

# Macular Pigment Optical Density in an Ageing Irish Population: The Irish Longitudinal Study on Ageing

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## Key Words

Age-related macular degeneration · Irish Longitudinal Study on Ageing · Macular pigment optical density

## Abstract

**Purpose:** The 3 carotenoids lutein, zeaxanthin, and *meso*-zeaxanthin, which account for the 'yellow spot' at the macula and which are referred to as macular pigment (MP), are believed to play a role in visual function and protect against age-related macular degeneration (AMD) via their optical and antioxidant properties. This study was undertaken to compare MP optical density (MPOD) in a population aged  $\geq 50$  years with MPOD values from a normative database of subjects aged 18–60 years. **Methods:** Seventy-nine subjects were recruited into this pilot study (The Irish Longitudinal Study on Ageing-TILDA). MPOD was measured using heterochromatic flicker photometry. Retinal fundus photographs, lifestyle data and general health data, were also obtained. **Results:** The mean  $\pm$  SD age of the 79 subjects recruited into this study was  $65 \pm 11$  years. There was a moderate, but statistically significant, age-related decline in MPOD at  $0.5^\circ$  in the TILDA data ( $r = -0.251$ ,  $p = 0.045$ ), which remained upon merging with a normative database of an additional 462 subjects aged between 18 and 67 years ( $r = -0.179$ ,  $p = 0.000$ ). **Conclusions:** We report an inverse associa-

tion between MPOD and increasing age. Longitudinal data in a larger cohort of participants are required to satisfactorily investigate the relationship between the optical density of this pigment and age, and with risk for development and/or progression of AMD. This pilot study represents a first step in this endeavour.

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## Introduction

Age-related macular degeneration (AMD) is the advanced form of age-related maculopathy (ARM), and is the leading cause of blindness in people over 50 years of age in the developed world [1, 2]. The number of adults registered blind as a result of AMD in industrialized countries continues to rise, primarily due to increasing longevity [3, 4]. Beyond its inevitable impact on the individual sufferer, AMD poses a growing socioeconomic challenge to modern society [5–7].

Three dietary carotenoids, lutein, zeaxanthin and *meso*-zeaxanthin, accumulate at the macula, where they are collectively referred to as macular pigment (MP). Lutein and zeaxanthin are present in many foods, whereas *meso*-zeaxanthin is not found in a conventional diet, al-

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though it is found in certain types of seafood [8, 9]. In recent years, the anatomic, biochemical and optical properties of MP have provoked interest in its putative protection for ARM [10].

The prevalence, incidence and progression of ARM have been shown to rise exponentially with increasing age [11, 12]. The free radical theory of ageing is consistent with the notion that oxidative stress contributes to age-related disorders, including ARM [10]. Given that MP is an antioxidant present in the macula, and that ARM is an age-related eye disease, MP's association with age is of particular interest.

To date, studies that have reported on the relationship between age and MP have been inconsistent due to the differing methodologies used and differences in sample size (table 1). However, the largest study to date to investigate this relationship across an appropriate age distribution (i.e. 20–60 years) found a modest, but statistically significant, age-related decline in MP levels [13]; however, in that study, the oldest subject recruited was 60 years of age.

The Irish Longitudinal Study on Ageing (TILDA) is a prospective cohort study led by Trinity College Dublin aimed at providing valid and reliable data relating to older people and ageing in Ireland. It will describe the social, economic and health status of older Irish adults and try to identify the factors that influence healthy ageing. TILDA plans to recruit a nationally representative sample of a minimum of 8,000 participants aged 50 years and over, with detailed clinical assessments taking place in 3 waves. Interviews will take place on a 2-yearly basis with a comprehensive health assessment at baseline and every 4 years thereafter.

The Macular Pigment Research Group (MPRG) at the Waterford Institute of Technology ([www.wit.ie/mprg](http://www.wit.ie/mprg)) is collaborating with TILDA, and has designed a study which is primarily aimed at answering the following question: does the amount of MP in an eye protect against the commonest cause of blind registration in the western world? To answer this question, we propose to measure MP levels in TILDA participants at baseline, and again at years 4 and 8, and correlate the findings with prevalence and incidence of AMD. In this way, and uniquely, this collaborative approach will be able to determine whether baseline and serial MP levels relate to ultimate risk of developing AMD. Such a finding could possibly defer the onset of the condition. Also, a finding that retinal nutritional status relates to the risk for developing AMD will have major implications for health policy planners and health care providers over the coming years.

In this paper, we present baseline data from the TILDA pilot study phase 1 population, thus allowing us to compare MP optical density (MPOD) in a population aged  $\geq 50$  years with values from a normative database (subjects aged 18–60 years).

## Methods

### Subjects

The sample for this pilot study was selected using the updated RANSAM sampling system developed by the Economic and Social Research Institute [14].

The sampling frame on which this system is based is the Irish Geodirectory, a comprehensive and up-to-date listing and mapping of all residential addresses in the Republic of Ireland compiled by Ordnance Survey Ireland. The target area for the sample comprised Dublin City and the county of Dun Laoghaire-Rathdown.

Addresses were randomly selected within this area by means of a 3-stage process, incorporating proportionate stratification of the primary sampling units (geographical units containing at least 500 addresses) by socio-economic status (percent in professional/managerial occupations and per cent with third level education), age structure (percent of population aged 50 or over), and geographical location. The sample is, therefore, a probability sample of addresses, where each address has a probability of selection proportionate to the percentage of adults aged 50 or over in the electoral division in which it is located.

