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

Emma J. Berrow [a,b], Hannah E. Bartlett [a], Frank Eperjesi[a]

[a]Ophthalmic Research Group, School of Life and Health Sciences, Aston University, Birmingham, United Kingdom, [b]Heart of England NHS Trust, Birmingham, United Kingdom

Correspondence to Emma J. Berrow, Ophthalmic Research Group, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, United Kingdom
Tel: +44 121 204 4208
email: berrowej@aston.ac.uk

Contact details:

Hannah E. Bartlett – h.e.bartlett@aston.ac.uk

Frank Eperjesi – f.eperjesi@aston.ac.uk

## 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) 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.


Comment [EJB1]: Now corrected

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

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

## 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 [1]. It is the leading cause of visual loss within western industrialised countries [2]. Numbers of blind registrations attributable to the disease increased by 30-40% between 1950-1990 [2] 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 [1]. Drusen are comprised of membranous debris which accumulates between the retinal pigment epithelium (RPE) and Bruch's membrane within the retina [3]. 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 [4].

The xanthophyll carotenoids lutein (L) and zeaxanthin (Z) are antioxidants that can only be obtained through ingestion from the diet [5] and together with meso-zeaxanthin make up macular pigment (MP). Situated within the central 5-10 degrees of the retina [6], MP concentrations are highest within the photoreceptor axons of the fovea [7], 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

**Comment [EJB2]:** Written in full as it is the start of a sentence

damaging short-wavelength blue light irradiation with a peak absorbance spectrum of 460nm [8], thus reducing the amount of harmful light irradiation reaching the photoreceptoral 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 [9]. The antioxidant properties of carotenoids quench reactive oxygen species and singlet oxygen, thus reducing oxidative stress and lipid peroxidation within the retina [10].

Augmented dietary L and Z levels have been associated with a reduced risk of developing ARMD in some studies[11, 12] but not in others[13, 14]. Dietary intervention and supplementation with L and Z have also been associated with improved measures of vision, including visual acuity, contrast sensitivity and electroretinographic measures in eyes with ARMD[15, 16]. Because L and Z are the only carotenoids found within the retina, with meso-zeaxanthin being synthesized from L [17], their tentative role in reducing risk for ARMD development remains of interest.

Ageing[18-21], smoking [22-26] and genetics [27-29] 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 ARM 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 [1].

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 ($\chi^2 = 10.56$, p = 0.01, p = 0.01). There was no significant difference between gender and groups ($\chi^2 = 0.14$, 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 [1].

Comment [EJB3]: Now included

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

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 ($\chi^2$ = 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

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 [32]. 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 [33]. 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 [34] 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.

Comment [EJB4]: Now amended

Conversely and consistent with our study, another study by Bartlett *et al.,* demonstrated no correlation between MPOD and age [31], 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 [35] without reporting dietary or supplemental L or Z. Conversely some studies have found an increase in MPOD with age [36], 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

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-

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.

References

1. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Surv Ophthalmol* 1995;39:367-374.
2. Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? *Br J Ophthalmol* 1996;80:9-14.
3. Curcio C, Millican C. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Archives of Ophthalmology* 1999;117:329-339.
4. The AREDS Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss - AREDS Report No. 8. *Archives of Ophthalmology* 2001;119:1417-1436.
5. Bone R, Landrum J, Tarsis S. Preliminary identification of the human macular pigment. *Vision Res* 1985;25:1531-1535.
6. Hammond BR, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *Journal of the Optical Society of America a-Optics Image Science and Vision* 1997;14:1187-1196.
7. Snodderly DM, Auran J, Delori F. The macular pigment II. Spatial distribution in primate retinas. *Investigative Ophthalmological Vision Science* 1984;25:674-685.
8. Snodderly DM, Brown B, Delori F. The macular pigment I. Absorbance spectra, localisation, and discrimination from other yellow pigments in primate retinas. *Investigative Ophthalmological Vision Science* 1984;25:660-673.
9. Organisciak DT, Vaughan DK. Retinal light damage: mechanisms and protection. *Prog Retin Eye Res* 2009;29:113-134.
10. Krinsky NID, S.M. Interaction of oxygen and oxy-radicals with carotenoids. *J Natl Cancer Inst* 1982;69:205-210.
11. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci* 2001;42:235-240.
12. Delcourt C, Carriere I, Delage M, Barberger-Gateau P, Schalch W. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest Ophthalmol Vis Sci* 2006;47:2329-2335.
13. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-controlled study. *Archives of Ophthalmology* 1995;113:1518-1523.
14. MaresPerlman JA, Klein R, Klein BEK, et al. Association of zinc and antioxidant nutrients with age-related maculopathy. *Archives of Ophthalmology* 1996;114:991-997.
15. Richer S. ARMD--pilot (case series) environmental intervention data. *J Am Optom Assoc* 1999;70:24-36.
16. Parisi V, Tedeschi M, Gallinaro G, Varano M, Saviano S, Piermarocchi S. Carotenoids and antioxidants in age-related maculopathy italian study: multifocal electroretinogram modifications after 1 year. *Ophthalmology* 2008;115:324-333 e322.
17. Bone R, Landrum J, Friedes L, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res* 1997;64:211-218.
18. Smith W, Assink J, Klein R, et al. Risk factors for age related macular degeneration - Pooled findings from three continents. *Ophthalmology* 2001;108:697-704.
19. The AREDS Research Group. Risk factors associated with age-related macular degeneration - A case-control study in the Age-Related Eye Disease Study: Age- Related Eye Disease Study report number 3. *Ophthalmology* 2000;107:2224-2232.

