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Risk factors for age-related maculopathy are associated with a relative lack of macular pigment☆

John M Nolan [a,*], Jim Stack [b], Orla O' Donovan [a], Edward Loane [a], Stephen Beatty [a,c]

[a] Macular Pigment Research Group, Department of Chemical and Life Sciences, Waterford Institute of Technology, Cork Road, Waterford, Ireland
[b] Department of Physical and Quantitative Sciences, Waterford Institute of Technology, Cork Road, Waterford, Ireland
[c] Department of Ophthalmology, Waterford Regional Hospital, Waterford, Ireland

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Abstract

Macular pigment (MP) is composed of the two dietary carotenoids lutein (L) and zeaxanthin (Z), and is believed to protect against age-related maculopathy (ARM). This study was undertaken to investigate MP optical density with respect to risk factors for ARM, in 828 healthy subjects from an Irish population. MP optical density was measured psychophysically using heterochromatic flicker photometry, serum L and Z were quantified by HPLC, and dietary intake of L and Z was assessed using a validated food-frequency questionnaire. Clinical and personal details were also recorded, with particular attention directed towards risk factors for ARM. We report a statistically significant age-related decline in MP optical density ($r^2 = 0.082$, $p < 0.01$). Current and past smokers had lower average MP optical density than never smokers and this difference was statistically significant ($p < 0.01$). Subjects with a confirmed family history of ARM had significantly lower levels of MP optical density than subjects with no known family history of disease ($p < 0.01$). For each of these established risk factors, their statistically significant negative association with MP persisted after controlling for the other two, and also after controlling for other potentially confounding variables such as sex, cholesterol, dietary and serum L ($p < 0.01$). In the absence of retinal pathology, and in advance of disease onset, the relative lack of MP seen in association with increasing age, tobacco use and family history of ARM supports the hypothesis that the enhanced risk that these variables represent for ARM may be attributable, at least in part, to a parallel deficiency of macular carotenoids.

Keywords: age-related macular degeneration; age-related maculopathy; antioxidant; blue light damage; lutein; macular pigment; oxidative stress; zeaxanthin

1. Introduction

The macula is a specialised part of the retina, and is responsible for central and colour vision. Age-related macular degeneration (AMD) is the advanced form of age-related maculopathy (ARM), and results in loss of central vision (Bird et al., 1995), and is the leading cause of severe visual impairment in elderly white populations in the Western World. The prevalence of AMD, also known as late ARM, is likely to rise as a consequence of increasing longevity (Klein et al., 1992). Although the pathogenesis of ARM (early and/or late) remains unclear, it is plausible that cumulative blue light damage and/or oxidative stress play a role (Beatty et al., 2000).

A pigment, composed of two hydroxycarotenoids, lutein (L) and zeaxanthin (Z), accumulates at the macula where it is known as macular pigment (MP). MP is a blue light filter (Snodderly et al., 1984) and a powerful antioxidant (Khachik et al., 1997), and may therefore protect against ARM.

Indeed, there is a growing body of circumstantial evidence in support of the view that MP protects against ARM (Beatty et al., 1999). In this context, the term circumstantial evidence refers to parallels between a ''relative lack'' (see statistical analysis) of MP and risk factors (putative and/or established) for ARM. These risk factors include: increasing age; cigarette

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* Corresponding author. Tel.: +353 51 845505; fax: +353 51 302679.
E-mail address: jnolan@wit.ie (J.M. Nolan).

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smoking; family history of ARM; sex; light iris colour; obesity; low carotenoid diet; low serum carotenoid levels; disease in fellow eye (Broekmans et al., 2002; Curran-Celentano et al., 2001; Hammond et al., 1996a,b; Hammond et al., 2002; Nolan et al., 2004). To date, however, the results of studies are inconsistent (possibly due to small sample sizes and/or differing methodologies), and therefore it is difficult to draw a firm conclusion regarding the protection, if any, that MP confers against ARM.

Our study was undertaken to investigate MP optical density in 828 healthy Irish subjects (largest sample studied to date), and to relate our findings to putative and established risk factors for ARM.

2. Methods

2.1. Subjects

Eight hundred and twenty-eight healthy subjects volunteered to participate in this study, which was endorsed by the Research Ethics Committee of the Waterford Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Subjects were recruited to this single-visit study by one of two means. First, a self-selected sample population volunteered as a result of posters, newsletters and word of mouth in the local community (Group 1: $n = 647$). And second, patients attending the Department of Ophthalmology at Waterford Regional Hospital with ARM (early and/or late) were encouraged to invite their offspring to participate (Group 2: $n = 181$). Any bias arising from the different method of recruitment for the subjects with a clinically confirmed family history of ARM was likely to be minimal, as all subjects came from the same homogeneous population in the south-east of Ireland. Also, the same large number of possible explanatory variables were collected from both cohorts, enabling us to control (statistically) for differences between the two cohorts with respect to these variables.

The inclusion criteria were Caucasian race and age between 20 and 60 years, and exclusion criteria comprised any demonstrable ocular pathology (visible on screening undilated fundus photography), visual acuity less than 6/18 in both eyes, or pregnancy. Best-corrected visual acuity (Snellen and LogMAR) was measured for each eye using a Lighthouse Distance Visual Acuity Test (second edition). Photographic documentation of the macula was obtained from each subject using a NIDEK handheld fundus camera. An experienced ophthalmologist inspected all images and no subject was excluded on the basis of fundus findings.

Eighteen subjects were excluded from Group 1 and a further six subjects were excluded from Group 2, as they were unable to use the Maculometer during MP testing (coefficient of variation [CoV] $> 20\%$). A further four subjects were excluded from Group 1 as their visual acuity was less than 6/18 in both eyes.

2.2. Personal details questionnaire/risk factor index

The following details were recorded for each volunteer using a personal details questionnaire: demographic data; general health status, with particular attention directed towards cardiovascular disease; blood pressure levels; cholesterol levels; eye disease; refractive status; use of medications. Also, average weekly alcohol consumption was estimated for each subject (ranging from 0 to 20+ units), as was tobacco use which was recorded as follows: never; current [measure per day]; past [measure per day and date stopped smoking]. Body mass index (BMI) was obtained by recording the subject's height (m) and weight (kg). BMI was calculated as kg/m^2. Photographic documentation of the iris was obtained from each subject (using a NIDEK handheld camera) and was graded by matching to colours on an Iris Cooper Vision Chart. In addition, iris colour was graded based on self-report.

2.3. Food-frequency questionnaire

A self-administered, semi-quantitative food-frequency questionnaire (FFQ) developed by the Scottish Collaborative Group was used for dietary analysis.

This FFQ was developed based on FFQs used in the Scottish Heart Health Study (Bolton-Smith et al., 1991), and was previously validated (Bodner et al., 1998; Masson et al., 2003). The questionnaire was designed to estimate habitual diet over the previous 2–3 months, which included 166 commonly eaten types of food or drink, grouped into 19 selections. A portion or measure for each food was specified and subjects were asked to record how many measures per day and how many days per week they consumed the food ranging from "rarely or never" to "7 days per week". Also, this FFQ was designed to monitor and quantify a subject's supplement usage (e.g. if a subject was supplementing with L and/or Z, this would have been added to their dietary intake values). The questionnaire was completed by the volunteer in the presence of the primary investigator (JN), and took between 25 and 35 min to complete.