Nineteen clusters of 40 addresses were selected, giving an initial sample of 760 addresses. Each address was visited by a field-worker and one (randomly selected) household member aged 50 or over was selected as primary respondent for the survey. This person's spouse (of any age) was also selected for interview. A total of 443 addresses were found to be ineligible (dwelling vacant or did not contain a person aged 50 or over), leaving 317 valid addresses. Within these households, a total of 143 respondents completed an interview in their home. All 143 respondents were invited to attend Trinity College Dublin for a full health assessment, of which, 79 accepted, and their data is presented in this paper.

This study was approved by the research ethics committee of Trinity College Dublin, and subjects were required to sign an informed consent document prior to participation. All experimental procedures adhered to the tenets of the Declaration of Helsinki. Inclusion criteria for participation in this study were age 50 years or older, and being resident in the Republic of Ireland. Institutionalized people were excluded from recruitment.

Of particular interest to the macular assessment part of the TILDA investigation, the following information was recorded for each subject: demographic details; general health status and medication use; family history of AMD; personal smoking history. The examination included: visual acuity (LogMAR); body mass index [BMI (calculated as  $\text{kg}/\text{m}^2$ )]; MPOD measurement by heterochromatic flicker photometry (HFP; using the Macular Densitometer™, see below); undilated fundus photography, using a non-mydratric NIDEK AFC210 auto fundus camera.

**Table 1.** Previous studies which reported on the relationship between age and MP levels

Reference	Technique	Test stimulus	Parafoveal stimuli (eccentricity)	Sample number	Age range, years	Age effect	Correlation coefficient	p
Werner, 1987 [40]	HFP	1°	5°	50	10–90	decline	–0.21	n.s.
Hammond, 2000 [22]	HFP	1°	4°	217	18–90	decline	–0.14	<0.05
Ciulla, 2001 [21]	HFP	1°	4°	280	18–50	none	–	n.s.
Delori, 2001 [41]	HFP	0.8°	5.5°	30	15–80	none	–	n.s.
Beatty, 2001 [37]	HFP	0.95°	6°	46	21–81	decline	–0.48	<0.05
Mellerio, 2002 [23]	HFP	1°	5°	124	18–84	none	–0.06	n.s.
Nolan, 2004 [34]	HFP	1°	5°	100	22–60	decline	–0.359	<0.01
Neelam, 2005 [42]	HFP	1°	5°	125	20–60	decline	–0.181	<0.05
Ciulla, 2004 [43]	HFP	1°	4°	390	18–88	none	0.04	n.s.
Bernstein, 2004 [44]	HFP	1.5°	8°	40	18–61	decline	–0.279	<0.05
Liew, 2005 [45]	HFP	1°	5°	150	18–50	none	–	n.s.
Berendschot, 2005 [25]	HFP	1°	5°	53	18–70	decline	–0.42	<0.01
Nolan, 2007 [13]	HFP	1°	5°	800	20–60	decline	–0.286	<0.01
Iannaccone, 2007 [46]	HFP	1°	7°	222	69–86	decline	–0.026	>0.05
Richer, 2007 [47]	HFP	1°	7°	90	–	none	–	–
Nolan, 2007 [29]	HFP	0.5°	7°	59	19–57	decline	–0.252	<0.05
van der Veen, 2009 [48]	HFP	0.5°	8°	26	22–64	increase	0.083	<0.1
Kilbride, 1989 [49]	Ref.			7	–	decline	–	–
Delori, 2001 [41]	Ref.			159	15–80	increase	–	–
Chen, 2001 [50]	Ref.			54	20–84	none	–	–
Berendschot, 2002 [51]	Ref.			435	60–91	increase	0.15	–
Broekmans, 2002 [28]	Ref.			376	18–75	none	0.035	n.s.
Wustemeyer, 2003 [52]	Ref.			109	16–76	decline	–0.44	<0.01
Berendschot, 2004 [53]	Ref.			138	18–76	increase	0.14	–
Zagers, 2004 [54]	Ref.			38	18–64	none	0.058	–
Berendschot, 2005 [25]	Ref.			134	18–70	none	0.05	n.s.
Berendschot, 2005 [25]	Ref.			133	19–70	increase	0.13	n.s.
Berendschot, 2005 [25]	Ref.			52	18–70	none	0.03	n.s.
Berendschot, 2005 [25]	Ref.			52	19–70	increase	0.16	n.s.
Berendschot, 2005 [25]	Ref.			53	19–70	decline	–0.22	n.s.
Delori, 2001 [41]	AF			159	15–80	increase	–	–
Wustemeyer, 2003 [52]	AF			109	16–76	none	0.03	n.s.
Liew, 2005 [45]	AF			150	18–50	increase	0.17	<0.05
Berendschot, 2005 [25]	AF			53	18–70	decline	–0.08	n.s.
Trieschmann, 2007 [55]	AF	1°	6°	108	51–87	decline	–	0.17
Gellermann, 2002 [56]	RS			140	21–84	decline	–0.664	<0.01
Bernstein, 2004 [44]	RS			40	18–61	decline	–0.467	<0.01
Neelam, 2005 [42]	RS			125	20–60	decline	–0.433	<0.01
Bone, 1988 [57]	HPLC			87	3–95	none	–	n.s.
Handelman, 1988 [58]	HPLC			16	1 week to 81	none	–	n.s.
Bone, 2001 [59]	HPLC			56	58–98	increase	0.34	0.01

AF = Autofluorescence; HFP = heterochromatic flicker photometry; HPLC = high-performance liquid chromatography; RS = Raman spectroscopy; Ref. = reflectance; n.s. = not statistically significant at 0.05 level. Table updated from previous publication by Nolan et al. [34].

