Macular Pigment and its Contribution to Visual Performance and Experience

Mukunda Chaitanya Akkali

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Mukunda Chaitanya Akkali, BS Optom,
Macular Pigment Research Group,
Waterford Institute of Technology,
Waterford.

Supervised by:

Dr. John M Nolan, PhD and Professor Stephen Beatty, MD,
Macular Pigment Research Group,
Waterford Institute of Technology,
Waterford.

Dr. James Loughman, PhD,
Department of Optometry,
Dublin Institute of Technology,
Dublin.

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Declaration

No part of the work described in this thesis, or the thesis itself, was submitted previously for a degree at this or any other institution. This M.Sc. (by Research) thesis comprises one review article and three original investigations. The principal authors and investigators of the review article and of the original investigations were Dr. James Loughman, Dr. John M Nolan, Dr. Peter A Davison and Professor Stephen Beatty. My role was one of supervised research, including the collection and archiving of data for the original investigations, and presentation of the data in this thesis.

Signature:

Date:

Dedication

To God,

To my supervisors Dr. John M Nolan, Dr. James Loughman and Professor Stephen Beatty for their assistance, support and guidance,

To my parents Alivelamma and Krishnaiah Akkali for their love, kindness and support.

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I remain highly indebted to the entire MPRG staff (Ms. Lorna Rushe for project coordination, Ms. Grainne Scanlon for data collection in DIT, Ms. Eithne Connolly for her help in serum analysis, Dr. Edward Loane and Dr. Mark Kirby for their friendly advices, and Ms. LeighAnne Maddock for her initial project coordination) for their continued support during the course of this project. Besides, many thanks to my fellow post-grads, and I wish them all the best in their own endeavours. I would like to express my sincere gratitude to the entire WIT staff (especially the science office, research support unit, international office, inter library loans, porters office, educational services and medical centre) who were always there to lend a hand of support. In addition, warm thanks to all the volunteers, without whom this project would not have been possible.

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LIST OF ABBREVIATIONS

Active	A
Age-Related Eye Diseases Study	AREDS
Age-related macular degeneration	AMD
Age-related maculopathy	ARM
Analysis of variance	ANOVA
Association for Research in Vision and Ophthalmology	ARVO
Best corrected visual acuity	BCVA
Body mass index	BMI
Candela per square metre	cd m^{-2}
Collaborative Optical Macular Pigment ASsessment Study	COMPASS
Compact fluorescent lamp	CFL
Confidence interval	CI
Contrast acuity threshold	CAT
Contrast sensitivity function	CSF
Critical flicker fusion frequency	CFF
Customised heterochromatic flicker photometry	cHFP
Cycles per degree	cpd
Cystic fibrosis	CF
Decibel	dB
Dioptre	D
Dublin Institute of Technology	DIT
Electroretinogram	ERG
European association for vision and eye research	EVER

Farnsworth-Munsell 100 hue test	FM100
Food frequency questionnaire	FFQ
Heterochromatic flicker photometry	HFP
High performance liquid chromatography	HPLC
Intra-ocular lens	IOL
International Standard Randomised Controlled Trial Number	ISRCTN
Laser-assisted in situ keratomileusis	LASIK
Light emitting diode	LED
Liquid crystal display	LCD
Logarithm of the minimum angle of resolution	LogMAR
Lutein	L
Lutein Antioxidant Supplementation Trial	LAST
LUtein Nutrition effects measured by Autofluorescence	LUNA
Macular automated photostress	MAP
Macular pigment	MP
Macular pigment optical density	MPOD
Macular pigment research group	MPRG
meso-zeaxanthin	MZ
Modulation transfer function	MTF
National Institute of Standards and Technology	NIST
Optimal flicker fusion frequency	OFF
Pattern electroretinogram	PERG
Pattern visual evoked potential	PVEP
Photostress recovery time	PRT
Placebo	P

Randomised controlled trial	RCT
Reactive oxygen intermediate	ROI
Red-green	RG
Response amplitude density	RAD
Retinal pigment epithelium	RPE
Retinitis pigmentosa	RP
Seasonal affective disorder	SAD
Standard deviation	SD
Standard operating procedure	SOP
Temporal contrast sensitivity function	TCSF
Ultra violet	UV
Visit	V
Visual acuity	VA
Visual evoked potential	VEP
Waterford Institute of Technology	WIT
Xanthophyll-binding proteins	XBP
Yellow-blue	YB
Zeaxanthin	Z