The FFQs were scanned and verified by a trained dietary data coder using optical recognition software (Teleform Version 7 Cardiff Software, Vista, CA, USA) at the Medical Research Council Human Nutrition Research, Cambridge. Nutrient analysis was conducted using Oracle Relational Database Management System (Version 7) (Holland et al., 1991). Dietary intake of L and Z was calculated using food composition data from UK, European and US data sources (O'Neill et al., 2001; United States Department of Agriculture, 1998) using standard principles or criteria for the matching of food items and standardised recipes or manufacturer ingredient information where necessary (Foods Standards Agency, 2002; Rand et al., 1987, 1991).

2.4. Serum carotenoid analysis

Blood samples (6–8 ml) were collected from all subjects, on the same day as the dietary and MP optical density

analysis. Serum was separated from blood by centrifugation, and then aliquoted into three light-sensitive micro-centrifuge tubes and stored at −76 °C until time of analysis.

Duplicate extractions were carried out for each serum sample. A 0.4 ml aliquot of serum was pipetted into a light-sensitive micro-centrifuge tube (2 ml total capacity). Ethanol (0.30 ml) containing 0.25 g/l butylated hydroxytoluene (BHT) and internal standard (tocopherol acetate) were added to each tube. Heptane (0.5 ml) was then added and samples were vortexed vigorously for 1 min followed by centrifugation at 2000 rpm for 5 min (MSC Micro Centaur, Davison & Hardy Ltd., Belfast, UK). The resulting heptane layer was retained and transferred to a second labelled, light-sensitive micro-centrifuge tube, and a second heptane extraction was performed. The combined heptane layers were immediately evaporated to dryness under nitrogen. These dried samples were reconstituted in methanol (200 µl), and 150 µl was injected for high-performance liquid chromatography (HPLC) analysis.

We used a Hewlett–Packard (HP 1090 LC; Agilent, Dublin, Ireland) system with photodiode array detection. A 5-µm analytical/preparative 4.6 × 250 mm 201TP speciality reverse phase column (Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran. The flow rate was 1 ml/min, and the total run time was 15 min. All carotenoid peaks were integrated and quantified using Agilent Chem Station software.

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards, which were used to generate standard curves for quantification of these carotenoids. This assay was validated against the National Institute of Standards and Technology (NIST) Standard.

2.5. Measurement of macular pigment

MP was measured psychophysically by heterochromatic flicker photometry (HFP) with a portable screening instrument which has been described in more detail elsewhere (Mellerio et al., 2002).

2.5.1. Principle

The concentration of MP peaks at the centre of the fovea, and is optically undetectable at an eccentricity of 5° (Snodderly et al., 1984). In a test field which alternates between blue light (470 nm, max MP absorption is at 460 nm) and green light (530 nm, not absorbed by MP), the luminance of the blue is varied until there is no or minimal flicker which means the lights are isoluminant. Isoluminance matches are made with the test field at 0.5° (foveal) eccentricity and at 5.5° (parafoveal) eccentricity, so the logarithm of the ratio of the blue luminances in the fovea to that in the parafovea gives the MP optical density.

2.5.2. Light sources

The maculometer uses light emitting diodes (LEDs) as light sources and Fig. 1 shows their normalised spectral power distribution (SPD) together with the normalised absorption spectrum of MP.

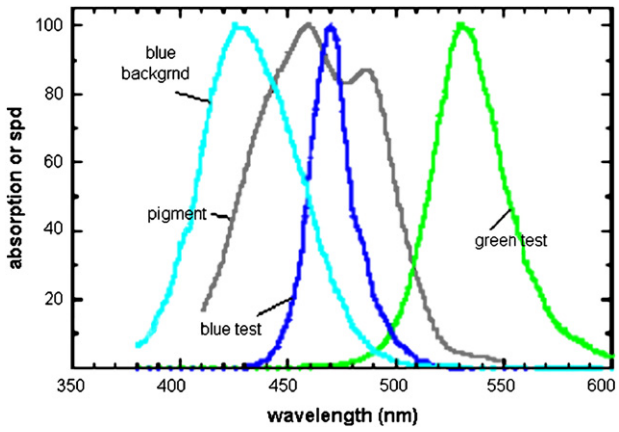

Fig. 1. Normalised spectral power distribution of the LEDs used in the maculometer together with the normalised spectrum of MP.

2.5.3. Test fields

The test fields are viewed at a distance of 330 mm and the foveal field subtends a diameter of 1° at the eye. The parafoveal test field comprises two 90° sector arcs of 10° inner diameter and of 1° width concentric with the foveal field with one horizontally on each side of the central fixation point.

2.5.4. Background adapting field

To ensure the S cones play no part in the minimum flicker match they are saturated by a circular 15° background field of blue light; the normalised SPD is shown in Fig. 1.

2.5.5. Frequency of flicker

Not only must the S cones be prevented from participating in making the isoluminance matches, the rods must also be excluded. This is achieved by arranging the blue/green alternation frequency to be above the critical fusion frequency (CFF) for rods. In the parafoveal arcuate fields, this frequency is set to 13 Hz and in the foveal field to 18 Hz.

The HFP technique we used (Mellerio et al., 2002) did not have the option to adjust flicker frequency. This inability to customise flicker frequency for each subject is a limitation of the current device, as it may lead to increased uncertainty of the match endpoint, particularly in older people as the CFF reduces with age (Snodderly et al., 2004). However, all our subjects were reasonably young (age range: 20–60 years) and were given detailed instructions by the primary investigator (JN) prior to performing the task. Also, any subject whose CoV of blue luminance settings exceeded 20% was not included in the study.

2.5.6. Procedure

Subjects were allowed to make two or three trial minimum flicker matches before measurements were recorded. When the subject was satisfied that minimum flicker had been achieved, he/she pressed the sample and hold button and the blue luminance was recorded. After each match, the investigator set the blue luminance control to some new random position. Six foveal readings were obtained, followed by six parafoveal readings, for each eye.

The readout for the foveal and the parafoveal conditions were entered into a spreadsheet, which contained the appropriate calibration relationships and calculated the MP optical density.

The reproducibility and test–retest variability for the maculometer has recently been assessed and is reported elsewhere (Nolan et al., 2004).

2.6. Statistical analysis

The statistical software package SPSS (Version 11) was used for analysis. Our main method of analysis was multiple linear regression, with MP optical density as the dependent variable, and known and putative risk factors for ARM as potential explanatory variables. Boxplots were used for graphical analysis of scores between different categories of subjects; analytically, these differences were investigated using one-way analysis of variance or independent samples *t*-tests, as appropriate. Pearson correlation coefficients were calculated to investigate the relationship between bivariables, and partial correlations when controlling for confounding variables.

A goal of this study was to compare MP optical density across various subject categories (e.g. males vs. females, smokers vs. non-smokers, etc.), and also to relate MP optical density to other continuous explanatory variables (e.g. age). Where the relationship between such variables reached statistical significance ($p < 0.05$), we used the term ''relative lack'' to denote the group, or variable, which demonstrated a statistical ''lack''.

3. Results

The demographic, medical, lifestyle, anthropometric, and visual data of the 800 subjects who met the inclusion criteria are summarized in Table 1.

