Do lutein, zeaxanthin and macular pigment optical density differ with age or age-related maculopathy?

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Abstract

Background and aims

Current age-related macular disease (ARMD) treatment includes antioxidant supplementation. Lutein (L) and zeaxanthin (Z) are antioxidants that make up macular pigment within the retina and may reduce the risk of developing ARMD. Ageing and smoking are leading risk factors for developing ARMD. We investigated differences in dietary, supplemental and retinal L and Z, and smoking habits in healthy younger eyes (HY), healthy older eyes (HO) and eyes with an early form of ARMD called age-related maculopathy (ARM).

Methods

HO, HY and ARM groups were assessed for dietary intakes of L and Z using food diaries. Smoking habits and self-administered quantities of L and Z were obtained via questionnaire. Retinal L and Z levels (macular pigment optical density, or MPOD) were determined using heterochromatic flicker photometry.

Results

No significant difference was demonstrated for dietary L and Z intake ($\chi^2 = 4.983, p=0.083$) or for MPOD between groups (F=0.40, p=0.67). There was a significant difference between the HY (mean ± sd: 1.20 ± 2.99), HO (4.51 ± 7.05) and ARM groups (9.15 ± 12.28) for pack years smoked ($\chi^2 = 11.61, p = 0.03$).

Conclusions

Our results do not support the theory that ARM develops as a result of L and Z deficiency. Higher pack years smoked may be a factor in disease development. Dietary and supplementary L and Z levels must be obtained when assessing MPOD between groups or over time.

Keywords: Macular, macular pigment, MPOD, age-related macular disease, lutein
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Introduction

Age-related macular disease (ARMD) is a degenerative disease of the central part of the retina, called the macula, most common over the age of 50 years. It is the leading cause of visual loss within western industrialised countries. Numbers of blind registrations attributable to the disease increased by 30-40% between 1950-1990 and cases each year are continuing to rise as these populations have an increasing longevity. Age related maculopathy is an early stage of this disease, characterised by the clinical appearance of drusen with or without hyperpigmentation or hypopigmentation within the retina. Drusen are comprised of membranous debris which accumulates between the retinal pigment epithelium (RPE) and Bruch’s membrane within the retina. There are several postulations for the aetiology of the ARMD including genetics, deterioration of Ruysch’s complex (RPE, Bruch’s membrane and choriocapillaris) and oxidative stress, although the aetiology is currently unclear.

The limited treatments available for delaying the course of ARMD at present, has prompted interest into how modifiable risk factors may play a role in reducing the incidence and progression of the disease. Because oxidative stress is a proposed factor in the pathogenesis of ARMD, the function of antioxidant supplementation in this disease is of interest. Although the evidence on the effects of nutritional supplementation in ARMD has been conflicting, current recommendation for the treatment of ARMD includes nutritional supplementation with antioxidants, vitamins and zinc.

The xanthophyll carotenoids lutein (L) and zeaxanthin (Z) are antioxidants that can only be obtained through ingestion from the diet and together with meso-zeaxanthin make up macular pigment (MP). Situated within the central 5-10 degrees of the retina, MP concentrations are highest within the photoreceptor axons of the fovea, declining with eccentricity. Macular pigment has also been found in the inner layers of the retina and the photoreceptor outer segments. There is evidence to suggest that MP acts as a filter to...
damaging short-wavelength blue light irradiation with a peak absorbance spectrum of 460nm, thus reducing the amount of harmful light irradiation reaching the photoreceptor layer. In conjunction with MP, outer segments of photoreceptors also contain polyunsaturated fatty acids (PUFA) and vitamin A. Under high oxygen tension and light irradiation lipid peroxidation of the photoreceptor outer segments occurs, especially within the macular area, inducing photoreceptor damage. The antioxidant properties of carotenoids quench reactive oxygen species and singlet oxygen, thus reducing oxidative stress and lipid peroxidation within the retina.