*Measurement of Macular Pigment Optical Density Using Customized HFP in TILDA Subjects*

MPOD was measured using the Macular Densitometer by HFP (Providence, R.I., USA). The device was modified specifically for TILDA study from the one originally described by Wooten et al. in 1999 [15]. Corrected distance visual acuity of at least

6/18 was an inclusion criterion for MP measurement (note, only one of the 79 subjects was excluded from testing based on this requirement). The eye with better vision was chosen for measurement. Of the 79 subjects recruited into the pilot study, 64 subjects were able to complete full MPOD assessment at 0.5° of eccentricity. Reasons for subjects not being able to complete full MPOD

**Table 2.** Frequency settings used to estimate optimal HFP flicker frequencies, by age, for both foveal and parafoveal measurements when using the Densitometer

Age	Flicker frequency at 0.5°	Flicker frequency at 7°
50–60	14	9
61–70	13	8
71–80	12	7
81+	11	6

assessment can be summarized as follows: too poor fixation, too poor visual acuity, not able to follow instructions, and retinal pathology.

In order to measure MPOD, the subject views a stimulus that alternates between a wavelength band absorbed by MP and one that is not. The radiance of the wavelength band absorbed by MP is adjusted in order to minimize the subjects' perception of flicker. The range of alternation rates where flicker is not perceived is called the null zone. Primarily because of inter-individual differences in temporal (e.g. flicker) sensitivity, it is optimal to customize the HFP task for each subject by selecting the alternation rate to achieve a null zone and a precise setting. This has been termed as customized HFP [16].

The first methodological consideration when using customized HFP is selecting the appropriate flicker rate. Selecting the best flicker rate for each subject enables one to accommodate the variation in flicker sensitivity due to factors such as age and disease [17, 18]. If differences among subjects in flicker sensitivity are not accounted for (i.e. a fixed flicker frequency is used), then a subject with low flicker sensitivity (i.e. low critical flicker fusion frequency) will most likely experience a large null flicker zone. Although the subject may be able to complete the task by eliminating flicker from the test target, the settings are likely to be variable, and subjects may exhibit systematic bias toward one end of the null range, resulting in either over- or underestimation of MPOD. Alternatively, a subject with a high critical flicker fusion frequency may not be able to eliminate flicker from the test target, which would make the task difficult to complete. Given that the MP investigation part of the TILDA study is only one of many parameters under investigation, we were aware of time constraints and therefore developed a 'bracketing' protocol (described below) to allow us to obtain quick, but accurate and customized, MPOD values. The sampling target used is a 1° diameter disk with 5 min fixation point at the centre. The reference target is a 2° diameter disk located 7° nasally with reference to a 5 min red fixation point. The background is a 5° disk generated by a light-emitting diode (468 nm with 20 nm half pass). Predicted optimal HFP flicker frequencies were estimated in order to facilitate good subject performance and reduce measurement error. To achieve this, we used an age-guided algorithm to estimate optimal HFP flicker frequencies for both the measurements performed (i.e. the measurement locus at 0.5° and reference locus at 7°; see below and table 2). This algorithm was informed by many years' experience with the Densitometer at several different laboratories. If the subject reports that there is no null flicker zone, the flicker frequency is increased by 2 Hz and the examiner proceeded with MP measurement. This step was repeated if necessary.

The second methodological consideration involves a test stimulus configuration in which the radiances of the 2 alternating components are inverse-yoked. In other words, when the blue component is adjusted to be more intense, the luminance of the green component is commensurately decreased, and vice versa. This procedure keeps the brightness of the test stimulus relatively constant. This approach is regarded as an improvement because some subjects find changes in brightness distracting when they perform the task.

As mentioned above, the procedure used in the present study has been termed as a 'bracketing' procedure and was developed specifically for TILDA study. This procedure was developed by members of Macular Pigment Research Group, Waterford, Ireland (J.M.N. and E.L.) and Prof. Wooten of Brown University, USA (inventor of the Densitometer), and is described below.

A description of the first part of the test (i.e. fovea, 0.5°) is achieved using diagrams, to familiarize the subject with the task ahead. The examiner selects the target required to measure MPOD at 0.5° (i.e. 1° stimulus) using an accompanying standard operating procedure developed for this device. The subject is instructed to place his/her test eye (the eye with best visual acuity, or else their right eye if visual acuity is equal in both eyes) at the viewing eyepiece and the investigator ensures that the tilt of the main unit allows comfortable viewing for the subject. The appropriate flicker frequency is set for the subject's age (table 2). The examiner sets the radiance dial all the way to the left (i.e. lowest blue light intensity). The examiner then pushes a button that electronically, smoothly and continuously alters the blue/green ratio until the subject reports that there is no flicker. The radiance value obtained is recorded and this same procedure is repeated on 2 more occasions (3 in total) and recorded in the MPOD log form. The examiner sets the radiance dial all the way to the right (i.e. highest blue light intensity) and repeats the test 3 times as above. Again, the radiance values obtained are recorded in the MPOD log form. This completes the first part of the measurement (6 radiance values obtained in total, 3 approaching from the lowest blue light intensity and 3 approaching from highest blue light intensity).

Then, a description of the second part of the measurement (parafovea, 7°), using diagrams, is given to the subject by the trained TILDA nurse. The examiner selects the target and fixation point required to measure MPOD at 7° using the accompanying standard operating procedure developed for this method and the procedure is repeated for this target arrangement as described above for the 0.5° measurement. All radiance values obtained (12 in total) are then inputted into the MPOD calculator, and an MPOD value at 0.5° is generated for that subject. It should be noted that previous investigations into the spatial profile have shown a secondary peak that occurs between 0.5° and 1° retinal eccentricity in some subjects [19, 20]; however, it is important to note that the value at 0.5° captures about 95% of the variance of the entire MP distribution.