Table 1
Demographic, medical, lifestyle, anthropometric, and visual data of the 800 subjects who met the inclusion criteria

Characteristic	*n*	%
Age (y)		
20–35	261	33
36–48	287	35
49–60	252	32
Sex		
Male	288	36
Female	512	64
Smoking status		
Current smokers (≥1 cigarette per day)	155	19
Past smokers (stopped smoking for at least 1 month)	205	26
Never smoked (never smoked in entire lifetime)	440	55
Family history (FH) of ARM		
No known FH of ARM	625	78
Clinically confirmed FH of early ARM	41	5
Clinically confirmed FH of geographic atrophy	55	7
Clinically confirmed FH of choroidal neovascularisation	79	10
Body mass index (BMI)		
Desirable weight (BMI < 25)	456	57
Overweight (BMI 25 - 30)	243	30
Obese (BMI > 30)	101	13
Alcohol consumption (units per week)		
0	134	17
0–1	121	15
2–5	252	32
6–10	155	19
>10	138	17
Iris colour		
Light (blue, green, grey)	691	86
Dark (brown, hazel)	109	14
Cholesterol levels (self-reported)		
Normal	699	87
High (>6.2 mmol/L)	101	13
Blood pressure (self-reported)		
Normal	726	91
High	74	9
Visual acuity ≥ 6/9 in the study eye	800	100

3.1. MP optical density

The optical density of the MP in 800 healthy subjects had a mean (±SD) value of 0.299 (±0.169), with a range of 0 to 0.868. For 49 subjects, MP optical density was measured in one eye only as the other eye's visual acuity was less than 6/18 and therefore did not fit the required criteria for testing. For the remaining 751 subjects, the 95% CI for difference in MP optical density (right–left) was −0.018 to −0.0046. This interval does not include 0, indicating that right eye MP was slightly lower than left eye MP, on average. However, for 49.8% of the subjects the difference was negative, and for 41.9% this difference was positive, with zero difference found for the remaining 8.3% of subjects. For the remainder of analysis, we report on right eye MP optical density.

3.2. MP optical density and its relationship with risk factors for ARM as assessed by multiple linear regression analysis

Multiple linear regression analysis (using indicator variables for the categorical variables) was performed to analyze the relationship between MP optical density and the following known and putative risk factors for ARM: dietary L; dietary Z; dietary fat; dietary *n*−3 docosahexaenoic acid (DHA); serum L, serum Z; age; sex; smoking habits; family history of early ARM; family history of geographic atrophy (GA); family history of choroidal neovascularisation (CNV); BMI; alcohol consumption; iris colour; cholesterol levels (self-reported); blood pressure (self-reported).

Variables for all these factors were included initially in a multiple regression model (with MP optical density as the dependent variable). Statistically non-significant variables were then removed, one by one, using the 5% level of significance as the criterion for removal. The regression model eventually obtained is presented in Table 2; this model explains about 25% of the variation in MP. The cholesterol variable in this model is of marginal significance; had we (to allow for multiple testing) used the more stringent 1% significance level, this variable would not appear in the final model.

A comparison of descriptive characteristics was carried out for the following groups: subjects with and without a clinically confirmed family history of ARM; current smokers, past smokers and never smokers; males and females. Results are presented in Tables 3–5, respectively. As seen from all three Tables, the percentages for categorical variables under investigation (e.g. sex, smoking status, family status) were similar across all sub-groups tested, as confirmed by the chi-squared statistic ($p > 0.05$, for all). However, in some cases, continuous variables (e.g. age, serum L) were significantly different between sub-groups tested [Tables 3–5]. Multiple linear regression analysis provides a means of dealing with this potential confounding.

3.3. Age and MP optical density

The subjects ranged in age from 20 to 60 years, with a mean (±SD) of 41.94 (±11.62). A statistically significant age-related decline in MP optical density was observed. The regression equation for MP optical density vs. age was:

$$\mathrm{MP} = 0.475 - 0.0042\mathrm{Age}$$

and was significant at the 0.001 level. The associated r^2 value was 0.082, indicating a lot of unexplained variation. Nevertheless, the graph in Fig. 2, which shows average MP optical density in each 10-year age group, strongly suggests a systematic decline with increasing age.

Table 2
Association between medical, lifestyle and anthropometric variables and MP optical density, for the 800 subjects

Model	Unstandardised coefficients		Standardised coefficients		
	B	Std. error	B	*t*	Sig.
Constant	0.363	0.024		14.854	0.000
Age (years)	−0.004	0.000	−0.251	−7.466	0.000
Sex	0.047	0.011	0.132	4.207	0.000
Serum L (μg/day)	0.822	0.134	0.204	6.134	0.000
Confirmed family history of early ARM[a]	−0.105	0.024	−0.138	−4.418	0.000
Confirmed family history of GA[b]	−0.105	0.021	−0.157	−4.98	0.000
Confirmed family history of CNV[c]	−0.103	0.018	−0.18	−5.664	0.000
Dietary L (mg/day)	0.03	0.006	0.174	5.361	0.000
High cholesterol (self-reported)	−0.033	0.017	−0.064	−1.961	0.049
Smoking habits	−0.019	0.007	−0.086	−2.735	0.006

$n = 800$, $r^2 = 0.250$.
Dependant variable = MP optical density; sex (indicator variable: 1 = male, 0 = female); smoking status (0 = never smoked, 1 = past smoker, 2 = current smoker); confirmed family history of early ARM, GA or CNV (each coded 1 for membership of that group, otherwise 0); cholesterol (1 = high, 0 = low).
[a] Confirmed family history of early ARM = subjects with a clinically confirmed FH of early ARM (offspring only).
[b] Confirmed family history of GA = subjects with a clinically confirmed family history of geographic atrophy/late ARM (offspring only).
[c] Confirmed family history of CNV = subjects with a clinically confirmed family history of choroidal neovascularisation/late ARM (offspring only).

Of note, age remained as a significant negative predictor of MP optical density, with virtually the same coefficient (−0.004) when analysed in a variety of multiple linear regression models, i.e. after controlling for cigarette smoking, family history status, and other variables such as sex, dietary and serum L, cholesterol, etc. [Table 2, age variable has coefficient −0.004, $p < 0.01$].

3.4. Family history of ARM and MP optical density

Initially, for this analysis we divided our data into two groups [Group 1: subjects with no known family history (FH) of ARM, $n = 625$; Group 2: subjects with a clinically confirmed FH of ARM (early and/or late), $n = 175$]. The mean MP optical density for Group 2 was significantly lower than Group 1 ($p < 0.01$) [Table 3].

Sub-dividing the healthy subjects with a clinically confirmed FH of ARM into three sub-groups (Group A: FH of early ARM, $n = 41$; Group B: FH of GA, $n = 55$; Group C: FH of CNV, $n = 79$) [Table 3] we found that the average MP levels in each of these sub-groups was significantly lower than the average MP levels for 625 healthy subjects with no known family history of disease ($p < 0.01$) [Fig. 3]. Of note, groups A, B and C remained as significant negative predictors of MP optical density even after controlling for other variables [Table 2, $p < 0.01$, for all].

