relationship between smoking and AMD but the mechanism by which smoking influenced the risk of AMD is not known.

We have previously shown that plasma lutein concentrations were depressed in smokers compared with non-smokers⁶⁸ in data from The Survey of British Adults in 1988–9. However, it seems unlikely that the lower MPOD in smokers are just a consequence of lower circulating concentrations of lutein and zeaxanthin since, as explained elsewhere, the absolute amount of xanthophylls in serum exceeds by several thousand-fold the absolute amount of xanthophylls in the macula. It seems more likely that other factors associated with smoking influence selective uptake mechanisms. The higher concentration of the zeaxanthin isomers relative to lutein in the macula of the eye and the different distribution of the isomers in the macula in comparison with serum suggests that specific uptake mechanisms for the different xanthophyll carotenoids must exist in the eye. In addition, studies in primates suggest that the uptake and assimilation of the macular carotenoids are biologically regulated by selective mechanisms, as comparative studies on the macular pigment concentrations in the left and right eyes of individual monkeys showed

Table 5. Age-related macular degeneration (AMD) and plasma zeaxanthin and lutein concentrations

AMD ...	Number and type of subjects	Absent			Present			Reference				
		Zeaxanthin ($\mu\text{mol/l}$)	Lutein ($\mu\text{mol/l}$)	Difference (<i>P</i>)	Zeaxanthin ($\mu\text{mol/l}$)	Lutein ($\mu\text{mol/l}$)	Difference (<i>P</i>)					
		Median	IQR	Median	IQR	Median	IQR					
Descriptive study	Age 66–75 years; AMD present in seventy-eight subjects and absent in 302	0.036	0.024–0.055	0.177	0.130–0.232	0.030	0.02–0.046	0.153	0.118–0.215	0.16	0.02	Gale <i>et al.</i> ⁷²

IQR, interquartile range.

excellent agreement in both zeaxanthin (within 5%) and lutein (within 11%)⁷⁶. However, there were large differences in macular pigment concentrations between different monkeys (up to 4-fold for zeaxanthin), suggesting, as is observed in human subjects, that phenotype as well as diet and serum may all influence the pigmentation of macular tissue.

Differences between men and women in the relationships between MPOD and serum carotenoids indicate the importance of biological control of retinal uptake of the macular pigments. In a study on non-smoking American women (*n* 48) and men (*n* 40), there was no difference in the dietary intake of carotenoids or of the combined plasma concentrations of lutein and zeaxanthin, yet MPOD was 38% higher in men than women ($P < 0.001$)⁷⁷. Furthermore, dietary intake of carotenoids and serum concentrations of lutein and zeaxanthin were both positively related to MPOD in men but only serum concentrations of the xanthophyll carotenoids and MPOD were related in the women. We also observed differences in the plasma responses to supplements of lutein (10.2 mg/d) and MZ (8 mg/d) between men and women⁴⁶. Following 21 d of supplementation, plasma responses of both lutein ($P = 0.013$) and MZ ($P = 0.001$) were higher in the women than the men but there was no difference in the case of 3*R*,3'*R*-zeaxanthin (1.2 mg/d; $P = 0.95$). Both groups appeared to have similar intakes of fruit and vegetables, and baseline concentrations of plasma lutein and 3*R*,3'*R*-zeaxanthin were not different between the sexes. Women are reported to have a higher risk of AMD than men^{78,79} but whether the higher plasma responses indicate a higher demand for the macular pigments in women than men is not yet known.

Smokers frequently display evidence of sub-clinical inflammation^{80,81} and inflammation in both smokers and non-smokers is inversely related to serum carotenoid concentrations^{82,83}. Seddon *et al.*⁸⁴ reported concentrations of the systemic inflammatory marker C-reactive protein to be an independent risk factor for AMD. Therefore an additional factor to consider in interpreting the reason for non-response to dietary supplemental carotenoids may also be inflammation. The relationship was explored in the participants of the Age-Related Eye Disease Study because C-reactive protein is elevated in CVD and some of the risk factors for the latter are known to be associated with AMD. Smoking is associated with an increased risk of AMD^{74,75} and smokers often have elevated C-reactive protein (i.e. inflammation) and depressed serum xanthophyll concentrations^{82,83}. It is not known if inflammation explains the non-response in the studies of Hammond *et al.*¹⁴ and Wenzel *et al.*⁴⁰. Individuals with known diseases were excluded but the subjects were aged 24–65 years and the prevalence of sub-clinical inflammation increases with age. But, if inflammation is an independent risk factor for AMD, inflammation markers should be included when workers explore relationships between the risk of AMD and serum carotenoid concentrations.

The recent study of factors influencing MPOD in healthy Irish individuals confirmed several of the points made regarding smoking and MPOD outlined above. MPOD and serum lutein concentrations were significantly lower in the

heavy smokers (> twenty cigarettes/d) than both the light (< twenty cigarettes/d) and non-smokers, although serum zeaxanthin concentrations were not different between the groups⁶⁷. In non-smokers and current light smokers, MPOD was positively correlated with both lutein and zeaxanthin concentrations in serum. However, in current heavy smokers there were no correlations with either xanthophyll in spite of serum lutein and MPOD being lowest in this group⁶⁴. Inflammation is likely to be higher in the heavy than mild smokers or non-smokers⁸⁰, thus the lack of correlations between MPOD and serum lutein or zeaxanthin in the heavy smokers may be due to inflammation-related disturbances in serum xanthophyll concentrations and/or interference with uptake mechanisms as suggested earlier.

Conclusions

A number of factors indicate that specific uptake mechanisms for the different xanthophyll carotenoids must exist in the eye. The recent report of a binding protein in optical tissue with similar binding capacity for the two zeaxanthin isomers but not for lutein is no doubt part of the explanation. The binding protein may respond differently in men and women and may be impaired by external factors such as inflammation. The relatively high binding capacity for MZ by this protein is surprising in view of the fact that MZ is not a normal component of the diet or serum in man. Whether MZ supplements to increase plasma concentrations of MZ will be accessible to this protein and will influence MPOD and eye health still needs to be determined.

The optimal plasma response obtained with long-term feeding of pure lutein or zeaxanthin is approximately 0.1 $\mu\text{mol/l}$ per mg xanthophyll supplement and is achieved after approximately 21 d. Vegetable supplements of both lutein and zeaxanthin are both far less efficient in increasing plasma concentrations than the pure supplements but sometimes even the pure materials are poorly absorbed even when administered in the same vehicle. Plasma responses to xanthophyll supplements given as eggs were particularly high and in spite of their low content of xanthophyll carotenoids compared with vegetables, there was a significant increase in MPOD in adult female subjects given < one egg/d. Eggs are a particularly useful source of xanthophyll pigments as they naturally contain a relatively high proportion of zeaxanthin. Laying hens divert both endogenous and dietary xanthophylls to their eggs and the amount of pigment in the egg can be manipulated by the concentration of pigment in the feed. Currently a high proportion of the eggs consumed in Mexico will contain MZ obtained from their feed but the impact of this on eye health is not known. Lastly, long-term studies with pure lutein, mixtures of pure xanthophylls, lutein-rich foods and eggs have been shown to increase MPOD. Once increased, several studies have shown that MPOD remains high even though plasma xanthophyll concentrations fall relatively rapidly when intervention ceases. There is some evidence to suggest that a previously high dietary intake of lutein (and by proxy zeaxanthin) may optimise MPOD and prevent any further increase in macular pigment optical density with xanthophyll supplements. Such observations are important as they may help to quantify the amount of dietary

xanthophyll needed for optimal macular pigment density, but much more work in this area is still needed.

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