Previous models of the Densitometer (and most other similar devices) control the blue/green energy ratio with a rotary knob. Thus, the subject (if using self adjustment) or the experimenter (if using bracketing) turns the knob until the desired point of no-flicker is reached. This works well for most subjects. However, some are prone to adjust the knob much too slowly. Others, on the other hand, make their adjustments too quickly. In the bracketing procedure, there are individual differences in the way different experimenters control the knob. The current version of the Den-

sitometer avoids these potential sources of variability by substituting the knob with 2 push buttons: one button when depressed and held down causes the blue/green ratio to increase, whereas the other causes the blue/green ratio to decrease. Unlike a subject or experimenter turning a knob, the rate of blue/green change is controlled entirely by the Densitometer's electronics and was determined to be optimal (neither too fast nor too slow) at 7 s for a sweep from one extreme to the other of the blue/green ratio. Preliminary studies have shown that this new procedure not only removes the aforementioned variability, but the task is qualitatively easier for the subjects.

#### *Statistical Analysis*

The statistical software package SPSS (version 15) was used for statistical and graphical analysis. In addition, the software package Sigma plot (version 8) was used for some graphical analysis.

All variables investigated exhibited a typical normal distribution. Means  $\pm$  SDs are presented in the text. Pearson correlation coefficients were calculated to investigate bivariate relationships. The significance between group differences was determined by 1-way ANOVA or independent samples t test, depending on the analysis in question. We used the 5% level of significance throughout our analysis.

## **Results**

### *Demographics and Variables of Relevance to AMD*

The mean  $\pm$  SD age of the 79 subjects recruited into the TILDA pilot I study was  $65 \pm 11$  years. Twenty-nine of the subjects were male and 50 were female. The mean  $\pm$  SD BMI of the study group was  $28 \pm 6$ . Of the 72 subjects that reported their smoking habits, 39 reported that they smoked cigarettes, cigars, cigarillos or a pipe daily for a period of at least 1 year during their lifetime, and 33 subjects reported that they had not. Of the 70 subjects that reported presence or absence of a family history of AMD in a first degree relative (i.e. parent or sibling), 9 (12.9%) reported a positive family history of AMD, whereas 61 (87.1%) reported no known family history of AMD. The mean  $\pm$  SD MPOD at  $0.5^\circ$  of eccentricity for the TILDA pilot I study group was  $0.23 \pm 0.17$ .

### *AMD Prevalence in Study Group*

Undilated retinal fundus photographs were obtained (non-mydratic NIDEK AFC210 auto fundus camera) on both eyes of all subjects recruited into the study. Of the 158 eyes photographed, 123 pictures were deemed of sufficient quality for retinological assessment with respect to the presence or absence of AMD by a retinologist (S.B.). It is perhaps unsurprising that 22% of photographs were of insufficient quality, given the impact of age-related miosis and lens opacity on a study population with this age profile, and given that this was a pilot study. Of the 123

eyes suitable for assessment, 29 eyes (24%) exhibited signs of early AMD (i.e. soft drusen, hyper- and/or hypo-pigmentary changes). In terms of subjects, 17 (28%) of the 61 subjects whose photographs were suitable for grading had evidence of early disease (as above), with bilateral disease evident in 12 (70%) of these subjects. Of the photographs upon which comment could be reliably made, no eyes exhibited signs of advanced AMD.

The large percentage (22%) of retinal photographs that were deemed of insufficient quality for retinal assessment in this pilot was an important learning outcome from the study. Indeed, the TILDA research teams have now taken appropriate measures to address this issue. For example, collaboration between the Retinal Reading Center, University of Wisconsin, and the TILDA research team has now been established, and will include: quality control of AMD grading and implementation of standard operating procedures and training and certifying the TILDA nurses (to optimize the number of 'gradable images' compared to the results presented here).

### *MPOD with Respect to Variables Relevant to AMD*

#### *MPOD and Age*

There was a moderate, but statistically significant, age-related decline in MPOD at  $0.5^\circ$  ( $r = -0.251$ ,  $p = 0.045$ ; fig. 1). Also, using a normative database of an additional 462 subjects, aged between 18 and 67 years (data previously collected and archived by the MPRG using the same measuring device as that used in this study) allowed for further, and more detailed (greater age spread for comparison), investigation of the relationship between MPOD and age. Upon merging the databases, the significant inverse relationship between age and MPOD remained ( $r = -0.179$ ,  $p = 0.000$ ; fig. 2).

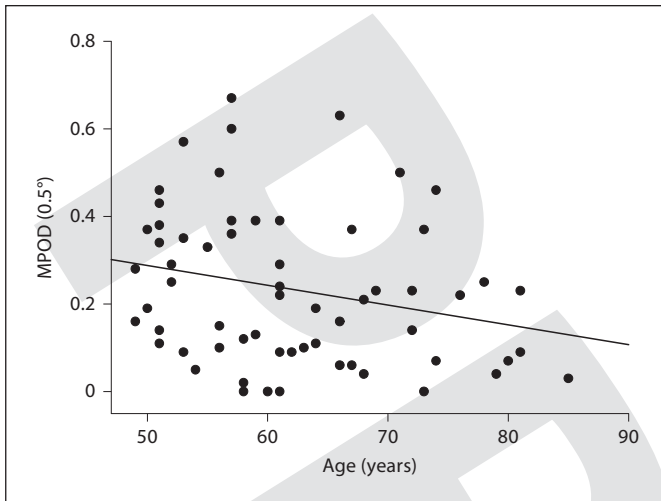
Reporting the data in terms of MPOD change per age decade, we see a significant stepwise reduction in MPOD (ANOVA;  $p = 0.000$ ; fig. 3).