ABSTRACT

Title

Macular pigment and its contribution to visual performance and experience

Introduction

The carotenoids lutein (L), zeaxanthin (Z) and *meso*-Z (MZ) accumulate at the macula where they are collectively referred to as macular pigment (MP). Scientific research continues to explore the function(s) of MP in the human retina, with two main hypotheses premised on its putative capacity to (1) protect the retina from (photo)-oxidative damage by means of its optical filtration and/or antioxidant properties, the so-called protective hypothesis and (2) influence the quality of visual performance by means of selective short wavelength light absorption prior to photoreceptor light capture, thereby attenuating the effects of chromatic aberration and light scatter, the so-called acuity, visibility and glare hypotheses. The current epidemic of age-related macular degeneration (AMD) has directed researchers to investigate the protective hypothesis of MP, while there has been a conspicuous lack of work designed to investigate the role of MP in visual performance

Objectives

This M.Sc. (by Research) thesis has four primary objectives:

1. To present and critically appraise the current literature germane to the contribution of MP, if any, to visual performance and experience
2. To assess whether MP optical density (MPOD) is associated with visual performance **(Study One)**
3. To investigate whether augmentation of MPOD enhances visual performance in normal subjects **(Study Two)**
4. To assess whether MPOD is associated with colour discrimination and matching **(Study Three)**

Methods

Study One:

The Relationship between Macular Pigment and Visual Performance

One hundred and forty-two young healthy subjects were recruited. MPOD was assessed by customised heterochromatic flicker photometry (cHFP). Visual performance was assessed by psychophysical tests including best corrected visual acuity (BCVA), mesopic and photopic contrast sensitivity, glare disability, photostress recovery time (PRT).

Study Two:

The Impact of Macular Pigment Augmentation on Visual Performance in Normal Subjects

One hundred and twenty-one normal subjects were recruited. The active (A) group consumed 12mg of L and 1mg of Z daily. MPOD was assessed by cHFP. Visual performance was assessed as BCVA, mesopic and photopic contrast sensitivity, glare disability, photostress, and subjective visual function. Subjects were assessed at baseline; three; six; twelve months (V1, V2, V3 and V4, respectively).

Study Three:

Macular Pigment: its Associations with Colour Discrimination and Matching

One hundred and two normal subjects were recruited. Colour vision was assessed with the Farnsworth-Munsell 100 Hue test (FM100), Moreland match on the Heidelberg Multi Colour (HMC) Anomaloscope, and a customised short wavelength automated perimetry (cSWAP) technique at the foveola and at 1°, 2°, 3°, 4° and 5° of retinal eccentricity. MPOD spatial profile was measured using cHFP.

Results

Study One:

The Relationship between Macular Pigment and Visual Performance

We report a positive and statistically significant relationship between BCVA and MPOD across its full spatial profile (r = 0.237 to 0.308, $P < 0.01$). MPOD was also positively and significantly related to both mesopic and photopic contrast sensitivity (at 7.5 cycles per degree (cpd) and 11.8 cpd), but was confined to the central MPOD at 0.25° and 0.50° of retinal eccentricity (r = 0.167 to 0.220, $P < 0.05$, for all). Glare disability and PRT were unrelated to MPOD spatial profile ($P > 0.05$).

Study Two:

The Impact of Macular Pigment Augmentation on Visual Performance in Normal Subjects

Central MPOD increased significantly in the A group ($P < 0.05$) but not in the placebo (P) group ($P > 0.05$). This statistically significant increase in MPOD in the A group was not, in general, associated with a corresponding improvement in visual performance ($P > 0.05$, for all variables), with the exception of a statistically significant time/treatment effect in "daily tasks comparative analysis" ($P = 0.03$). At V4, we report statistically significant differences in mesopic contrast sensitivity at 20.7 cpd, mesopic contrast sensitivity at 1.5 cpd under high glare conditions, and light/dark adaptation comparative analysis between the lower and the upper MP tertile groups ($P < 0.05$).