Augmented dietary L and Z levels have been associated with a reduced risk of developing ARMD in some studies but not in others. Dietary intervention and supplementation with L and Z have also been associated with improved measures of vision, including visual acuity, contrast sensitivity and electrophotographic measures in eyes with ARMD.

Because L and Z are the only carotenoids found within the retina, with meso-zeaxanthin being synthesized from L, their tentative role in reducing risk for ARMD development remains of interest.

Ageing, smoking and genetics appear to be the leading risk factors for developing ARMD. Other inconsistently proposed risk factors include female gender, white ethnicity, cataracts, intraocular lenses, cognitive impairment, arthritis, light iris pigmentation, hypermetropia, attenuated optic disc appearance, decreased hand grip strength, medication (statins, aspirin, antacids and thiazide diuretics), higher birth weight, lower socioeconomic status, increased alcohol intake, low antioxidant intake, high body mass index, high fat intake, cardiovascular disease, high cholesterol levels, type II diabetes, hormones (hormone replacement therapy, thyroid and antithyroid medication), and parity greater than zero.

Because MP is a modifiable factor potentially linked with reduced risk for ARMD, and ageing is a predominant risk factor for developing ARMD, the aim of this study was to determine whether there were differences in dietary and supplemented L and Z, and macular pigment optical density (MPOD, the amount of retinal MP) between young eyes, old eyes and eyes with ARMD using heterochromatic flicker photometry (HFP).
Materials and methods

Eighty one eyes from 81 participants aged between 18-83 (mean ± sd; 50.3 ± 18.1 years) were recruited over a nine month period from Aston University (Birmingham, UK) optometry department patients, and from staff and students from within the university. They were divided into three groups: a healthy younger (HY) group of 37 participants aged between 18-48, (mean age ± sd; 32.9 ± 9.0 years), a healthy older (HO) cohort of 28 participants aged between 50-77, (mean age ± sd; 63.4 ± 8.1 years) and an age-related maculopathy (ARM) cohort of 16 participants aged between 52-83 (mean age 67.2 ± 8.5 years). Age-related maculopathy was defined as per the international classification system.

All participants (including those with eyes affected by ARM) had a logarithmic minimum angle of resolution of visual acuity 0.2 or better to ensure good fixation, no ocular disease (other than ARM in the ARM group) determined by health questionnaire and fundus photography, normal blood pressure, no intraocular lenses, good general health, clear optical media or minimal opacity as determined by ophthalmic photography, and on no medication that affects the retina.

Research procedures followed the tenets of the Declaration of Helsinki and were approved by the Aston University Ethics Committee. Informed consent was obtained from all participants after they were given an explanation of the study.

Colour fundus photographs (Topcon TRC-NW8, Topcon, Newbury, Berkshire, UK) of the central 45º of the posterior pole were obtained. One eye per participant was chosen to eliminate intraclass correlation; environmental and genetic risk factors for ARMD such as smoking, age and genetic disposition, act on the individual and thus have an impact on the probability of the disease occurring in both eyes, even if not clinically visible in both eyes. Significance testing where total sample size (number of eyes) exceeds the number of participants is considered to be invalid and prone to false positive findings 30.

Macular pigment optical density using HFP with the MPS 9000 (also recognised as the M:Pod and the QuantifEYE; Topcon, Newbury, UK) was measured for each group. The testing
environment was identical for each subject. Untested eyes were occluded and tested eyes
were corrected with the subjects distance glasses if worn. Each participant undertook a
practice run was before the main test commenced. A stimulus consisting of a blue light
(465nm), and green light (530nm) stimuli were flicker matched by the subject pressing a
buzzer as soon as flicker was observed. This was done for the central one degree of visual
field. Blue light was absorbed by MP, thus a high intensity of blue light was necessary to
discriminate minimum flicker when the central value was being obtained. The test was
repeated to determine peripheral minimum flicker at eight degrees of retinal eccentricity
where MP is absent. Hence, the blue light had higher luminance and minimum flicker value is
different from that at the fovea. Both the background and target luminance was set to 250
cd/m². Subjects wore distance glasses for the test and were instructed to blink frequently,
especially when obtaining the peripheral value to reduce Troxler’s effect. Instructions were
given to the participant prior to the test and a practice run was undertaken for each subject
before undertaking the main test. Macular pigment was determined by dividing the central
blue light intensity by peripheral blue light intensity and log10 of this value. The study had
80% power at the 5% significance level to detect a change in MPOD of 0.33. This is based on
Bartlett et al’s work who found that a difference in MPOD of 0.33 or greater can be classed as
clinically significant 31.