3.5. Cigarette smoking and MP optical density

Of the 800 volunteers that met the inclusion criteria, 440 were subjects who never smoked, 205 were past smokers, and 155 were current smokers. The current and past smokers had lower average MP optical density [mean (±SD)] [0.268 (±0.162) and 0.290 (±0.166), respectively] than the never smokers [0.315 (±0.171)], and this difference was statistically significant ($p < 0.01$) [Table 4, Fig. 4]. As with age and family history of ARM, cigarette smoking remained as a significant negative predictor of MP optical density in our final multiple

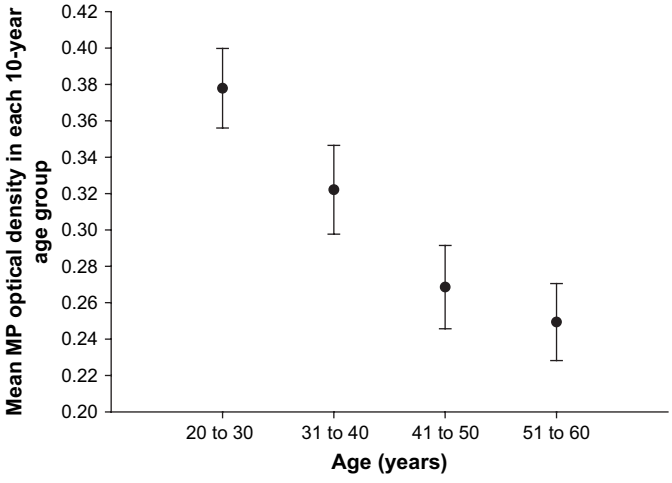

Fig. 2. Mean MP optical density by 10-year age groups; including 95% confidence intervals for the mean denoted by error bars.

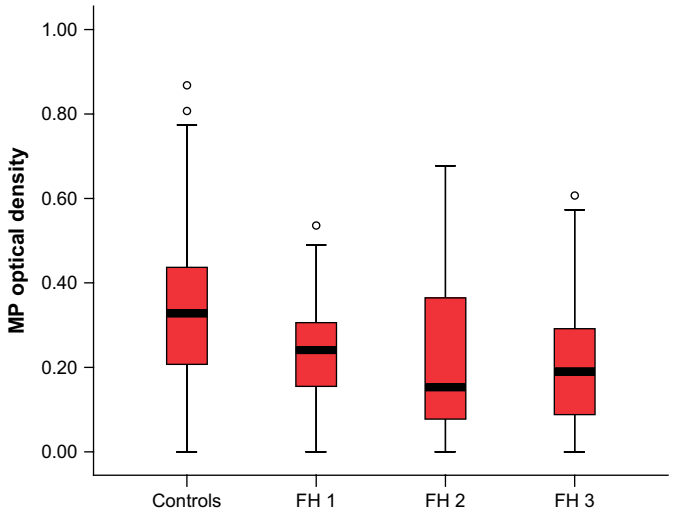

Fig. 3. Boxplots showing MP optical density for subjects with and without a clinically confirmed family history of age-related maculopathy (ARM). Controls: subjects with no known family history of ARM ($n = 625$); FH 1: subjects with a clinically confirmed family history of early ARM ($n = 41$); FH 2: subjects with a clinically confirmed family history of geographic atrophy ($n = 51$); FH 3: subjects with a clinically confirmed family history of choroidal neovascularisation ($n = 79$).

linear regression model ($p < 0.01$) i.e. after controlling for other explanatory variables (including dietary and serum L, sex, etc.) [Table 2]. There was a statistically significant inverse relationship between smoking frequency for the current smokers (cigarettes per day) and MP optical density ($r = -0.239$, $p < 0.01$) [Fig. 5], which remained significant after adjusting for dietary intake of L and Z ($r = -0.205$, $p < 0.01$).

3.6. Sex and MP optical density

The MP optical density [mean (±SD)] for males ($n = 288$) and females ($n = 512$) was 0.331 (±0.161) and 0.282 (±0.171), respectively, and this difference was statistically significant ($p < 0.01$) [Table 5]. Also, female sex was a significant negative predictor of MP optical density when analysed in a multiple linear regression model, i.e. controlling for other explanatory variables ($p < 0.01$) [Table 2].

3.7. Dietary and serum L (and Z), and MP optical density

Dietary and serum concentrations of L and Z were positively related to MP optical density ($r = 0.185$–0.230, $p < 0.01$, for all). However, in the multiple linear regression

Table 3
Comparison of descriptive characteristics for subjects with and without a clinically confirmed family history of ARM[a]

Characteristic	No known FH of ARM ($n = 625$)[b]	FH of early ARM, GA, & CNV combined ($n = 175$)[c]	Subdivision of family history group		
			FH of early ARM ($n = 41$)[d]	FH of GA ($n = 55$)[e]	FH of CNV ($n = 79$)[f]
MP optical density**	0.322 ± 0.166	0.219 ± 0.15	0.237 ± 0.12	0.214 ± 0.18	0.207 ± 0.16
Age**	41.20 ± 12.16	44.31 ± 9.15	38.70 ± 9.46	46.01 ± 7.06	46.62 ± 8.53
BMI*	25.19 ± 4.43	24.81 ± 4.12	24.13 ± 4.43	23.95 ± 3.13	25.82 ± 4.59
Diet					
Lutein (mg/day)	1.37 ± 0.95	1.50 ± 1.08	1.61 ± 0.99	1.53 ± 0.90	1.39 ± 1.11
Zeaxanthin (mg/day)	0.198 ± 0.118	0.203 ± 0.117	0.235 ± 0.144	0.195 ± 0.095	0.187 ± 0.109
Fat (g/day)	103 ± 37	107 ± 43	119 ± 55	102 ± 31	103 ± 42
DHA (mg/day)	349 ± 294	387 ± 400	366 ± 407	413 ± 347	388 ± 440
Serum					
Lutein (μg/ml)**	0.084 ± 0.040	0.095 ± 0.047	0.086 ± 0.050	0.098 ± 0.040	0.099 ± 0.050
Zeaxanthin (μg/ml)	0.026 ± 0.016	0.027 ± 0.015	0.026 ± 0.013	0.027 ± 0.013	0.029 ± 0.013
Sex					
Male	232 (37)	56 (32)	15 (37)	13 (24)	28 (35)
Female	393 (63)	119 (68)	26 (63)	42 (76)	51 (65)
Smoking status					
Current smokers	123 (20)	32 (18)	8 (20)	7 (13)	17 (21)
Past smokers	159 (26)	46 (26)	9 (22)	12 (22)	25 (32)
Never smoked	339 (54)	101 (56)	24 (58)	36 (65)	37 (47)
Cholesterol levels					
High	77 (12)	24 (14)	3 (7)	7 (13)	14 (18)
Normal	548 (87)	151 (86)	38 (93)	48 (87)	65 (82)

* Significant difference between groups at 0.05 level (2-tailed).
** Significant difference between groups at the 0.01 level (2-tailed).
[a] Mean ± SD for continuous data, number (%) for categorical data.
[b] No known FH of ARM = subjects who reported having no known family history of ARM.
[c] FH of early ARM, GA, & CNV combined = subjects with a clinically confirmed family history of early ARM, geographic atrophy or choroidal neovascularisation (offspring of ARM sufferers only).
[d] FH of early ARM = subjects with a clinically confirmed family history of early ARM (offspring only).
[e] FH of GA = subjects with a clinically confirmed family history of geographic atrophy/late ARM (offspring only).
[f] FH of CNV = subjects with a clinically confirmed family history of choroidal neovascularisation/late ARM (offspring only).

