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Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin

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Abstract

Observational evidence is accumulating that the onset of age-related maculopathy, the leading cause of legal blindness in the Western World, could be delayed, or even averted, with antioxidant supplements. Lutein (L) and zeaxanthin (Z) are two hydroxy-carotenoids with antioxidant activity which accumulate at the macula, where they are collectively known as macular pigment (MP). It has been shown that MP is entirely of dietary origin, and that L and Z levels in serum, diet, and retina correlate. However, the nature of the relationships between L and Z in foodstuffs, blood, and macula is confounded by many variables including processes which influence digestion, absorption, and transport of the compounds in question, and accumulation and stabilization of the carotenoids in the tissues. If macular pigment is protective for age-related maculopathy, a clear understanding of the mechanisms whereby L and Z arrive at the target tissue (retina) from their source (foodstuff) is essential. In this paper, we review the literature germane to this growing area of interest.

Keywords: Age-related maculopathy; Carotenoids; Lutein; Zeaxanthin

The central retina subserves central and colour vision, and is known as the macula. Macular pigment (MP)[1] refers to the presence of lutein (L) and zeaxanthin (Z), two hydroxy-carotenoids, at the macula, and accounts for the yellow colouration of this retinal region [1]. Of note, MP is entirely of dietary origin [2].

Age-related maculopathy, or ARM, is the commonest cause of blind registration in the Western World [3], and its prevalence is likely to rise as a consequence of increasing longevity. The aetiological mechanisms underlying ARM continue to elude us, but there is a growing body of evidence implicating cumulative blue light damage and/or oxidative stress in the process [4].

Interestingly, MP is a blue-light filter at pre-receptorial level [1], and is a powerful antioxidant [5]. It is unsurprising, therefore, to learn that attention has recently focussed on the possibility that macular pigment may protect against ARM. Indeed, several investigators have found an association between risk-factors for age-related maculopathy and a relative lack of MP [6]. The hypothesised protective effect of macular pigment for ARM is rendered all the more provocative because of its dietary origins, and, by extension, the implications for potentially simple and safe public health measures to prevent, or even delay, the most common cause of blind registration in the developed world.

In this paper, we explore the relationships between: (a) dietary intake of L and Z and macular pigment; (b) serum levels of L and Z and macular pigment; and (c) serum levels of L and Z and dietary intake of L and Z. Furthermore, we review the literature germane to macular and serum responses to supplemental lutein and/or zeaxanthin.

Dietary lutein and zeaxanthin

Mean daily intake of L and Z, combined, varies from 0.8 to 4 mg per day, depending on the population

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[1] *Abbreviations used:* MP, macular pigment; L, lutein; Z, zeaxanthin; HDL, high-density lipoproteins; LDL, low-density lipoproteins; ROS, rod outer segments; RPE, retinal pigment epithelium.

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studied and the method of dietary assessment employed [7–9]. However, daily intake of carotenoids such as L varies widely between individuals, as illustrated by a standard deviation of 2.45 mg/day in a recently published study [9]. Approximately 78% of dietary L and Z is sourced from vegetables, spinach (30 g contains 3659 mg of lutein and zeaxanthin), and orange pepper being particularly rich source of these carotenoids, but a high mole percentage is also found in egg yolk [10,11]. A typical Western diet contains between 40 and 50 carotenoids, with significantly more L than Z (represented by an estimated ratio of 7:1) [11]. Of note, although using different sources of carotenoid data does influence estimates of absolute carotenoid intake, the relative values for individuals are not significantly affected [12].

Effective absorption of L and Z from the alimentary tract depends on many processes, including digestion of the food matrix, the formation of lipid micelles, uptake of the carotenoids by mucosal cells, and transport of the carotenoids to the lymphatic or portal circulation [13]. Digestion of carotenoids, in turn, depends on the manner in which the foodstuff is delivered because of the protein complexes (carotenoproteins) in which carotenoids are found in nature. Carotenoproteins inhibit optimal digestion, thus accounting for the enhanced absorption of lycopene from tomato juice after heating. The inhibitory effect of the carotenoproteins is also believed to account for the disparity in intestinal absorption of β-carotene, which is as low as 2% from raw vegetables, but up to 50% when delivered in oil solutions, aqueous dispersions or antioxidant-protected commercial beadlets [13].

Reduced intestinal absorption of carotenoids has been observed in fat-deficient diets, as the formation of lipid micelles requires emulsification of the fat-soluble vitamins by bile flow which, in turn, is stimulated by dietary fat [14].

