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 (χ^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) ARM groups (9.15 ± 12.28) for pack years smoked (χ^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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77 maculopathy?

78

79 Introduction

80 Age-related macular disease (ARMD) is a degenerative disease of the central part of the

81 retina, called the macula, most common over the age of 50 years [1]. It is the leading cause of

82 visual loss within western industrialised countries [2]. Numbers of blind registrations attributable

83 to the disease increased by 30-40% between 1950-1990 [2] and cases each year are

84 continuing to rise as these populations have an increasing longevity. Age related maculopathy

85 is an early stage of this disease, characterised by the clinical appearance of drusen with or

86 without hyperpigmentation or hypopigmentation within the retina [1]. Drusen are comprised of

87 membranous debris which accumulates between the retinal pigment epithelium (RPE) and

88 Bruch's membrane within the retina [3]. There are several postulations for the aetiology of the

89 ARMD including genetics, deterioration of ruysch's complex (RPE, Bruch's membrane and

90 choriocapillaris) and oxidative stress, although the aetiology is currently unclear.

91

92 The limited treatments available for delaying the course of ARMD at present, has prompted

93 interest into how modifiable risk factors may play a role in reducing the incidence and

94 progression of the disease. Because oxidative stress is a proposed factor in the pathogenesis

95 of ARMD, the function of antioxidant supplementation in this disease is of interest. Although

96 the evidence on the effects of nutritional supplementation in ARMD has been conflicting,

97 current recommendation for the treatment of ARMD includes nutritional supplementation with

98 antioxidants, vitamins and zinc [4].

99

100 The xanthophyll carotenoids lutein (L) and zeaxanthin (Z) are antioxidants that can only be

101 obtained through ingestion from the diet [5] and together with meso-zeaxanthin make up

102 macular pigment (MP). Situated within the central 5-10 degrees of the retina [6], MP

103 concentrations are highest within the photoreceptor axons of the fovea [7], declining with

104 eccentricity. Macular pigment has also been found in the inner layers of the retina and the

105 photoreceptor outer segments. There is evidence to suggest that MP acts as a filter to

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106 damaging short-wavelength blue light irradiation with a peak absorbance spectrum of 460nm

107 [8], thus reducing the amount of harmful light irradiation reaching the photoreceptoral layer. In

108 conjunction with MP, outer segments of photoreceptors also contain polyunsaturated fatty

109 acids (PUFA) and vitamin A. Under high oxygen tension and light irradiation lipid peroxidation

110 of the photoreceptor outer segments occurs, especially within the macular area, inducing

111 photoreceptor damage [9]. The antioxidant properties of carotenoids quench reactive oxygen

112 species and singlet oxygen, thus reducing oxidative stress and lipid peroxidation within the

113 retina [10].

114 Augmented dietary L and Z levels have been associated with a reduced risk of developing

115 ARMD in some studies[11, 12] but not in others[13, 14]. Dietary intervention and supplementation

116 with L and Z have also been associated with improved measures of vision, including visual

117 acuity, contrast sensitivity and electroretinographic measures in eyes with ARMD[15, 16].

118 Because L and Z are the only carotenoids found within the retina, with meso-zeaxanthin being

119 synthesized from L [17], their tentative role in reducing risk for ARMD development remains of

120 interest.

121 Ageing[18-21], smoking [22-26] and genetics [27-29] appear to be the leading risk factors for

122 developing ARMD. Other inconsistently proposed risk factors include female gender, white

123 ethnicity, cataracts, intraocular lenses, cognitive impairment, arthritis, light iris pigmentation,

124 hypermetropia, attenuated optic disc appearance , decreased hand grip strength, medication

125 (statins, aspirin, antacids and thiazide diuretics), higher birth weight, lower socioeconomic

126 status, increased alcohol intake, low antioxidant intake, high body mass index, high fat

127 intake, cardiovascular disease, high cholesterol levels, type II diabetes, hormones (hormone

128 replacement therapy, thyroid and antithyroid medication), and parity greater than zero.