#### *MPOD and Cigarette Smoking*

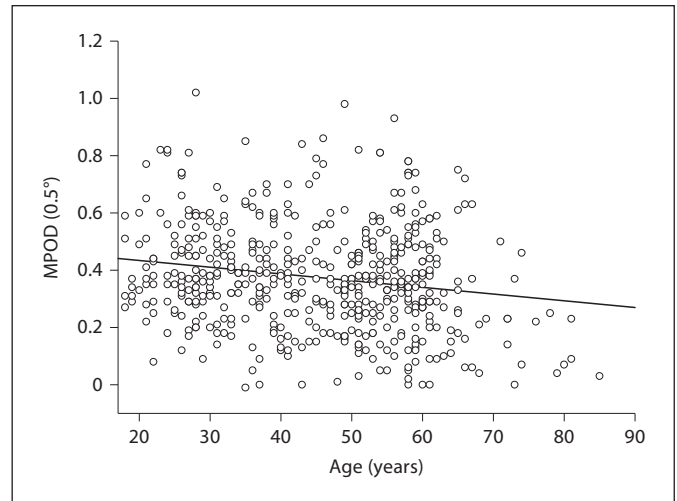
The 39 subjects who reported that they smoked cigarettes, cigars, cigarillos or a pipe daily for a period of at least 1 year during their lifetime had significantly lower average MPOD values when compared to the 33 subjects who reported that they had not (mean  $\pm$  SD MPOD at  $0.5^\circ = 0.18 \pm 0.14$  and  $0.28 \pm 0.19$ , respectively; fig. 4).

#### *MPOD and Sex*

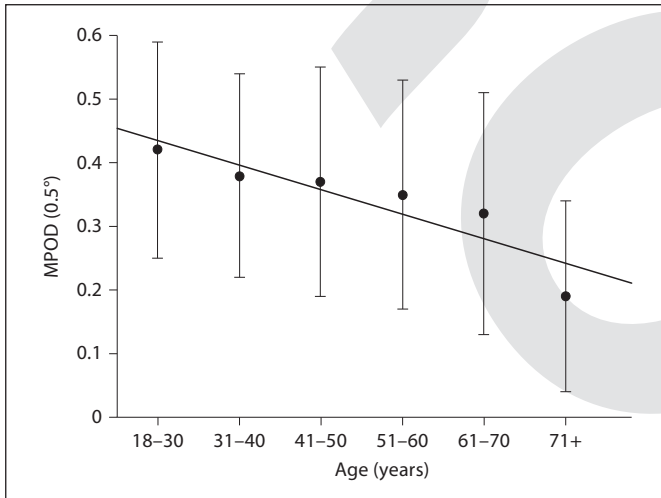
Females had significantly higher MPOD values when compared to their male counterparts (mean  $\pm$  SD MPOD at  $0.5^\circ = 0.27 \pm 0.17$  and  $0.18 \pm 0.15$ , respectively,  $p = 0.032$ ).



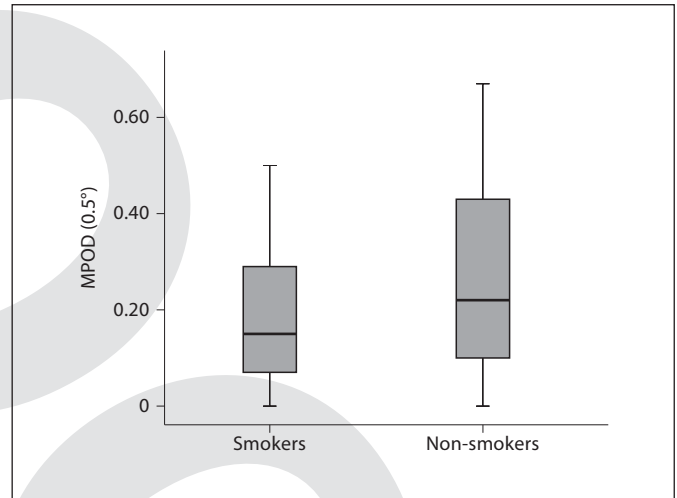
**Fig. 1.** Age-related decline in MPOD for TILDA subjects (n = 64).



**Fig. 2.** Age-related decline in MPOD for 521 Irish subjects.



**Fig. 3.** Age-related decline in MPOD for 521 subjects (per decade). Values presented = mean  $\pm$  SD MPOD (per decade).



**Fig. 4.** MPOD and smoking habits.

### MPOD and BMI

There was no significant relationship found between MPOD and BMI in the TILDA study group ( $r = -0.26$ ,  $p = 0.836$ ).