To date, carotenoid transfer into intestinal mucosal cells has not been attributed to specific cell membrane and/or intracellular transport mechanisms, and the process is assumed to be a passive one. The simultaneous appearance of β-carotene and newly absorbed fat in lymph following a meal suggests that carotenoids and fatty acids are co-transported from micelle to the plasma membrane and/or cytoplasm [13].

Whatever the mechanism of uptake of carotenoids by intestinal mucosal cells, there is some, albeit conflicting, evidence to suggest there is competition between carotenoids for absorption. For example, it has been observed that serum levels of L are reduced following short-term supplementation with β-carotene by some, but not all, investigators [15–17].

Serum lutein and zeaxanthin

Carotenoids enter the circulation via the lymphatic duct as a component of the chylomicrons formed in the enterocyte, and analysis of the chylomicron composition is required if intestinal absorption of the carotenoids is to be studied prior to hepatic metabolism, uptake into tissues, and exchange with other lipoproteins. Gartner et al. [18] have demonstrated a peak rise in carotenoid composition of the chylomicron fraction 9 h following a loading dose of multiple carotenoids, with preferential absorption of L and Z compared with β-carotene.

Following uptake by the liver, carotenoids are re-secreted on plasma lipoproteins. High-density lipoproteins (HDL) carry primarily L and Z, whereas low-density lipoproteins (LDL) transport hydrocarbon carotenoids (e.g., lycopene, β-carotene) [19]. Indeed, some investigators have suggested that the low particle contents of L and Z in LDL may underlie reduced tissue targeting of antioxidants in subjects with a dense LDL phenotype [20]. Also, the preferential uptake of HDL by the retina may explain the selective uptake of L and Z to the exclusion of hydrocarbon carotenoids [21]. Fluctuations in serum levels of L, but not Z, have been observed, with peaks in summer and spring for males and females, respectively, and this has been attributed to perennial changes in dietary intake of these carotenoids [22].

The most prominent plasma carotenoids include lycopene, α-carotene, β-carotene, β-cryptoxanthin, L, and Z [5]. In blood, only a single isomer of each macular carotenoid is found (L: [{3*R*,3′*R*,6′*R*}-β,e-carotene-3,3′diol]; Z, or RRZ: [{3*R*,3′*R*}-β,β-carotene-3,3′diol]), and this contrasts with the macula where all three possible stereoisomers of Z are present [23]. As RRZ is the only form of Z found in the human diet and serum, it has been hypothesised that *meso*-zeaxanthin ([{3*R*,3′*S*}-β,β-carotene-3,3′diol]), which is found in the macula, represents the product of chemical processes involving lutein within the retina. This hypothesis is supported by the observation that L can be isomerised to *meso*-zeaxanthin by a base-catalysed reaction, and an approximate 2 to 1 predominance of Z and *meso*-zeaxanthin over L in the retina and a L–Z ratio close to 3 in human plasma [23].

Macular pigment optical density

Macular pigment (MP) refers to the accumulation at the macula of a single isomer of L, and 3 stereoisomers of Z (RRZ, *meso*-Z, and SSZ or [{3*S*,3′*S*}-β, β-carotene-3,3′diol]), to the exclusion of all other carotenoids which are found in human blood [1,23]. Snodderly and coworkers [1,24,25] have eloquently described the anatomical distribution of MP in the primate retina, and demonstrated that its optical density peaks at the centre of the fovea, representing a concentration of almost 1 mM, and 3 orders of magnitude above that found in normal serum. Although MP becomes optically unde-

tectable at an eccentricity of 1.5 mm, L and Z are found throughout the whole retina with the total mass pigment per unit area decreasing from macula to peripheral retina by a factor of almost 300 [25]. Peak concentrations of both L and Z are found at the centre of the fovea where Z is the dominant carotenoid, whereas L is typically dominant in the perifoveal region [25,26]. Of note, there is good interocular agreement, and significant individual variability, of MP [27–29].

Within the layer structure of the retina, the greatest concentrations of MP are found in the photoreceptor axons of the fovea, with relatively high concentrations in the receptor axon and inner plexiform layers outside the foveola [1]. Of note, the concentrations of L and Z in most retinal layers are equivalent to that of the receptor axon layer at an eccentricity of only 400 μm, reflecting the more rapid decline in MP concentration of inner retina, when compared with outer retina, with eccentricity [1].