Table 4
Comparison of descriptive characteristics in current smokers, past smokers and never smokers[a]

Characteristic	Current smokers[b] ($n = 155$)	Past smokers[c] ($n = 205$)	Never smokers[d] ($n = 440$)
MP optical density**	0.268 ± 0.162	0.290 ± 0.166	0.315 ± 0.171
Age**	38 ± 11.45	45 ± 10.19	41 ± 11.95
BMI	25.1 ± 4.93	25.63 ± 4.78	24.8 ± 3.92
Diet			
Lutein (mg/day)	1.412 ± 1.02	1.383 ± 0.861	1.407 ± 1.02
Zeaxanthin (mg/day)	0.204 ± 0.139	0.203 ± 0.116	0.195 ± 0.110
Fat (g/day)**	112 ± 47	106 ± 37	101 ± 36
DHA (mg/day)	357 ± 375	392 ± 320	342 ± 300
Serum			
Lutein (μg/ml)*	0.073 ± 0.042	0.086 ± 0.037	0.091 ± 0.043
Zeaxanthin (μg/ml)	0.025 ± 0.165	0.024 ± 0.014	0.027 ± 0.015
Sex			
Male	49 (32)	80 (39)	159 (36)
Female	106 (68)	125 (61)	281 (64)
Family history of ARM[e]			
No family history of ARM	123 (79)	159 (77)	339 (77)
Confirmed family history of ARM	32 (21)	46 (23)	101 (23)
Cholesterol levels			
High	17 (11)	26 (13)	58 (13)
Normal	138 (89)	179 (87)	382 (87)

* Significant difference between groups at 0.05 level (one-way ANOVA).
** Significant difference between groups at the 0.01 level (one-way ANOVA).
[a] Mean ± SD for continuous data, number (%) for categorical data.
[b] Current smokers = subjects who smoke (on average) at least one cigarette per day.
[c] Past smokers = subjects who have given up smoking for at least one month.
[d] Never smokers = subjects who never smoked cigarettes in their lifetime.
[e] Family history of ARM: No family history of ARM = subjects who reported having no known family history of any type of ARM (early or late). Confirmed family history of ARM = subjects with a clinically confirmed family history of any type of ARM, early ARM, geographic atrophy/late ARM or choroidal neovascularisation/late ARM (offspring of ARM sufferers only).

models in which dietary and serum L and Z were both included, only dietary and serum L were found to be positive predictors of MP optical density, possibly due to collinearity between these variables (diet L/diet Z: $r = 0.697$; serum L/serum Z: $r = 0.469$, $p < 0.01$, for both). We intend to report on the relationships between MP optical density and serum concentrations of L (and Z) and dietary intake of L (and Z) in a separate paper.

3.8. Serum cholesterol levels and MP optical density

The mean (±SD) optical density of MP for subjects who self-reported having high levels of cholesterol (>6.2 mmol/L) was 0.234 (±0.153), which was significantly lower when compared to subjects who self-reported having normal cholesterol levels 0.309 (±0.169) ($p < 0.01$). Also, self-reported high serum cholesterol levels remained as a significant negative predictor of MP optical density when analysed in a multiple linear regression model ($p < 0.05$) [Table 2].

3.9. Other variables and MP optical density

None of the other possible explanatory variables (BMI; iris colour; alcohol consumption; blood pressure; dietary fat) remained in our final regression model for MP. In fact, with the exception of BMI ($r = -0.078$, $p = 0.028$), we obtained no significant relationship of any of these variables to MP, even without controlling for other variables.

4. Discussion

This study reports on MP optical density and its relationship with risk factors for ARM, in 800 healthy subjects from an Irish population.

4.1. MP optical density

Mean MP optical density amongst our subjects was 0.299, and this is comparable with values ranging from 0.211 to 0.44 for populations of similar age groups, using similar testing conditions (Berendschot and van Norren, 2005; Ciulla et al., 2001; Hammond and Caruso-Avery, 2000; Liew et al., 2005a; Mellerio et al., 2002). Similarly, we found good interocular symmetry of MP optical density, with a mean difference of 0.011, and this is comparable with previously published data (Hammond and Fuld, 1992; Liew et al., 2005a).

Although optimal MP values have yet to be established, there is a growing body of evidence in support of the view that MP protects against ARM. This evidence includes

Table 5
Comparison of descriptive characteristics for male and female subjects[a]

Characteristic	Male ($n = 288$)	Female ($n = 512$)
MP optical density**	0.33 ± 0.161	0.282 ± 0.171
Age*	40.61 ± 11.82	42.68 ± 11.45
BMI**	26.03 ± 0.22	24.58 ± 4.59
Diet		
Lutein (mg/day)*	1.30 ± 0.957	1.459 ± 0.994
Zeaxanthin (mg/day)	0.194 ± 0.114	0.202 ± 0.119
Fat (g/day)**	117 ± 40	97.30 ± 36.30
DHA (mg/day)	349 ± 342	373 ± 280
Serum		
Lutein (μg/ml)*	0.081 ± 0.040	0.089 ± 0.043
Zeaxanthin (μg/ml)	0.025 ± 0.013	0.026 ± 0.016
Family history of ARM[b]		
No family history of ARM	229 (80%)	392 (77%)
Confirmed family history of ARM	59 (20%)	120 (23%)
Smoking status		
Current smokers	49 (17%)	106 (21%)
Past smokers	80 (28%)	125 (24%)
Never smokers	159 (55%)	281 (55%)
Cholesterol levels		
High	36 (12%)	65 (13%)
Normal	256 (88%)	447 (87%)

* Significant difference between groups at 0.05 level (2-tailed).
** Significant difference between groups at the 0.01 level (2-tailed).
[a] Mean ± SD for continuous data. Number (%) for categorical data.
[b] Family history of ARM: No family history of ARM = subjects who reported having no known family history of any type of ARM (early or late). Confirmed family history of ARM = subjects with a clinically confirmed family history of any type of ARM, early ARM, geographic atrophy/late ARM or choroidal neovascularisation/late ARM (offspring of ARM sufferers only).

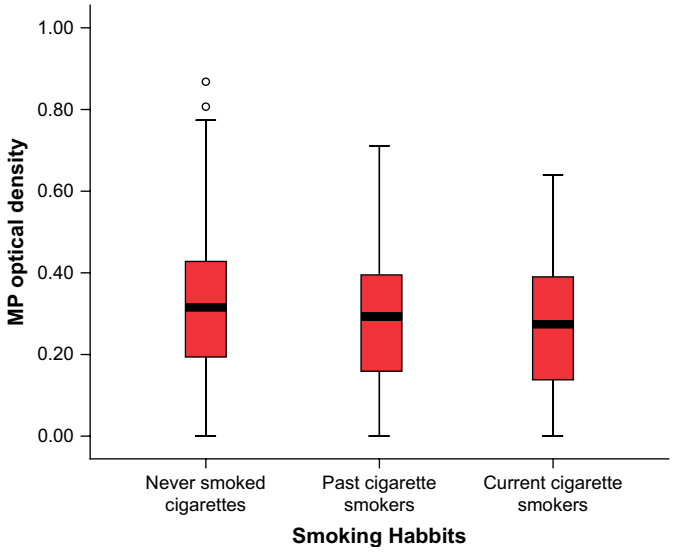

Fig. 4. Boxplots showing MP optical density for subjects who never smoked ($n = 440$), past smokers ($n = 205$) and current smokers ($n = 155$). Never smoked cigarettes: subjects never smoked cigarettes in entire lifetime; past cigarette smokers: subjects who stopped smoking cigarettes for at least one month; current cigarette smokers: subjects who currently smoke cigarettes.