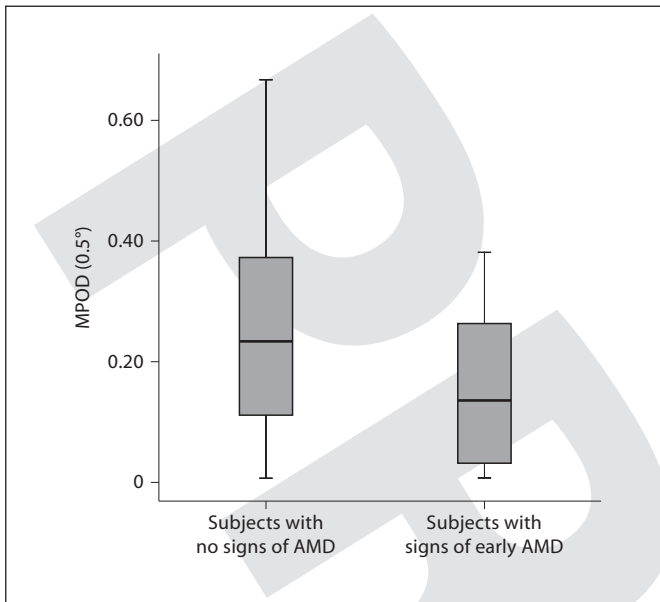
### MPOD and Its Relationship with Risk Factors for AMD as Assessed by Multiple Linear Regression Analysis

Multiple linear regression analysis was performed to analyze the relationship between MPOD and the following known and suggested risk factors for AMD: age; sex;

smoking habits; family history of early AMD, and BMI. 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 explains about 11% of the variation in MP, with the variables age and smoking habits remaining significant independent predictors of MP ( $p < 0.05$ , for both).

### MPOD with Respect to Early AMD Prevalence

Both MPOD measurement and retinal assessment were successfully completed in 64 of the 79 subjects re-



**Fig. 5.** MPOD in subjects with and without early stage AMD.

cruited into the study. Twelve of the 17 (70%) subjects with early AMD were able to complete the test for MPOD measurement, whereas 52 of the 62 (84%) subjects with no signs of AMD were able to complete the test for MPOD measurement. Mean  $\pm$  SD MPOD in subjects with no signs of AMD was significantly higher when compared to subjects with early stage AMD (mean MPOD at  $0.5^\circ = 0.25 \pm 0.17$  and  $0.14 \pm 0.13$ , respectively; fig. 5).

## Discussion

TILDA represents a unique opportunity to investigate the prevalence and incidence of AMD, as well as the risk factors for and impact of this condition in a longitudinal fashion, in a naturally representative sample of at least 8,000 participants.

This pilot study reports on MPOD in an ageing randomly selected sample of the Irish population (TILDA pilot phase I). Mean MPOD in this ageing adult population was 0.23, which is low when compared to different study populations across all ages (18–87, years inclusive) [13, 21–25]. 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 AMD via its antioxidant and short-wavelength filtering properties [10, 26, 27]. This evidence is primarily available from observational and supplementation studies [13, 28–35]. For

a critical review of the scientific literature on this topic, see the recent publication by Loane et al. in 2008 [26].

There are numerous risk factors for AMD, and it has been established that age, cigarette smoking and a family history of AMD are the most important and undisputed. Of the risk factors that were investigated in the TILDA pilot study, increasing age and smoking habits (2 of the 3 established risk factors for AMD) were independently, and significantly, associated with a relative lack of MP. No meaningful comment can be made with regards to family history of AMD, as this data was self-reported and is therefore of limited value, especially when one considers that all subjects enrolled were  $>50$  years of age and therefore their parents probably did not enjoy the longevity seen today and associated with the current epidemic of AMD.

The age-related decline in MPOD and the relative lack of MP in association with tobacco use are consistent with previous findings by our research group, and are in support of the hypothesis that a relative lack of MP is associated with risk for AMD [13], but are not in agreement with data reported from the ‘Carotenoids in Age-Related Eye Disease Study – CAREDS’ investigation which measured MPOD in 1,698 women aged 53–86 years [36]. Possible explanations for the outcome discrepancies between these studies may be differences in the sample populations studied, the fact that CAREDS included female subjects only, and differences in dietary and lifestyle habits between the populations studied.

The age-related decline in MPOD observed here represents an association between the most universal risk factor for AMD and a relative lack of the macular carotenoids, and is consistent with the view that the increasing vulnerability of the ageing macula to AMD may be attributable, at least in part, to a parallel and age-related decline in the optical density of MP at the central retina. The observed age-related drop 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 the macular carotenoids in response to the age-related increase in oxidant load, but could also be attributable to age-related changes in dietary intake, absorption, transport in serum, and/or capture by retinal tissue of these carotenoids [13, 37, 38].

It should be emphasized that our observations are associative only, as are all the published data investigating the relationship between age and MPOD. Indeed, addressing this issue by analysing serial MPOD measurements in subjects over time is a key goal of the collaborative research between the MPRG and TILDA, and the

subject of future work. Nevertheless, the data presented here represent the first analysis of MPOD values in subjects randomly selected according to best practice in terms of epidemiologic study design, where a validated technique (refined for elderly subjects) of measuring the optical density of MP was used, and where a large normative database of MP values (assessed using the same technique) of a similar but younger Irish population was readily available for comparison.

Not only do the TILDA pilot data exhibit an age-related decline in MPOD in subjects over 50 years, the observed and modest, but statistically significant, age-related decline in MPOD remained when we merged the data with a larger database with a wider age spread ( $n = 541$ , aged 18–89 years in total, additional data collected by the MPRG using the same measuring device as the one used in this study). Indeed, comparing the oldest age group available (aged 70 years or more) to the youngest age group available (aged 18–30 years), we see a mean difference in MPOD of 0.23 optical density units ( $p = 0.000$ ). It is also important to note that, while the literature is conflicting with respect to the relationship between age and MP (table 1), of the 17

published studies that used HFP to measure MPOD and report on its association with age, 10 report an age-related decline in MPOD (7 of which were statistically significant), 6 report no age effect in MPOD, and only 1 study reported an age-related increase in MPOD.