To assess differences in dietary L and Z levels between HO, HY and ARM groups, food
diaries were given to participants to complete over two weekdays and one weekend day.
Standard L and Z content of foods were taken from the United States Department of
Agriculture (USDA) national nutrient database. The nutrients from the food diaries were
analysed using Weighted Intake Software Program (WISP) version 3.0 (Tinuviel software,
Llanfechell, Anglesey, UK). Participants were also asked about self administration of lutein-
based supplements.

Smoking history was established using questionnaires. Former and current smokers were
asked about their total number of smoking years and the average number of cigarettes
smoked per day. To calculate pack years of smoking, the average of number of cigarettes
smoked per day was multiplied by the total number of years of smoking and divided by 20:
Pack years smoked = (cigarettes smoked per day x years smoked) / 20

Results

An independent-samples t-test demonstrated no significant difference in age between the ARM (mean ± sd: 67.2 ± 8.5 years) and HO (63.4 ± 8.1 years) groups; t = 1.45, p = 0.16.

There was a significant difference using ANOVA in spherical equivalent refraction (F = 3.43, p = 0.04), between the HY (mean ± sd: -0.23 ± 1.90D), HO (0.78 ± 2.39D) and the ARM (1.29 ± 2.17D) groups with post hoc analysis demonstrating a difference between ARM and HY groups; p=0.02 but no difference between HY and HO eyes, or between HO and ARM eyes.

A Chi-squared test for independence using SPSS 16.0 software (SPSS UK ltd, West Street, Woking, Surrey) indicated a significant difference between ethnicity and groups, with HO and ARM groups exclusively containing 28 and 16 Caucasians respectively and the HY group containing 8 Asians and 29 Caucasians (χ² = 10.56, p = 0.01, p = 0.01). There was no significant difference between gender and groups (χ² = 0.14, p= 0.93) with 13 males and 24 females in the HY group, 9 males and 19 females in the HO group and 6 males and 10 females in the ARM group. The ARM group were as classified as per the international classification system – drusen with or without hyperpigmentation / hypopigmentation.

The data was checked for normality using the Shapiro-Wilk test which assesses the normality of distribution of the data. A non-significant result indicated normality for the data. Therefore a one-way ANOVA was used for analysis with Tukey’s post-hoc range test using SPSS 16.0 software to explore the impact of age and ARM on MPOD. No statistically significant disparity was established in MPOD between younger, older or diseased eyes in this study (F=0.40, p=0.67).

There was a food diary return rate of 17 (46%) in the HY group, 18 (64%) in the HO group and 13 (81%) in the ARM group, giving an overall return rate of 48 (59%). As parametric assumptions were not met with statistical significance for normality using Shapiro-Wilks test, differences between the three groups for dietary lutein and zeaxanthin intake were assessed using the Kruskal-Wallis test with SPSS 16.0 software. No significant difference was
demonstrated between groups for dietary lutein and zeaxanthin intake ($\chi^2=4.983, p=0.083$) when analysed using food diaries.