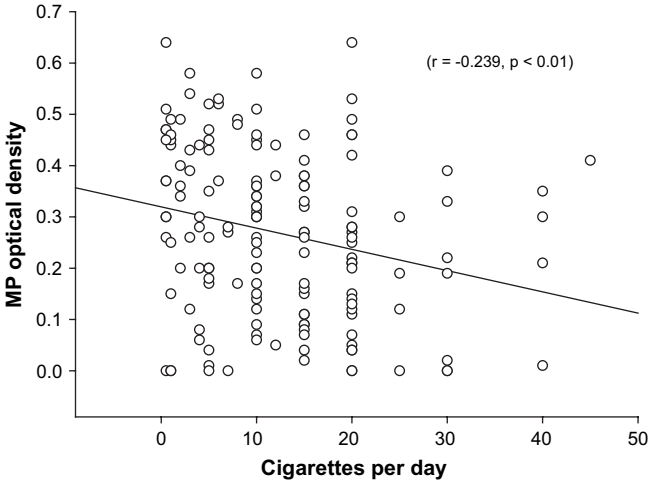

Fig. 5. The dose–response relationship between MP optical density and the number of cigarettes current smokers ($n = 155$) smoked per day.

parallels between a relative lack of MP and risk factors for ARM (Broekmans et al., 2002; Curran-Celentano et al., 2001; Hammond et al., 1996a,b, 2002; Nolan et al., 2004). However, as the results of studies to date are somewhat inconsistent, it has been difficult to draw definite conclusions regarding the role of MP, if any, in preventing ARM.

One limitation of studies to date rests on the fact that they have measured MP in a relatively small number of individuals (n ranging from 7 to 390) (Beatty et al., 2001; Berendschot et al., 2002; Berendschot and van Norren, 2004, 2005; Bernstein et al., 2002, 2004; Broekmans et al., 2002; Burke et al., 2005; Chen et al., 2001; Ciulla et al., 2001; Ciulla and Hammond, 2004; Curran-Celentano et al., 2001; Delori et al., 2001; Gellermann et al., 2002; Hammond et al., 1995a,b, 1996a,c, 1997, 2002; Hammond, 2002; Hammond and Caruso-Avery, 2000; Hammond and Fuld, 1992; Kilbride et al., 1989; Liew et al., 2005a; Mellerio et al., 2002; Neelam et al., 2005; Nolan et al., 2004; Snodderly et al., 2004; Werner et al., 1987; Wustemeyer et al., 2003; Zhao et al., 2003). Also, it is difficult to compare the results of these studies due to the differing methodologies used to measure MP (all with their own merits and limitations) (Hammond et al., 2005). The present study, however, utilised a sample size large enough to assess and compare MP among different groups in the normal population (e.g. males, females, smokers, non-smokers, etc.), and allowed for a simultaneous assessment of MP predictors in a general linear model, thereby enhancing the validity of our results ($n = 800$).

There are numerous risk factors for ARM, although there is a variable degree of consensus regarding the validity and relative importance of many of these. Of the risk factors that we investigated (using multiple linear regression), increasing age, female sex, smoking habits, high cholesterol (reported, not measured) and family history of ARM (early and/or late) were significantly associated with a relative lack of MP, and dietary and serum L were positively correlated with MP.

4.2. Age and MP optical density

Since age is an established risk factor for ARM, a fundamental question to be answered is whether there is an age-related decline in MP, in healthy subjects. Although there is no paucity of studies in the published literature that have reported on the relationship between age and MP levels, it is difficult to draw a firm conclusion regarding the age relationship, if any, with MP because of inconsistencies in the design and results of the reports [Table 6; study number 1–23].

We report a significant and inverse relationship between MP optical density and age, which persisted in our multiple linear regression model ($p < 0.01$). As seen from Table 6, our finding is consistent with some, but not all, published studies. However, of the 12 studies where HFP was used to measure MP [Table 6; study number 1–12], 7 (58%) have reported a trend towards an age-related decline in MP optical density [Table 6; study number 1, 2, 5, 7, 8, 10, and 12], with a significant age-related decline found in 6 (50%) of these studies [Table 6; study number 2, 5, 7, 8, 10, and 12]. However, it should be noted that two of the six studies which found a significant age-decline in MP optical density where from the same Irish population reported in this study (but not the same sample).

Of interest, a recent study by Berendschot et al. (2002) measured MP using a variety of techniques (Berendschot and van Norren, 2005). These investigators found no association between age and MP, when measured using the objective techniques, whereas MP values obtained with HFP (same device as used in this study) showed a significant decrease with age ($r = -0.42$, $p < 0.01$). However, that report included only 53 subjects, with only four subjects aged between 28 and 50 years.

Table 6
Previous studies which reported on the relationship between age and MP levels