129 Because MP is a modifiable factor potentially linked with reduced risk for ARMD, and ageing

130 is a predominant risk factor for developing ARMD, the aim of this study was to determine

131 whether there were differences in dietary and supplemented L and Z, and macular pigment

132 optical density (MPOD, the amount of retinal MP) between young eyes, old eyes and eyes

133 with ARM using heterochromatic flicker photometry (HFP).

134   Materials and methods

135   Eighty one eyes from 81 participants aged between 18-83 (mean ± sd; 50.3 ± 18.1 years)

136   were recruited over a nine month period from Aston University (Birmingham, UK) optometry

137   department patients, and from staff and students from within the university. They were divided

138   into three groups: a healthy younger (HY) group of 37 participants aged between 18-48,

139   (mean age ± sd; 32.9 ± 9.0 years), a healthy older (HO) cohort of 28 participants aged

140   between 50-77, (mean age ± sd; 63.4 ± 8.1 years) and an age-related maculopathy (ARM)

141   cohort of 16 participants aged between 52-83 (mean age 67.2 ± 8.5 years). Age-related

142   maculopathy was defined as per the international classification system [1].

143

144   All participants (including those with eyes affected by ARM) had a logarithmic minimum angle

145   of resolution of visual acuity 0.2 or better to ensure good fixation, no ocular disease (other

146   than ARM in the ARM group) determined by health questionnaire and fundus photography,

147   normal blood pressure, no intraocular lenses, good general health, clear optical media or

148   minimal opacity as determined by ophthalmic photography, and on no medication that affects

149   the retina.

150

151   Research procedures followed the tenets of the Declaration of Helsinki and were approved by

152   the Aston University Ethics Committee. Informed consent was obtained from all participants

153   after they were given an explanation of the study.

154

155   Colour fundus photographs (Topcon TRC-NW8, Topcon, Newbury, Berkshire, UK) of the

156   central 45º of the posterior pole were obtained. One eye per participant was chosen to

157   eliminate intraclass correlation; environmental and genetic risk factors for ARMD such as

158   smoking, age and genetic disposition, act on the individual and thus have an impact on the

159   probability of the disease occurring in both eyes, even if not clinically visible in both eyes.

160   Significance testing where total sample size (number of eyes) exceeds the number of

161   participants is considered to be invalid and prone to false positive findings [30].

162   Macular pigment optical density using HFP with the MPS 9000 (also recognised as the M:Pod

163   and the QuantifEYE; Topcon, Newbury, UK) was measured for each group. The testing

164 environment was identical for each subject. Untested eyes were occluded and tested eyes

165 were corrected with the subjects distance glasses if worn. Each participant undertook a

166 practice run was before the main test commenced. A stimulus consisting of a blue light

167 (465nm), and green light (530nm) stimuli were flicker matched by the subject pressing a

168 buzzer as soon as flicker was observed. This was done for the central one degree of visual

169 field. Blue light was absorbed by MP, thus a high intensity of blue light was necessary to

170 discriminate minimum flicker when the central value was being obtained. The test was

171 repeated to determine peripheral minimum flicker at eight degrees of retinal eccentricity

172 where MP is absent. Hence, the blue light had higher luminance and minimum flicker value is

173 different from that at the fovea. Both the background and target luminance was set to 250

174 cd/m². Subjects wore distance glasses for the test and were instructed to blink frequently,

175 especially when obtaining the peripheral value to reduce Troxler's effect. Instructions were

176 given to the participant prior to the test and a practice run was undertaken for each subject

177 before undertaking the main test. Macular pigment was determined by dividing the central

178 blue light intensity by peripheral blue light intensity and log10 of this value. The study had

179 80% power at the 5% significance level to detect a change in MPOD of 0.33.This is based on

180 Bartlett *et al's* work who found that a difference in MPOD of 0.33 or greater can be classed as

181 clinically significant [31].

182 To assess differences in dietary L and Z levels between HO, HY and ARM groups, food

183 diaries were given to participants to complete over two weekdays and one weekend day.