Of the total 81 subjects who were questioned about their current and previous smoking habits, 75 replied (37 in the HY, 23 in the HO and 15 in the ARM group). Because a significant result for the Shapiro-Wilk test indicated non-normality for smoking for each group the Kruskal-Wallis non-parametric ANOVA was used in place of the one-way ANOVA. There was a significant difference between the HY (mean ± sd: 1.20 ± 2.99), HO (4.51 ± 7.05) and ARM (9.15 ± 12.28) groups for pack years smoked ($\chi^2 = 11.61, p = 0.03$) with post hoc analysis demonstrating a difference between HY and HO groups (Z = -2.56, p = 0.01) and between HY and ARM groups (Z = -3.06, p < 0.01), but not between HO and ARM groups.

Because a significant result for the Shapiro-Wilk test indicated non-normality for self-administered lutein-based supplementation the Kruskal-Wallis non-parametric ANOVA was used in place of the one-way ANOVA. There was a significant difference between the three groups with both HY and HO groups not taking any self-administered lutein-based supplementation whereas 3 of the 16 in the ARM group were taking a supplement (mean ± sd: 2.75 ± 7.72 µg, $\chi^2 = 11.58, p = 0.003$).

Further analysis after removal of the 3 ARM participants taking the L and Z supplement from the data also showed no statistical significance between ARM, HO or HY groups ($F = 0.688, p = 0.506$).

Discussion
The aim of this study was to assess the effects of age and ARM on dietary and supplementary L and Z, and MPOD levels. This study found no difference in dietary L and Z or MPOD between young, old and diseased retinae using this subjective measure.

Nolan et al., found an age-related decline in MPOD in a study of healthy subjects up to the age of 60 years. They did not assess the effects of age after the age of 60 years. They also reported a significantly lower than average MPOD in healthy subjects with a family history of ARM, exudative age-related macular degeneration (AMD) or geographic atrophy. They did assess dietary and supplement usage but they grouped supplement quantities together with dietary intake values. We have statistically analysed and reported dietary and supplemented L and Z separately here. A study by Beatty et al., also found an inverse relationship between age and MPOD in healthy eyes. They also compared 9 eyes at risk of developing ARMD (contralateral to an eye with advanced AMD) to 9 age-matched healthy eyes in this study. At risk eyes had a lower MPOD than age-matched healthy eyes although they did not specify whether the at risk eyes had any signs of drusen or ARM. They reported dietary L and Z but did not report data on supplementary forms. The Irish Longitudinal Study on Ageing did find an inverse relationship between age and MPOD when comparing a group aged 50 years and older with a group aged 18-60 years although they did not report dietary or supplemented L and Z values.

Conversely and consistent with our study, another study by Bartlett et al., demonstrated no correlation between MPOD and age, although the age range was limited (18-50 years, mean age 25.4 ± 8.2 years) when compared to our study (18-83 years, mean ± sd; 50.3 ± 18.1 years) and no dietary or supplementary L and Z data was reported. Other studies have also shown a lack of variation in MPOD with age without reporting dietary or supplemental L or Z. Conversely some studies have found an increase in MPOD with age, but again dietary and supplementary L and Z were not reported.