Other interesting observations, which have emanated from this pilot study, include the prevalence of early AMD (28%) in a randomly selected sample of Irish subjects over 50 years of age and the statistically significant relative lack of MP seen in subjects who displayed early signs of AMD. This latter finding is consistent with a previous report by Beatty et al. [37] and is also consistent with the hypothesis that a relative lack of MP is associated with disease. However, and again, this observation remains associative only, and causality cannot be inferred.

In conclusion, we report an association between declining MPOD and increasing age. Ultimately, however, longitudinal data in a larger cohort of participants are required to satisfactorily investigate the relationship between the optical density of this pigment and age, and with risk for development and/or progression of AMD. This pilot study represents a first step in this endeavour.

## References

- Bressler NM: Age-related macular degeneration is the leading cause of blindness. *JAMA* 2004;291:1900–1901.
- Congdon NG, Friedman DS, Lietman T: Important causes of visual impairment in the world today. *JAMA* 2003;290:2057–2060.
- Kelliher C, Kenny D, O'Brien C: Trends in blind registration in the adult population of the Republic of Ireland 1996–2003. *Br J Ophthalmol* 2006;90:367–371.
- Bunce C, Wormald R: Causes of blind certifications in England and Wales: April 1999–March 2000. *Eye* 2008;22:905–911.
- van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, de Jong PT: Epidemiology of age-related maculopathy: a review. *Eur J Epidemiol* 2003;18:845–854.
- Augustin A, Sahel JA, Bandello F, Dardennes R, Maurel F, Negrini C, et al: Anxiety and depression prevalence rates in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2007;48:1498–1503.
- Bandello F, Lafuma A, Berdeaux G: Public health impact of neovascular age-related macular degeneration treatments extrapolated from visual acuity. *Invest Ophthalmol Vis Sci* 2007;48:96–103.
- Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM: Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol* 1998;82:907–910.
- Maoka T, Arai A, Shimizu M, Matsuno T: The first isolation of enantiomeric and meso-zeaxanthin in nature. *Comp Biochem Physiol B* 1986;83:121–124.
- Beatty S, Koh HH, Henson D, Boulton M: The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115–134.
- Klein R, Klein BEK, Linton KLP: Prevalence of age-related maculopathy – the Beaver Dam Eye Study. *Ophthalmology* 1992;99:933–943.
- Klein R, Klein BEK, Jensen SC, Meuer SM: The five-year incidence and progression of age-related maculopathy – The Beaver Dam eye study. *Ophthalmology* 1997;104:7–21.
- Nolan JM, Stack J, O'Donovan O, 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.
- Whelan BJ: RANSAM: a random sample design for Ireland. *Econ Soc Rev* 1979;10:161–174.
- Wooten BR, Hammond BR, Land RI, Snodderly DM: A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci* 1999;40:2481–2489.
- Stringham JM, Hammond BR, Nolan JM, Wooten BR, Mammen A, Smollon W, et al: The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res* 2008;87:445–453.
- Falsini B, Fadda A, Iarossi G, Piccardi M, Canu D, Minnella A, et al: Retinal sensitivity to flicker modulation: reduced by early age-related maculopathy. *Invest Ophthalmol Vis Sci* 2000;41:1498–1506.
- Tyler CW: Two processes control variations in flicker sensitivity over the life span. *J Opt Soc Am A* 1989;6:481–490.
- Kirby ML, Galea M, Loane E, Stack J, Beatty S, Nolan JM: Foveal anatomic associations with the secondary peak and the slope of the macular pigment spatial profile. *Invest Ophthalmol Vis Sci* 2009;50:1383–1391.
- Berendschot TTJM, van Norren D: Macular pigment shows ringlike structures. *Invest Ophthalmol Vis Sci* 2006;47:709–714.
- Ciulla TA, Curran-Celantano J, Cooper DA, Hammond BR, Danis RP, Pratt LM, et al: Macular pigment optical density in a mid-western sample. *Ophthalmology* 2001;108:730–737.
- Hammond BR, Caruso-Avery M: Macular pigment optical density in a southwestern sample. *Invest Ophthalmol Vis Sci* 2000;41:1492–1497.