Sommerburg et al. [30] have shown that a substantial proportion of total human retinal L and Z, approximately 25%, is found in the rod outer segments (ROS). Interestingly, the L/Z ratio in the ROS was comparable to that in the peripheral retina, and neither carotenoid was detectable in the ROS following removal of extrinsic membrane proteins. Also, L and Z were demonstrated in the retinal pigment epithelium (RPE), albeit in trace amounts, in similar proportions to the amounts in the retina, consistent with the view that the RPE carotenoids are derived from phagocytosed rod outer segments. This area of research has been advanced by Rapp et al. [31], who showed that the concentration of L and Z is 70% higher in human ROS than in residual membranes (ROS-depleted), and that perifoveal ROS membrane concentration is 2.7 times higher than that of the peripheral retina.

There is a growing body of evidence in support of the hypothesis that MP is bound by a retinal protein, possibly tubulin, in the macula [32]. The abundance of tubulin in the retina could explain the selective accumulation of L and Z at the macula to the exclusion of other carotenoids in serum, and is also consistent with the spatial distribution of MP. Furthermore, the results of modelling studies support the role of tubulin as the carotenoid-binding protein in the macula [33].

Lutein and zeaxanthin in adipose tissue, liver, and spleen

L and Z are known to accumulate in liver, spleen, and adipose tissue [21]. There is evidence of an inverse relationship between body fat and macular pigment optical density in humans [34,35], and a similar relationship with retinal L (but not Z) in female quail [21], suggesting that fat and retina compete for L. This hypothesis is supported by the observed preferential uptake by quail fat of serum lutein, when compared with zeaxanthin, by a margin of 4:1 [36].

Relationship between dietary intake, and serum levels, of L and Z

Observational studies

Of the seven observational studies analysing the relationship between dietary intake of L and Z and serum levels of these carotenoids, all have demonstrated significant and positive relationships ($p < 0.05$; $r = 0.21$–0.74) [34,37–42]. The largest of these studies included 2786 subjects, and found that demographic characteristics, dietary L and Z intake, serum cholesterol concentration, and lifestyle factors explained 24% of the variance in serum L concentration, and that every 10% increase in estimated dietary intake of L and Z was associated with a 2.4% increase in serum L concentration [42].

Supplement studies in non-humans

Dietary supplements of L and/or Z have resulted in rapid increases in plasma concentrations of these carotenoids in BALB/c mice [43], quail [21], and Rhesus monkeys [44].

The retina of quail on a low carotenoid diet supplemented with a Z isomer (RRZ or [{3*R*,3′*R*,6′*R*}-β, β-carotene-3,3′-diol]) accumulated Z, L, and cryptoxanthin, but preferentially absorbed Z [36]. In contrast, L was preferentially absorbed by liver and fat. In supplemented females, Z increased approximately 4-fold in retina, and 74-, 63-, and 22-fold in serum, liver, and fat, respectively. In males, Z was elevated approximately 3-fold in retina, and 42-, 17-, and 12-fold in serum, liver, and fat, respectively. Interestingly, birds supplemented with Z absorbed a higher fraction of L into serum, but L was reduced in the retina.

In a related study by the same investigators, similarly raised levels of Z were demonstrated in serum, liver, and fat by factors of 50, 43, and 6.5, respectively, after only 7 days of supplementation with Z. Supplementation with Z was associated with an enrichment of the Z fraction of total serum carotenoids, and serum L and Z concentrations were positively and significantly associated with concentrations of these carotenoids in liver, fat, and retina [21].

To better understand these differences in the L–Z ratio, the investigators coined the term ''capture efficiency,'' which refers to the ratio of tissue xanthophylls to serum xanthophylls, because it reflects both uptake and stabilization in the tissues. In brief, it was found that fat captured L far more efficiently, and liver only slightly more efficiently, than Z. Capture ef-

ficiencies for Z in retina and fat were significantly reduced in Z-supplemented birds, suggesting that serum Z concentration in these birds was sufficient to saturate the mechanisms responsible for capture of this carotenoid by those tissues. Further, capture efficiencies for L in retina and fat were also reduced by Z supplementation, suggesting that elevated serum Z interferes with uptake of L by these tissues. These findings are consistent with different binding proteins for the xanthophylls in retina and fat, those in the former preferentially binding Z and those in the latter preferentially binding L, thus explaining the phenomenon whereby high levels of one xanthophyll inhibit the binding of the other. However, other mechanisms to explain the differential concentrations of L and Z in tissues could exist, and these include shared uptake mechanisms but differential stabilization processes or preferential oxidative degradation of one carotenoid over the other [45].