184 Standard L and Z content of foods were taken from the United States Department of

185 Agriculture (USDA) national nutrient database. The nutrients from the food diaries were

186 analysed using Weighted Intake Software Program (WISP) version 3.0 (Tinuviel software,

187 Llanfechell, Anglesey, UK). Participants were also asked about self administration of lutein-

188 based supplements.

189 Smoking history was established using questionnaires. Former and current smokers were

190 asked about their total number of smoking years and the average number of cigarettes

191 smoked per day. To calculate pack years of smoking, the average of number of cigarettes

192 smoked per day was multiplied by the total number of years of smoking and divided by 20:

193   Pack years smoked = (cigarettes smoked per day x years smoked) / 20

194   Results

195   An independent-samples t-test demonstrated no significant difference in age between the

196   ARM (mean ± sd: 67.2 ± 8.5 years) and HO (63.4 ± 8.1 years) groups; t = 1.45, p = 0.16.

197   There was a significant difference using ANOVA in spherical equivalent refraction (F = 3.43, p

198   = 0.04), between the HY (mean ± sd: -0.23 ± 1.90D), HO (0.78 ± 2.39D) and the ARM (1.29 ±

199   2.17D) groups with post hoc analysis demonstrating a difference between ARM and HY

200   groups; p=0.02 but no difference between HY and HO eyes, or between HO and ARM eyes.

201   A Chi-squared test for independence using SPSS 16.0 software (SPSS UK ltd, West Street,

202   Woking, Surrey) indicated a significant difference between ethnicity and groups, with HO and

203   ARM groups exclusively containing 28 and 16 Caucasians respectively and the HY group

204   containing 8 Asians and 29 Caucasians (χ^2 = 10.56, p = 0.01, p = 0.01). There was no

205   significant difference between gender and groups (χ^2 = 0.14, p= 0.93) with 13 males and 24

206   females in the HY group, 9 males and 19 females in the HO group and 6 males and 10

207   females in the ARM group. The ARM group were as classified as per the international

208   classification system – drusen with or without hyperpigmentation / hypopigmentation [1].

> **Comment [EJB3]:** Now included

209   The data was checked for normality using the Shapiro-Wilk test which assesses the normality

210   of distribution of the data. A non-significant result indicated normality for the data. Therefore a

211   one-way ANOVA was used for analysis with Tukey's post-hoc range test using SPSS 16.0

212   software to explore the impact of age and ARM on MPOD. No statistically significant disparity

213   was established in MPOD between younger, older or diseased eyes in this study (F=0.40,

214   p=0.67).

215   There was a food diary return rate of 17 (46%) in the HY group, 18 (64%) in the HO group

216   and 13 (81%) in the ARM group, giving an overall return rate of 48 (59%). As parametric

217   assumptions were not met with statistical significance for normality using Shapiro-Wilks test,

218   differences between the three groups for dietary lutein and zeaxanthin intake were assessed

219   using the Kruskal-Wallis test with SPSS 16.0 software. No significant difference was

220   demonstrated between groups for dietary lutein and zeaxanthin intake (X^2=4.983, p=0.083)

221   when analysed using food diaries.

222   Of the total 81 subjects who were questioned about their current and previous smoking habits

223   75 replied (37 in the HY, 23 in the HO and 15 in the ARM group). Because a significant result

224   for the Shapiro-Wilk test indicated non-normality for smoking for each group the Kruskal-

225   Wallis non-parametric ANOVA was used in place of the one-way ANOVA. There was a

226   significant difference between the HY (mean ± sd: 1.20 ± 2.99), HO (4.51 ± 7.05) and ARM

227   (9.15 ± 12.28) groups for pack years smoked (χ^2 = 11.61, p = 0.03) with post hoc analysis

228   demonstrating a difference between HY and HO groups (Z = -2.56, p = 0.01) and between HY

229   and ARM groups (Z = -3.06, p = <0.01), but not between HO and ARM groups.