Although a statistically significant difference was seen between HY and ARM groups for distance refractive spherical equivalent in our study, participants wore their distance prescription when performing MPOD testing to counter any differences between groups. There is no evidence in the literature that ametropia affects MPOD. We also found a
statistically significant difference for ethnicity between groups, although there is no evidence
to suggest this would affect MPOD. There is paucity in the literature with regard to the MPOD
levels of Asians compared to Caucasians although one Chinese study found no difference
between MPOD levels between Chinese Asians and reported MPOD levels in Caucasians 37.
The ARM group in our study had the highest number of pack years smoked when compared
to the HY and HO groups. Post hoc analysis showed a statistically significant difference
between ARM and HY groups in pack years smoked and although no statistically significant
difference was found between ARM and HO groups, pack years smoked in the ARM group
was more than double that of the HO group. It is well documented that smokers have an
increased risk of developing ARMD 22-26 and previous studies have demonstrated lower
MPOD levels in smokers 32, 38. An inverse relationship between ARMD and dietary lutein and
zeaxanthin concentrations has also been reported 39. Thus it may be reasonable to suggest
that the combination of higher pack years smoked and ARM may be associated with lower
MPOD, although this was not the case in our study.
In our study supplementary L and Z were significantly higher in ARM eyes overall when
compared to the HY and HO groups who took no L and Z supplement. There was variation
within the ARM group with only 3 of the 16 ARM participants taking L and Z supplements
(intake range 0-30µg per day). Patient and GP awareness of ARMD and possible
preventative measures to reduce the risk of disease development may account for the
supplementary L and Z intake in this group. Higher L and Z supplementary levels in the ARM
group did not give rise to higher MPOD levels suggesting that either the mean supplement
throughout the group overall was not large enough to increase MPOD or that the retinal
processes do not metabolise L and Z supplementation in ARM eyes in the same way as
healthy eyes. It may also be that the higher pack years smoked in this group may counter any
effects of supplementary L and Z on MPOD levels. Further data analysis after removal of the
three ARM eyes taking a L and Z supplement continued to show no statistical significance
between HY, HO and ARM groups for MPOD.
Another study assessing spectral fundus reflectance found no differences in MPOD between
healthy and ARM eyes in a sample from a general population aged 55 years and older
although dietary and supplemental L and Z were not quantified in this study. This echoed our study but we quantified dietary and supplemental L and Z. Intervention with supplemented and dietary increases in L and Z have shown increases in MPOD in eyes with ARM. The Muenster ageing and retina study (MARS) showed increases in MPOD using autofluorescence with ARM stage but this association became non-significant when the influence of L and Z supplementation was adjusted for. The age group range for the MARS study was 60-80 years. It is still not clear whether decreased MPOD is related to an increased risk of developing ARMD.

The potential benefits of L and Z supplementation on visual function require further investigation. Long-term randomised-controlled trials are the most stringent methods to evaluate whether a cause-effect relationship exists between increased dietary or supplemented L and Z levels and improved MPOD and visual function in young, old and ARM eyes. However, it is imperative that dietary and supplementary L and Z values are obtained at baseline and throughout such studies and when comparing young, old and ARM eyes to ensure the validity of MPOD results. To the authors knowledge this is the first study to directly compare healthy young, healthy old and ARM eyes together with dietary and supplemented L and Z for each group. Supplementation studies often undertake dietary L and Z prior to, and during L and Z supplementation trials but we assessed differences between groups in a sample of a population and not as part of a L and Z supplementation trial for this study.

To summarise we found no statistical significance in dietary L and Z between HO, HY or ARM eyes in our study. There was a significant difference in supplementary L and Z in ARM eyes compared to HY and HO eyes but no statistical significance for MPOD between all three groups even when the three L and Z supplemented eyes in the ARM groups were removed from our analysis. Based on participant’s current intake of dietary and supplemental L and Z, our results do not support the theory that ARM develops as a result of L and Z deficiency because we have shown that the ARM group consume similar levels of L and Z as the other groups. It could be that historically our ARM participants consumed low levels of L and Z and that this predisposed them to ARM, although it seems likely that higher pack years smoked by this group could be a factor in the development of the disease. Long-term randomised-
controlled trials assessing the effects of supplementation with L and Z on visual function in healthy young, healthy old and eyes with ARMD may provide more tangible evidence and help resolve incompatible findings from other studies. However, it is crucial that dietary and supplementary L and Z levels are reported as standard when assessing MPOD between groups or over time.

Conflict of interest

Project funded by Bausch and Lomb

Acknowledgements

We thank Bausch and Lomb for funding the project.

Authors’ contributions

HB designed the study, HB and FE supervised the experiments. EB performed the experiments, acquired data, performed statistical analyses and wrote the manuscript. HB and FE assisted with manuscript preparation and critically reviewed the manuscript. All authors have read and approved the final manuscript.
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