Study Three:

Macular Pigment: its Associations with Colour Discrimination and Matching

Total error scores (TES) and % partial error scores (%PES) on the FM100 were uncorrelated to MPOD. Moreland matches shown a significant long wavelength shift with MPOD at between 1° and 3° (at 1.75°, r = 0.489, $P <$

0.001). Sensitivities on cSWAP using foveal targets were significantly inversely correlated with MPOD at both 1.75° (r = -0.461, $P < 0.001$) and 3° (r = -0.393, $P < 0.001$). Partial correlation analysis suggests that none of these findings can be attributed to age effects within the range 18 to 40 years.

Conclusions

Study One:

The Relationship between Macular Pigment and Visual Performance

Measures of central visual function, including BCVA and contrast sensitivity, were positively associated with MPOD. Glare disability and photostress recovery were unrelated to MPOD

Study Two:

The Impact of Macular Pigment Augmentation on Visual Performance in Normal Subjects

We report that a significant increase in central MP following L supplementation does not, in general, impact on visual performance in young normal subjects, and our pre-specified hypothesis that MP augmentation would result in improved visual performance and/or comfort by 12 months, in those randomised to the A arm, remains unproven. However, subjects with high MP following L supplementation demonstrate visual benefits with respect to glare disability and mesopic contrast sensitivity. Further study into MP and its relationship with visual performance is warranted to enhance our understanding of this pigment's role. However, in order to investigate the impact of MP augmentation on visual performance, the findings of our study suggest that we should direct our attention to, a) subjects with low baseline central MP levels, b) subjects with suboptimal visual performance and c) subjects with symptoms of glare disability

Study Three:

Macular Pigment: its Associations with Colour Discrimination and Matching

Our findings suggest that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. The association of MPOD with cSWAP may be a temporally limited effect to which the visual system normally adapts. We suggest that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity

-CHAPTER ONE-

INTRODUCTION

Macular Pigment and its Contribution to Visual Performance and Experience

1.1 MACULAR PIGMENT

The carotenoids lutein (L), zeaxanthin (Z) and *meso*-Z (MZ) accumulate at the macula where they are collectively referred to as macular pigment (MP).[1] L and Z are of dietary origin, whereas MZ is not normally found in a conventional diet, and is generated at the retina following L isomerisation.[1;2] Interestingly, it has been shown that L is the dominant carotenoid in the diet[3], whereas Z/MZ have been shown to be the dominant carotenoids at the macula.[4;5]

1.1.1 LOCATION OF MACULAR PIGMENT

MP is predominantly found in the photoreceptor axons of the foveola and the plexiform layers of the macula. (Figure 1.1).[6;7]