230   Because a significant result for the Shapiro-Wilk test indicated non-normality for self-

231   administered lutein-based supplementation the Kruskal-Wallis non-parametric ANOVA was

232   used in place of the one-way ANOVA. There was a significant difference between the three

233   groups with both HY and HO groups not taking any self-administered lutein-based

234   supplementation whereas 3 of the 16 in the ARM group were taking a supplement (mean ±

235   sd: 2.75 ± 7.72 µg, X^2 = 11.58, p = 0.003).

236

237   **Please place table 1 about here**

238

239   Further analysis after removal of the 3 ARM participants taking the L and Z supplement from

240   the data also showed no statistical significance between ARM, HO or HY groups (F = 0.688, p

241   = 0.506).

242   Discussion

243   The aim of this study was to assess the effects of age and ARM on dietary and

244   supplementary L and Z, and MPOD levels. This study found no difference in dietary L and Z

245   or MPOD between young, old and diseased retinae using this subjective measure.

246   Nolan et al., found an age-related decline in MPOD in a study of healthy subjects up to the

247   age of 60 years. They did not assess the effects of age after the age of 60 years [32]. They also

248   reported a significantly lower than average MPOD in healthy subjects with a family history of

249   ARM, exudative age-related macular degeneration (AMD) or geographic atrophy. They did

250   assess dietary and supplement usage but they grouped supplement quantities together with

251   dietary intake values. We have statistically analysed and reported dietary and supplemented

252   L and Z separately here. A study by Beatty et al., also found an inverse relationship between

253   age and MPOD in healthy eyes. They also compared 9 eyes at risk of developing ARMD

254   (contralateral to an eye with advanced AMD) to 9 age-matched healthy eyes in this study. At

255   risk eyes had a lower MPOD than age-matched healthy eyes although they did not specify

256   whether the at risk eyes had any signs of drusen or ARM [33]. They reported dietary L and Z but

257   did not report data on supplementary forms. The Irish Longitudinal Study on Ageing did find

258   an inverse relationship between age and MPOD [34] when comparing a group aged 50 years

259   and older with a group aged 18-60 years although they did not report dietary or supplemented

260   L and Z values.

261   Conversely and consistent with our study, another study by Bartlett *et al.,* demonstrated no

262   correlation between MPOD and age [31], although the age range was limited (18-50 years,

263   mean age 25.4 ±8.2 years) when compared to our study (18-83 years, mean ± sd; 50.3 ± 18.1

264   years) and no dietary or supplementary L and Z data was reported. Other studies have also

265   shown a lack of variation in MPOD with age [35] without reporting dietary or supplemental L or

266   Z. Conversely some studies have found an increase in MPOD with age [36], but again dietary

267   and supplementary L and Z were not reported.

268   Although a statistically significant difference was seen between HY and ARM groups for

269   distance refractive spherical equivalent in our study, participants wore their distance

270   prescription when performing MPOD testing to counter any differences between groups.

271   There is no evidence in the literature that ametropia affects MPOD. We also found a

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272 statistically significant difference for ethnicity between groups, although there is no evidence

273 to suggest this would affect MPOD. There is paucity in the literature with regard to the MPOD

274 levels of Asians compared to Caucasians although one Chinese study found no difference

275 between MPOD levels between Chinese Asians and reported MPOD levels in Caucasians [37].

276 The ARM group in our study had the highest number of pack years smoked when compared

277 to the HY and HO groups. Post hoc analysis showed a statistically significant difference

278 between ARM and HY groups in pack years smoked and although no statistically significant

279 difference was found between ARM and HO groups, pack years smoked in the ARM group

280 was more than double that of the HO group. It is well documented that smokers have an

281 increased risk of developing ARMD [22-26] and previous studies have demonstrated lower

282 MPOD levels in smokers [32, 38]. An inverse relationship between ARMD and dietary lutein and

283 zeaxanthin concentrations has also been reported [39]. Thus it may be reasonable to suggest

284 that the combination of higher pack years smoked and ARM may be associated with lower