- 23 Mellerio J, Ahmadi-Lari S, van Kuijk FJGM, Pauleikhoff D, Bird AC, Marshall J: A portable instrument for measuring macular pigment with central fixation. *Curr Eye Res* 2002;25:37–47.
- 24 Liew M, Gilbert C, Spector TD, Mellerio J, van Kuijk FJGM, Beatty S, et al: Central retinal thickness is positively related to macular pigment optical density. *Exp Eye Res* 2006;82:915–920.
- 25 Berendschot TTJM, van Norren D: On the age dependency of the macular pigment optical density. *Exp Eye Res* 2005;81:602–609.
- 26 Loane E, Kelliher C, Beatty S, Nolan JM: The rationale and evidence base for a protective role of macular pigment in age-related maculopathy. *Br J Ophthalmol* 2008;92:1163–1168.
- 27 Snodderly DM, Brown PK, Delori FC, Auran JD: The macular pigment.1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci* 1984;25:660–673.
- 28 Broekmans WMR, Berendschot TTJM, Klopping-Ketelaars IAA, de Vries AJ, Goldbohm RA, Tijburg LBM, et al: Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr* 2002;76:595–603.
- 29 Nolan JM, Stack J, O'Connell E, Beatty S: The relationships between macular pigment optical density and its constituent carotenoids in diet and serum. *Invest Ophthalmol Vis Sci* 2007;48:571–582.
- 30 Caruso-Averi M, Perez J, Myers-Evitt MJ, Crosley R, Nenez G, Alexander B, et al: Factors related to individual differences in macular pigment optical density in a southwestern sample. *Invest Ophthalmol Vis Sci* 1999;40:888B848.
- 31 Hammond BR, Ciulla TA, Snodderly DM: Macular pigment density is reduced in obese subjects. *Invest Ophthalmol Vis Sci* 2002;43:47–50.
- 32 Hammond BR, CurranCelentano J, Judd S, Fuld K, Krinsky NI, Wooten BR, et al: Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res* 1996;36:2001–2012.
- 33 Hammond BR, Fuld K, Snodderly DM: Iris color and macular pigment optical density. *Exp Eye Res* 1996;62:293–297.
- 34 Nolan J, O'Donovan O, Kavanagh K, Stack J, Harrison M, Muldoon A, et al: Macular pigment and percentage of body fat. *Invest Ophthalmol Vis Sci* 2004;45:3940–3950.
- 35 Curran-Celentano J, Hammond BR, Ciulla TA, Cooper DA, Pratt LM, Danis RB: Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am J Clin Nutr* 2001;74:796–802.
- 36 Mares JA, Larowe TL, Snodderly DM, Moeller SM, Gruber MJ, Klein ML, et al: Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. *Am J Clin Nutr* 2006;84:1107–1122.
- 37 Beatty S, Murray IJ, Henson DB, Carden D, Koh HH, 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.
- 38 Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S: Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration. *Surv Ophthalmol* 2008;53:68–81.
- 39 Beatty S, Koh HH, Murray IJ, Henson DB, Boulton M: Macular pigment optical density and the risk for age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;41:3187B285.
- 40 Werner JS, Donnelly SK, Kliegl R: Aging and human macular pigment density. Appended with translations from the work of Max Schultze and Ewald Hering. *Vision Res* 1987;27:275–268.
- 41 Delori FC, Goger DG, Hammond BR, Snodderly DM, Burns SA: Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J Opt Soc Am A Opt Image Sci Vis* 2001;18:1212–1230.
- 42 Neelam K, O'Gorman N, Nolan J, O'Donovan O, Wong HB, Eong KGA, et al: Measurement of macular pigment: Raman spectroscopy versus heterochromatic flicker photometry. *Invest Ophthalmol Vis Sci* 2005;46:1023–1032.
- 43 Ciulla TA, Hammond BR: 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.
- 44 Bernstein PS, Zhao DY, Sharifzadeh M, Ermakov IV, Gellermann W: Resonance Raman measurement of macular carotenoids in the living human eye. *Arch Biochem Biophys* 2004;430:163–169.
- 45 Liew SHM, Gilbert CE, Spector TD, Mellerio J, Marshall J, van Kuijk FJGM, et al: Heritability of macular pigment: a twin study. *Invest Ophthalmol Vis Sci* 2005;46:4430–4436.
- 46 Iannaccone A, Mura M, Gallaher KT, Johnson EJ, Todd WA, Kenyon E, et al: Macular pigment optical density in the elderly: findings in a large biracial Midsouth population sample. *Invest Ophthalmol Vis Sci* 2007;48:1458–1465.
- 47 Richer S, Devenport J, Lang JC: LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age-related macular degeneration to dietary supplementation with xanthophylls. *Optometry* 2007;78:213–219.
- 48 van der Veen RL, Ostendorf S, Hendrikse F, Berendschot TT: Macular pigment optical density relates to foveal thickness. *Eur J Ophthalmol* 2009;19:836–841.
- 49 Kilbride PE, Alexander KR, Fishman M, Fishman GA: Human macular pigment assessed by imaging fundus reflectometry. *Vision Res* 1989;29:663–674.
- 50 Chen SF, Chang Y, Wu JC: The spatial distribution of macular pigment in humans. *Curr Eye Res* 2001;23:422–434.
- 51 Berendschot TTJM, Willemse-Assink JJM, Bastiaanse M, De Jong PTVM, van Norren D: Macular pigment and melanin in age-related maculopathy in a general population. *Invest Ophthalmol Vis Sci* 2002;43:1928–1932.
- 52 Wustemeyer H, Moessner A, Jahn C, Wolf S: Macular pigment density in healthy subjects quantified with a modified confocal scanning laser ophthalmoscope. *Graefes Arch Clin Exp Ophthalmol* 2003;241:647–651.
- 53 Berendschot TTJM, van Norren D: Objective determination of the macular pigment optical density using fundus reflectance spectroscopy. *Arch Biochem Biophys* 2004;430:149–155.
- 54 Zagers NPA, van Norren D: Absorption of the eye lens and macular pigment derived from the reflectance of cone photoreceptors. *J Opt Soc Am A Opt Image Sci Vis* 2004;21:2257–2268.
- 55 Trieschmann M, Beatty S, Nolan JM, Hense HW, Heimes B, Austermann U, et al: Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res* 2007;84:718–728.
- 56 Gellermann W, Ermakov IV, Ermakov MR, McClane RW, Zhao DY, Bernstein PS: In vivo resonant Raman measurement of macular pigments in the young and the aging human retina. *J Opt Soc Am A Opt Image Sci Vis* 2002;19:1172–1186.
- 57 Bone RA, Landrum JT, Fernandez L, Tarsis SL: Analysis of the macular pigment by HPLC – retinal distribution and age study. *Invest Ophthalmol Vis Sci* 1988;29:843–849.
- 58 Handelman GJ, Dratz EA, Reay CC, van Kuijk FJGM: Carotenoids in the human macula and whole retina. *Invest Ophthalmol Vis Sci* 1988;29:850–855.
- 59 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.