Study number	Principal author	Journal published	Year	Technique	Test stimulus	Parafoveal stimuli (eccentricity)	Sample number	Age range (years)	Age effect	Correlation coefficient	Significance
1	Werner, J.S.	Vision Res.	1987	HFP	1°	5°	50	10–90	Decline	−0.21	$p =$ ns
2	Hammond, B.R.	Invest. Ophthal. Vis. Sci.	2000	HFP	1°	4°	217	18–90	Decline	−0.14	$p < 0.05$
3	Ciulla, T.A.	Ophthalmol.	2001	HFP	1°	4°	280	18–50	None	–	$p =$ ns
4	Delori	J. Opt. Soc. Am.	2001	HFP	0.8°	5.5°	30	15–80	None	–	$p =$ ns
5	Beatty, S.	Invest. Ophthal. Vis. Sci.	2001	HFP	0.95°	6°	46	21–81	Decline	−0.48	$p < 0.05$
6	Mellerio, J.	Curr Eye Res.	2002	HFP	1°	5°	124	18–84	None	−0.06	$p =$ ns
7	Nolan, J.	Invest. Ophthal. Vis. Sci.	2004	HFP	1°	5°	100	22–60	Decline	−0.359	$p < 0.01$
8	Neelam, K.	Invest. Ophthal. Vis. Sci.	2004	HFP	1°	5°	125	20–60	Decline	−0.181	$p < 0.05$
9	Ciulla, T.A.	Am. J. Ophthalmol.	2004	HFP	1°	4°	390	18–88	None	0.04	$p =$ ns
10	Bernstein, P.S.	Arch. Biochem. Biophys.	2004	HFP	1.5°	8°	40	18–61	Decline	−0.279	$p < 0.05$
11	Liew, M.	Invest. Ophthal. Vis. Sci.	2005	HFP	1°	5°	150	18–50	None	–	$p =$ ns
12	Berendschot, T.T.J.M.	Exp. Eye Res.	2005	HFP	1°	5°	53	18–70	Decline	−0.42	$p < 0.01$
13	Kilbride, P.E.	Vision Res.	1989	REF			7	–	Decline	–	–
3	Delori, F.C.	J. Opt. Soc. Am.	2001	REF			159	15–80	Increase	–	–
14	Chen, S.F.	Curr. Eye Res.	2001	REF			54	20–84	None	–	–
15	Berendschot, T.T.J.M.	Invest. Ophthal. Vis. Sci.	2002	REF			435	60–91	Increase	0.15	–
16	Brockmans, W.M.R.	Am. J. Clin. Nutr.	2002	REF			376	18–75	None	0.035	$p =$ ns
17	Wustemeyer, H.	G. Arch. Klin. Exp. Ophth.	2003	REF			109	16–76	Decline	−0.44	$p < 0.01$
18	Berendschot, T.T.J.M.	Arch. Biochem. Biophys.	2004	REF			138	18–76	Increase	0.14	–
19	Zagers	J. Opt. Soc. Am.	2004	REF			38	18–64	None	0.058	–
12	Berendschot, T.T.J.M.	Exp. Eye Res.	2005	REF			134	18–70	None	0.05	$p =$ ns
12	Berendschot T.T.J.M.	Exp. Eye Res.	2005	REF			133	19–70	Increase	0.13	$p =$ ns
12	Berendschot, T.T.J.M.	Exp. Eye Res.	2005	REF			52	18–70	None	0.03	$p =$ ns
12	Berendschot T.T.J.M.	Exp. Eye Res.	2005	REF			52	19–70	Increase	0.16	$p =$ ns
12	Berendschot, T.T.J.M.	Exp. Eye Res.	2005	REF			53	19–70	Decline	−0.22	$p =$ ns
3	Delori, F.C.	J. Opt. Soc. Am.	2001	AF			159	15–80	Increase	–	–
17	Wustemeyer, H.	G. Arch. Klin. Exp. Ophth.	2003	AF			109	16–76	None	0.03	$p =$ ns
11	Liew, M.	Invest. Ophthal. Vis. Sci.	2005	AF			150	18–50	Increase	0.17	$p < 0.05$
12	Berendschot, T.T.J.M.	Exp. Eye Res.	2005	AF			53	18–70	Decline	−0.08	$p =$ ns
20	Gellermann, W.	J. Opt. Soc. Am.	2002	RS			140	21–84	Decline	−0.664	$p < 0.01$
10	Bernstein, P.S.	Arch. Biochem. Biophys.	2004	RS			40	18–61	Decline	−0.467	$p < 0.01$
8	Neelam, K.	Invest. Ophthal. Vis. Sci.	2004	RS			125	20–60	Decline	−0.433	$p < 0.01$
21	Bone, R.A.	Invest. Ophthal. Vis. Sci.	1998	HPLC			87	3–95	None	–	$p =$ ns
22	Handelman, G.J.	Invest. Ophthal. Vis. Sci.	1998	HPLC			16	1 week to 81	None	–	$p =$ ns
23	Bone, R.A.	Invest. Ophthal. Vis. Sci.	2001	HPLC			56	58–98	Increase	0.34	$p = 0.01$

HFP = heterochromatic flicker photometry, REF = reflectance, AF = autofluorescence, RS = Raman spectroscopy, HPLC = high-performance liquid chromatography.

HFP is the most commonly used technique for measuring MP *in vivo* [Table 6], and has been validated against *in vitro* measurements (Hammond et al., 2005; Mellerio et al., 2002; Wooten and Hammond, 2005). However, as the majority of HFP techniques to date used reference points at 5° eccentricity (which is taken as a zero reference point), it is likely that such techniques result in underestimation of peak MP, as MP has been shown to extend beyond this reference point (Berendschot and van Norren, 2005). In addition, with age, there is an extension of the lateral extent of MP without, perhaps, a significant change in its peak density (Chang et al., 2002) and our findings (as well as other studies that used reference points of 5° eccentricity and below) must be interpreted with full appreciation of this.

In spite of the lack of consistency of the findings of previous investigators with respect to the relationship between age and MP, we believe that our study provides strong evidence of an age-related decline in MP optical density in healthy subjects between the ages of 20 and 60 years. Of note, our study is the largest of its kind to address the age/MP association in normal subjects ($n = 800$). Therefore, it seems plausible that our large sample enabled detection of an age/MP relationship, which previous and smaller studies were unable to detect. Also, only three other studies investigated the age/MP relationship amongst subjects with a similar age-spread to ours, and all found a significant age-related decline in MP optical density (two of these were from the same Irish population as in this study) (Bernstein et al., 2004; Neelam et al., 2005; Nolan et al., 2004). Furthermore, in our study, each of our three age tertiles (20–35, 36–48 and 49–60 years) accounted for approximately one-third (32–35%) of the total, which has not been the case in previous reports (Berendschot and van Norren, 2005).

We purposely excluded subjects over 60 years because a protective effect of MP against chronic and cumulative oxidative damage, if any, will need to be exerted over decades, and before the onset of ARM. In other words, an observed lack of MP in aged subjects, with or without ARM, would be difficult to interpret because such a finding could be the consequence of physiological and/or pathological aging processes within the retina. In contrast, however, the age-related decline in MP optical density prior to the age of 60 that we observed represents an association between the most universal risk factor for ARM and a relative lack of macular carotenoids, and suggests that the increasing vulnerability of the macula with age may be attributable to deficiency of the macular carotenoids.

An age-related decline in MP may be attributable to excessive depletion, or inadequate uptake, of the macular carotenoids in association with increasing age. A depletion of MP with age would be consistent with excessive utilization of L and/or Z, in response to the age-related increase in oxidant load. Inadequate accumulation of macular carotenoids in association with increasing age could, on the other hand, be attributable to age-related changes in dietary intake, absorption, transport in serum, and/or capture by retinal tissue of these carotenoids (Beatty et al., 2001).

4.3. Family history of ARM and MP optical density

Family history is an important and established risk factor for ARM, as shown by twin and family studies (Klein et al., 1994; Seddon et al., 2005). Indeed, a recent study investigating the impact of genetic and environmental factors on ARM concluded that genetic factors play a substantial role in the aetiology of ARM, explaining 46–71% of the variation in overall severity of the disease (Seddon et al., 2005). It is reasonable to hypothesise that MP levels would also be, to some degree at least, genetically determined (Handelman et al., 1991), in a fashion similar to that of other pigments (e.g. skin melanin, iris pigmentation) (Weiter et al., 1985). This view is consistent with that of a study involving monozygotic twins, which found that only five of the 10 twin pairs had statistically significant differences in MP optical density, suggesting that MP is not completely genetically determined (Hammond et al., 1995a). However, a more recent twin study investigating the heritability of MP optical density has shown genetic background to be a very important determinant of MP levels (Liew et al., 2005b). In that study, MP optical density was measured using HFP and dual wavelength autofluorescence (AF) in 76 monozygotic vs. 74 dizygotic twin pairs. That study reported that genetic background represents an important determinant of MP optical density, with heritabilities of 0.85 and 0.67 for AF and HFP values, respectively. Therefore, if MP is protective for ARM one might expect those genetically predisposed to this condition to exhibit a relative lack of MP. Also, one might expect the distribution of MP to be genetically determined, as its distribution is likely to reflect foveal architecture and microanatomy (e.g. size of the avascular zone, retinal thickness, receptoral topography), which are influenced by heredity (Liew et al., 2005b).

Our regression model [Table 2] suggests that subjects with a clinically confirmed family history of early ARM and/or late ARM had significantly lower levels of MP optical density than subjects with no known family history of disease ($p < 0.01$, for all).