20. Krishnaiah S, Das T, Nirmalan PK, et al. Risk factors for age-related macular degeneration: Findings from the Andhra Pradesh Eye Disease Study in South India. *Investigative Ophthalmology & Visual Science* 2005;46:4442-4449.
21. Song SJ, Youm DJ, Chang Y, Yu HG. Age-related macular degeneration in a screened South Korean population: prevalence, risk factors, and subtypes. *Ophthalmic Epidemiol* 2009;16:304-310.
22. EDCCS Group. Risk factors for neovascular age-related macular degeneration. The Eye Disease Case Control Study Group. *Archives of Ophthalmology* 1992;110:1701-1708.
23. Chakravarthy U, Augood C, Bentham GC, et al. Cigarette smoking and age-related macular degeneration in the EUREYE study. *Ophthalmology* 2007;114:1157-1163.
24. Klein R, Knudtson MD, Cruickshanks KJ, Klein BEK. Further observations on the association between smoking and the long-term incidence and progression of age-related macular degeneration. *Archives of Ophthalmology* 2008;126:115-121.
25. Neuner B, Komm A, Wellmann J, et al. Smoking history and the incidence of age-related macular degeneration-Results from the Muenster Aging and Retina Study (MARS) cohort and systematic review and meta-analysis of observational longitudinal studies. *Addictive Behaviors* 2009;34:938-947.
26. Coleman AL, Seitzman RL, Cummings SR, et al. The Association of Smoking and Alcohol Use With Age-related Macular Degeneration in the Oldest Old: The Study of Osteoporotic Fractures. *Am J Ophthalmol* 2010;149:160-169.
27. Yoshimura N. Age-related macular degeneration and genetics. *Clin Experiment Ophthalmol* 2010;38:1.
28. Katta S, Kaur I, Chakrabarti S. The molecular genetic basis of age-related macular degeneration: an overview. *J Genet* 2009;88:425-449.
29. Meyers S. A twin study on age-related macular degeneration. *Trans Am Ophthalmol Soc* 1994;92:775-844.
30. Newcombe RG, Duff GR. EYES OR PATIENTS - TRAPS FOR THE UNWARY IN THE STATISTICAL-ANALYSIS OF OPHTHALMOLOGICAL STUDIES. *Br J Ophthalmol* 1987;71:645-646.
31. Bartlett H, Stainer L, Singh S, Eperjesi F, Howells O. Clinical evaluation of the MPS 9000 Macular Pigment Screener. *Br J Ophthalmol* 2010;94:753-756.
32. Nolan JM, Stack J, O OD, Loane E, Beatty S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res* 2007;84:61-74.
33. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci* 2001;42:439-446.
34. Nolan JM, Kenny R, O'Regan C, et al. Macular pigment optical density in an ageing Irish population: The Irish Longitudinal Study on Ageing. *Ophthalmic Res* 2010;44:131-139.
35. Ciulla TA, Hammond BR, Jr. Macular pigment density and aging, assessed in the normal elderly and those with cataracts and age-related macular degeneration. *Am J Ophthalmol* 2004;138:582-587.
36. Berendschot TT, Willemse-Assink JJ, Bastiaanse M, de Jong PT, van Norren D. Macular pigment and melanin in age-related maculopathy in a general population. *Invest Ophthalmol Vis Sci* 2002;43:1928-1932.
37. Tang CY, Yip HS, Poon MY, Yau WL, Yap MK. Macular pigment optical density in young Chinese adults. *Ophthalmic Physiol Opt* 2004;24:586-593.
38. Kirby ML, Beatty S, Loane E, et al. A central dip in the macular pigment spatial profile is associated with age and smoking. *Invest Ophthalmol Vis Sci* 2010;51:6722-6728.

39. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamin A,C and E and advanced age-related macular degeneration. *Jama-Journal of the American Medical Association* 1994;272:1413-1420.
40. Koh HH, Murray IJ, Nolan D, Carden D, Feather J, Beatty S. Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp Eye Res* 2004;79:21-27.
41. Wenzel AJ, Gerweck C, Barbato D, Nicolosi RJ, Handelman GJ, Curran-Celentano J. A 12-wk egg intervention increases serum zeaxanthin and macular pigment optical density in women. *J Nutr* 2006;136:2568-2573.
42. Dietzel M, Zeimer M, Heimes B, Claes B, Pauleikhoff D, Hense HW. Determinants of Macular Pigment Optical Density and its Relation to Age-Related Maculopathy -- Results from the Muenster Aging and Retina Study (MARS). *Invest Ophthalmol Vis Sci* 2011.
43. Sibbald B, Roland M. Understanding controlled trials - Why are randomised controlled trials important? *Br Med J* 1998;316:201-201.