Supplement studies in humans

There have been several studies investigating plasma response to supplemental L and/or Z in humans. In the first of these, significant increases in serum L (mean ± SD: 33% ± 22%), but not Z, were observed in 8 of 11 volunteers on diets modified to deliver about four times as much L, and two to three times as much Z, as a typical diet [46]. Significant rises in serum levels of L were also demonstrated by Landrum et al. [47] following 140 days of supplementation with 30 mg of L per day in two subjects.

Handelman et al. [48] monitored the serum response of L and Z in 11 subjects on a beef tallow diet supplemented with cooked chicken egg yolks, and found mean plasma L and Z concentrations to increase by 28 and 142%, respectively. Also, plasma increases in Z were observed in all subjects. The authors suggest that the egg yolk provides a highly bioavailable source of L and Z because the lipid matrix of the egg yolk provides an ideal vehicle for the efficient absorption of these carotenoids, but warn that potential benefits could be offset by elevation of LDL–cholesterol.

Berendschot et al. [49] reported on male volunteers who took supplemental L (10 mg per day) for 4 weeks. They exhibited a 5-fold rise in mean blood level of L from 0.18 to 0.9 μM, and this declined to 0.28 μM 4 weeks following discontinuation of the supplements.

Johnson et al. [35] monitored seven subjects on a diet modified with additional quantities of spinach and corn representing a 7-fold increase in daily dietary intake of the macular carotenoids, and observed a 2-fold rise in serum L concentrations after 4 weeks, and significantly higher than baseline levels throughout the period of supplementation (15 weeks). However, serum responses of Z were significant at 4 weeks only, possibly reflecting the lower amounts of this carotenoid in the modified diet. Serum levels of both L and Z returned to baseline 2 months following discontinuation of the modified diet.

Relationship between serum levels of L and Z and macular pigment

Studies investigating the relationship between retinal and serum carotenoids should also be interpreted with caution, primarily because serum levels of L and Z reflect recent nutritional intake only. In contrast, MP has a slow biological turnover, and therefore reflects the local balance between pro-oxidant stresses and antioxidant defences in the retina. In other words, a dramatic change in diet is unlikely to affect MP for several weeks, but will be reflected in much more rapid changes of serum concentrations of L and Z.

Observational studies

Of the seven observational studies investigating the relationship between serum L and Z and MP optical density, six have found positive and significant correlations ($p < 0.05$; $r = 0.21$–0.82) [37–39,50–53]. Furthermore, the only non-significant finding was in a study of only 20 subjects (10 monozygotic twins) [52].

Supplement studies in non-humans

In the study of quail response to Z supplementation, described above, it was observed that retinal Z had increased significantly following 3 days, but returned to baseline levels following 7 days, of supplementation, despite a significant and persistent rise in serum Z beyond day 7. However, enrichment of the Z fraction of total retinal xanthophylls was observed, with consequential protection of photoreceptors [21].

Thomson et al. [21] speculated that the delay in retinal response to supplemental Z, when compared with the serum response, was attributable to preferential uptake of HDL in the retina, only 2.5% of which carry carotenoid compared with 100% for low-density lipoproteins.

Supplement studies in humans

Of Hammond et al.'s [46] 11 subjects, there were two "retinal non-responders" in whom a significant increase in serum L (mean: 31%) was not accompanied by a parallel rise in MP optical density in response to increased dietary intake of L and Z. Several investigators have also reported that serum levels of the carotenoids rapidly return to baseline levels upon discontinuation of dietary supplements whereas the MP remains aug-

mented for at least 100 days, suggesting that the retinal carotenoids have a slow biological turnover in the retina [46,47].

Berendschot et al. [49] supplemented eight males with 10 mg of lutein per day, and observed an increase in mean plasma lutein by a factor of 5, and a linear 4-week increase in relative MP optical density of 4–5%. These investigators also noted that plasma concentration of L was still elevated 1 month following discontinuation of the supplements, and that MP optical density was continuing to rise, thus suggesting that a high plasma lutein level is associated with a gradual increase in the accumulation of retinal carotenoids. It has since been shown, however, that MP optical density is reduced in subjects with a higher body mass index [34,35], possibly reflecting preferential uptake of serum L by adipose tissue over retina [21].