This finding warrants discussion in the context of the following relevant and associated findings. First, all subjects had healthy fundi, and therefore any relative lack of MP cannot be attributed to disease. Second, many subjects with a confirmed family history of ARM (early and/or late) had higher levels of MP than subjects with no known family history of disease, but the group mean was significantly lower for the former. Third, there was no significant difference between the two groups in terms of dietary intake, or serum concentrations, of the macular carotenoids [Table 3]. In fact, subjects with a confirmed family history of ARM had significantly higher levels of serum L when compared to subjects without such a family history ($p < 0.01$).

It would seem, therefore, that there is not a critical level of MP which negates the increased risk for ARM associated with family history, and given that those with a family history did not exhibit lower dietary intake, or serum levels, of the macular carotenoids, our finding of lower MP optical density amongst the offspring of ARM sufferers cannot be attributed

to dietary, digestive, absorptive or transport problems. Rather, the observed relative lack of MP in those with a confirmed history of ARM could be, in theory, due to a poor correlation between dietary, serum and retinal levels of L and/or Z, and/or excessive depletion of these carotenoids as a result of increased oxidative stress (alone or in combination with reduced levels of co-antioxidant defences), in the offspring of ARM sufferers. Given the genetic predisposition to ARM, and the protection that MP may afford against development of that condition, the mechanisms whereby genetic background influences MP optical density and distribution warrants further investigation.

4.4. Cigarette smoking and MP optical density

Cigarette smoking has recently been established as an important risk factor for ARM, with studies consistently demonstrating an increased prevalence of CNV and GA amongst smokers when compared with non-smokers (George et al., 2005; Tamakoshi et al., 1997). Several studies have reported on the association between cigarette smoking and MP (Beatty et al., 2001; Broekmans et al., 2002; Curran-Celentano et al., 2001; Hammond et al., 1996c; Hammond and Caruso-Avery, 2000), with only one study making this the primary outcome measure (Hammond et al., 1996c).

Our findings are identical to those reported by Hammond et al. (2000) who compared measurements of MP optical density in 34 current cigarette smokers to that of 34 non-smokers. They found that current cigarette smokers had significantly less MP optical density than the control subjects (mean MP optical density: 0.16 vs. 0.34, respectively, $p < 0.01$). Consistent with this, we found that 155 current smokers had significantly lower MP optical density than past smokers ($n = 205$) and that past smokers had significantly lower MP than never smokers ($n = 440$) [Tables 2 and 4, coefficient for smoking habits $= -0.019$]. Further, we demonstrated an inverse relationship between smoking frequency and MP optical density ($r = -0.239$, $p < 0.01$), which is again consistent with the report of Hammond and co-workers ($r = -0.448$, $p < 0.01$).

The possible explanations to account for a relative lack of MP amongst cigarette smokers include a poor diet (with consequentially reduced levels of antioxidants) (Dallongeville et al., 1998) and/or increased overall oxidant load associated with tobacco use (Beatty et al., 2000). Interestingly, however, the relative lack of MP that we found for the current smokers cannot be attributable to poor dietary intake of the relevant carotenoids, as the dietary intake of L and Z was statistically comparable for smokers and non-smokers [Table 4]. However, we did find that the current smokers had significantly lower serum levels of L than past and never smokers. It seems, therefore, that our finding of a relative lack of MP amongst smokers when compared to non-smokers may be attributable to at least one of the following explanations: a relative lack of serum L (but not Z); a poor serum macular correlation of L and/or Z; an increased pro-oxidant load associated with tobacco use, with a consequential excessive depletion of L and/or Z at the macula.

4.5. Sex and MP optical density

Although female gender has not been definitively shown to represent risk for ARM, the incidence of the neovascular form of this condition has been reported to be higher amongst women than men (Yannuzzi et al., 1992). Recently published studies pertaining to sex-related differences in MP optical density are conflicting (Beatty et al., 2001; Curran-Celentano et al., 2001; Hammond et al., 1996a; Hammond and Caruso-Avery, 2000). We have found that MP optical density was significantly higher for 288 male subjects when compared to 512 female subjects, consistent with the hypothesis that a relative lack of MP is associated with a risk factor for ARM. This observation is also consistent with work carried out by Hammond et al. (2000) who investigated the sex differences in MP optical density, and after adjusting for caloric intake, found that males had an average of 38% more MP than females ($p < 0.01$) (Hammond et al., 1996a). Also, and in a separate study, Hammond and Caruso-Avery (2000) reported MP optical density values to be significantly lower in 138 women when compared to 79 men ($p < 0.05$).

There are several possible explanations to account for a relative lack of MP in females when compared with males. First, hormonally controlled variations in the lipid transport system, which is utilised by carotenoids, could affect serum concentrations of the macular carotenoids, and their relationship with MP optical density (Schaefer et al., 1983). Second, it has been suggested that steroid hormones may affect the metabolism of L and/or Z directly; however, there is little data to support this (Hammond et al., 1996a). Finally, females are known to have a higher percentage body fat than males, and body fat is related to serum concentrations of L and Z, and with MP optical density, but in a manner which differs between the sexes (Hammond et al., 2002; Nolan et al., 2004).

4.6. BMI and MP optical density

Consistent with most previous reports (Burke et al., 2005; Hammond et al., 2002; Nolan et al., 2004), we found a significant (albeit slight) and inverse relationship between BMI and MP optical density ($r = -0.078$, $p = 0.028$). However, BMI was not included in our final multiple linear regression model, suggesting that this variable may be a weaker predictor of MP optical density in normal subjects than the other independent variables which remained significant in the model (e.g. age, family history of ARM, cigarette smoking). For a full discussion regarding BMI and its association with MP, see a recent publication by our research group (Nolan et al., 2004).

4.7. Serum cholesterol levels and MP optical density

Several studies have investigated the relationship between serum cholesterol levels and ARM; however, results have been inconsistent (van Leeuwen et al., 2004). A possible link between elevated cholesterol levels and decreased MP rests on the knowledge that high density lipoproteins (HDLs) are the primary carriers of L and Z (Clevidence and

Bieri, 1993), and this has prompted the suggestion that an individual's lipoprotein profile (high serum cholesterol levels are associated with low levels of HDL) (Sauar et al., 1980) may influence the transport and delivery of these carotenoids to the retina, with a consequential impact on MP optical density.

Consistent with this hypothesis, we found that subjects who reported having high cholesterol levels had significantly lower MP optical density than that of subjects who reported having normal cholesterol levels ($p < 0.01$). Furthermore, high cholesterol levels (albeit self-reported) were a negative predictor of MP optical density in the multiple linear regression model ($p < 0.05$). However, our findings should be interpreted with caution, as cholesterol levels were self-reported. Also, we did not investigate if these volunteers who reported having hypercholesterolemia were on prescribed statins, which are known to alter an individual's lipoprotein profile. To our knowledge, there are no published studies investigating, or even commenting upon, the relationship between serum cholesterol levels and MP. This is an area, which warrants a more comprehensive scientific investigation.

In conclusion, there is a relative lack of MP in association with the most important and established risk factors for ARM (age, cigarette smoking and family history of ARM), several decades before the onset of disease. The importance of this finding rests on the fact that any protective effect of MP depends on its ability to defend against chronic and cumulative retinal oxidative damage, whether induced by blue light (photochemical) or as a result of high oxygen metabolism, and will need to be exerted in young to middle-age.

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