285 MPOD, although this was not the case in our study.

286 In our study supplementary L and Z were significantly higher in ARM eyes overall when

287 compared to the HY and HO groups who took no L and Z supplement. There was variation

288 within the ARM group with only 3 of the 16 ARM participants taking L and Z supplements

289 (intake range 0-30µg per day). Patient and GP awareness of ARMD and possible

290 preventative measures to reduce the risk of disease development may account for the

291 supplementary L and Z intake in this group. Higher L and Z supplementary levels in the ARM

292 group did not give rise to higher MPOD levels suggesting that either the mean supplement

293 throughout the group overall was not large enough to increase MPOD or that the retinal

294 processes do not metabolise L and Z supplementation in ARM eyes in the same way as

295 healthy eyes. It may also be that the higher pack years smoked in this group may counter any

296 effects of supplementary L and Z on MPOD levels. Further data analysis after removal of the

297 three ARM eyes taking a L and Z supplement continued to show no statistical significance

298 between HY, HO and ARM groups for MPOD.

299 Another study assessing spectral fundus reflectance found no differences in MPOD between

300 healthy and ARM eyes in a sample from a general population aged 55 years and older

301 although dietary and supplemental L and Z were not quantified in this study [36]. This echoed

302 our study but we quantified dietary and supplemental L and Z. Intervention with supplemented

303 and dietary increases in L and Z have shown increases in MPOD in eyes with ARM [40, 41]. The

304 Muenster ageing and retina study (MARS) showed increases in MPOD using

305 autofluorescence with ARM stage but this association became non-significant when the

306 influence of L and Z supplementation was adjusted for. The age group range for the MARS

307 study was 60-80 years [42]. It is still not clear whether decreased MPOD is related to an

308 increased risk of developing ARMD.

> **Comment [EJB5]:** Now included

309 The potential benefits of L and Z supplementation on visual function require further

310 investigation. Long-term randomised-controlled trials are the most stringent methods to

311 evaluate whether a cause-effect relationship exists between increased dietary or

312 supplemented L and Z levels and improved MPOD and visual function in young, old and ARM

313 eyes [43]. However, it is imperative that dietary and supplementary L and Z values are obtained

314 at baseline and throughout such studies and when comparing young, old and ARM eyes to

315 ensure the validity of MPOD results. To the authors knowledge this is the first study to directly

316 compare healthy young, healthy old and ARM eyes together with dietary and supplemented L

317 and Z for each group. Supplementation studies often undertake dietary L and Z prior to, and

318 during L and Z supplementation trials but we assessed differences between groups in a

319 sample of a population and not as part of a L and Z supplementation trial for this study.

320 To summarise we found no statistical significance in dietary L and Z between HO, HY or ARM

321 eyes in our study. There was a significant difference in supplementary L and Z in ARM eyes

322 compared to HY and HO eyes but no statistical significance for MPOD between all three

323 groups even when the three L and Z supplemented eyes in the ARM groups were removed

324 from our analysis. Based on participant's current intake of dietary and supplemental L and Z,

325 our results do not support the theory that ARM develops as a result of L and Z deficiency

326 because we have shown that the ARM group consume similar levels of L and Z as the other

327 groups. It could be that historically our ARM participants consumed low levels of L and Z and

328 that this predisposed them to ARM, although it seems likely that higher pack years smoked by

329 this group could be a factor in the development of the disease. Long-term randomised-

330 controlled trials assessing the effects of supplementation with L and Z on visual function in

331 healthy young, healthy old and eyes with ARMD may provide more tangible evidence and

332 help resolve incompatible findings from other studies. However, it is crucial that dietary and

333 supplementary L and Z levels are reported as standard when assessing MPOD between

334 groups or over time.

335 Conflict of interest

337 Acknowledgements

339 Authors' contributions

340 HB designed the study, HB and FE supervised the experiments. EB performed the

341 experiments, acquired data, performed statistical analyses and wrote the manuscript. HB and

342 FE assisted with manuscript preparation and critically reviewed the manuscript. All authors

343 have read and approved the final manuscript.

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