Johnson et al. monitored MP and serum responses to a diet modified to result in a 7-fold increase in daily intake of L and Z and, consistent with Hammond et al.'s findings, observed significantly raised MP optical density at 4 weeks, which remained augmented after serum concentrations of L declined [35,46]. Interestingly, MP optical density peaked, and adipose L was decreased, at 4 weeks, whereas the reverse was seen at 8 weeks, suggesting competition between fat and retina for serum L. After 8 weeks, MP optical density was significantly higher than baseline until the last assessment which was 2 months following discontinuation of the diet [35].

The relationship between macular pigment optical density and dietary intake of lutein and zeaxanthin

Cross-sectional studies investigating the relationship between dietary intake of L and Z and MP should be interpreted with full appreciation of their limitations. First, dietary assessment by questionnaire is vulnerable to many sources of error including a subject's recall bias, as well as his/her digestive and absorptive idiosyncracies. Also, the use of different sources of carotenoid data by investigators can introduce inconsistencies which prevent meaningful comparisons between studies. Nevertheless, these observational studies have made a useful contribution to this area of research and warrant discussion.

Observational studies

Of the five observational studies investigating whether there was a demonstrable relationship between dietary intake of L and Z and MP optical density, three found such a relationship to be significant and positive [37,38,51,52,54]. The two studies failing to demonstrate a significant relationship comprised only small numbers of subjects ($n < 50$) [52,54].

Supplement studies in non-humans

Rhesus monkeys fed a Z-containing supplement from an extract of *Fructus lycii* (Gou Qi Zi) exhibited a rise in macular Z, but not L, concentrations [44].

And in quail, a 4-fold increase in retinal Z concentrations following 6 months of supplementation with Z has been demonstrated [21].

Supplement studies in humans

Of Hammond et al.'s [46] 11 subjects on a diet modified to augment intake of L and Z, 8 exhibited augmented MP optical density (mean ± SD: 19 ± 11%). Landrum et al. [47] also showed that MP optical density could be increased with appropriate supplements, and that interocular asymmetry of MP in one subject was maintained throughout the period of supplementation, suggesting that local retinal processes play a role in the accumulation and stabilization of MP.

Berendschot et al. [49] demonstrated a 4-week increase of 4–5% in MP optical density following supplementation with 10 mg of L per day, and that the levels of retinal carotenoids continued to rise following discontinuation of the supplements but in the presence of persistently augmented plasma levels of this carotenoid.

Johnson et al. [35] demonstrated a significantly higher than baseline MP optical density at 4, 12, 15, and 23 weeks, but not at 8 weeks, following commencement of a L- and Z-fortified diet. The investigators attributed the relative decrease in MP optical density at 8 weeks to a coincident and relative rise in adipose lutein at that time, suggesting preferential uptake of that carotenoid by fat tissue. This hypothesis is consistent with their finding of a significant and inverse relationship between percentage body fat and L concentrations in buccal mucosa, with body fat acting as a sink for L thus making less of this carotenoid available for other tissues [35].

Lutein supplementation in patients with retinal pathology has also been reported [55,56]. MP augmentation can be achieved in a substantial proportion of patients with retinitis pigmentosa and choroideremia, but this augmentation is not accompanied by detectable changes in central visual function within the period of follow-up (6 months) [55,56]. However, it is worth noting that Falsini et al. [57], have shown an improvement in retinal function using focal electroretinography in patients with ARM following supplementation with lutein and other antioxidants.

Conclusion

There is a growing body of scientific evidence which suggests that MP may protect against ARM, thus rendering our need to comprehend the relationships be-

tween L and Z concentrations in the diet, serum, and retina, as well as other tissues, all the more urgent. We need, and should support, studies designed to enhance our understanding of the bioavailability of carotenoids, and their distribution into various tissues. Such projects will require a team of researchers with a diverse array of interests including nutrition, epidemiology, ophthalmology, biochemistry, and vision science.

One of many lines of enquiry that should be pursued, in the context of the putative protective value of MP for ARM, is the role of tissue and serum carotenoids in the elderly population in whom dietary, digestive, and absorptive characteristics are likely to be compromised [58]. A rise in oxidant load, and reduced oxidant defences, associated with increasing age may well affect transport and stabilization of the macular carotenoids in tissues [40,59–61]. Particular attention should be directed toward the relationships between L and Z concentrations in diet, serum, and retina in a large number of elderly subjects on a typical diet, and taking account of recently identified variables such as adiposity. Then, the varying associations and interactions need to be re-explored in the context of L and/or Z supplementation, with and without co-antioxidants. And finally, an elderly population consisting of ARM sufferers and control subjects with healthy maculae will need to be studied to test whether the relationships hold true for the target population.

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