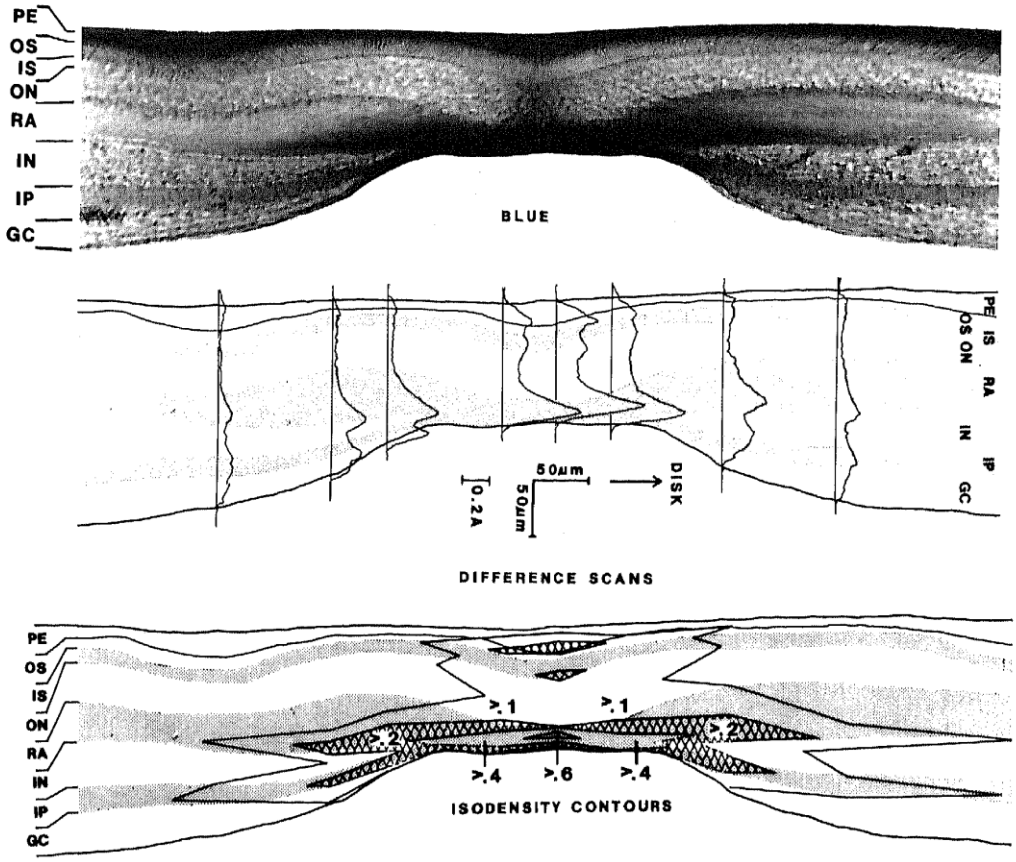

Figure 1.1 Cross sections through the macaque macula show the asymmetric distribution of MP (dark region, top). Contours (middle) show the variation in the absorbance graphically superimposed on the cross-section outline. Regions of isodensity are indicated and illustrate the anatomic fine structure of the MP distribution (bottom) [From Snodderly *et al*. 1984].[7]

1.1.2 DISTRIBUTION OF MACULAR PIGMENT

Studies have demonstrated that, MP generally peaks at the centre of macula, with a concentration of almost 1mM at this location and experiences an exponential decay with optically undetectable levels at 6° to 8° of retinal eccentricity.[8] However, significant deviations from this typical distribution have been reported in some studies.[8-12] Individual variations in peak optical density are large ranging from 0.175 up to 1.39 log units.[8] It reaches its half-

peak optical density at an average of only 1.03° (0.3 mm) of retinal eccentricity.[8] Though MP is optically undetectable at a retinal eccentricity of 7° (2mm), L and Z are present in the peripheral retina in minute concentrations (approximately 1/300 of that within 0.25 mm of the foveal centre).[4] The aggregate amount of total L and Z in peripheral retina, accounts for approximately 50% of the total amount of the carotenoids within the entire retina.[13;14] MZ and Z are the predominant carotenoids in the foveal region, whereas L predominates in parafoveal region.

It has been shown that, the combined concentration of L + Z is 70% higher in human rod outer segments (ROS) than in residual membranes. L+Z is 2.7 times higher in the human perifoveal ROS membrane than in the peripheral retina. L and Z are consistently detected in human retinal pigment epithelium (RPE) at relatively low concentrations.[15]

1.1.3 BIOCHEMICAL STRUCTURE OF CAROTENOIDS

Carotenoids have a basic 40-carbon polyene ($C_{40}H_{56}$) structure, forming the backbone of these molecules. The central carbon chain of alternating single and double bond carries cyclic or acyclic end groups. The colour of these carotenoids ranges from pale yellow to deep red and is directly linked to their structure. The extended system of conjugated double bonds is responsible for their colour, and also contributes to their major biochemical functions.

There are over 600 known carotenoids. Carotenoids are generally split into two classes: Carotenoids with molecules containing at least one oxygen atom, are termed as xanthophylls. L, Z, α-cryptoxanthin, β-cryptoxanthin and astaxanthin are important xanthophyll carotenoids. Carotenoids composed of only carbon and hydrogen atoms (oxygen free) termed as Carotenes. β-Carotene, α-carotene and lycopene are prominent members of the carotene group.

1.1.4 STEREOCHEMISTRY OF MACULAR PIGMENT

L, Z and MZ contain two hydroxyl groups, one on each side of the molecule. Structurally, L, Z, and MZ are isomers of one another (Figure 1.2), with L differing from Z/MZ in the position of double bond in the six-carbon (ionene) ring located on the right side of the carbon chain. Z and MZ differ in that the hydroxyl group is above the plane of the ionene ring in Z and below it in MZ.[1]

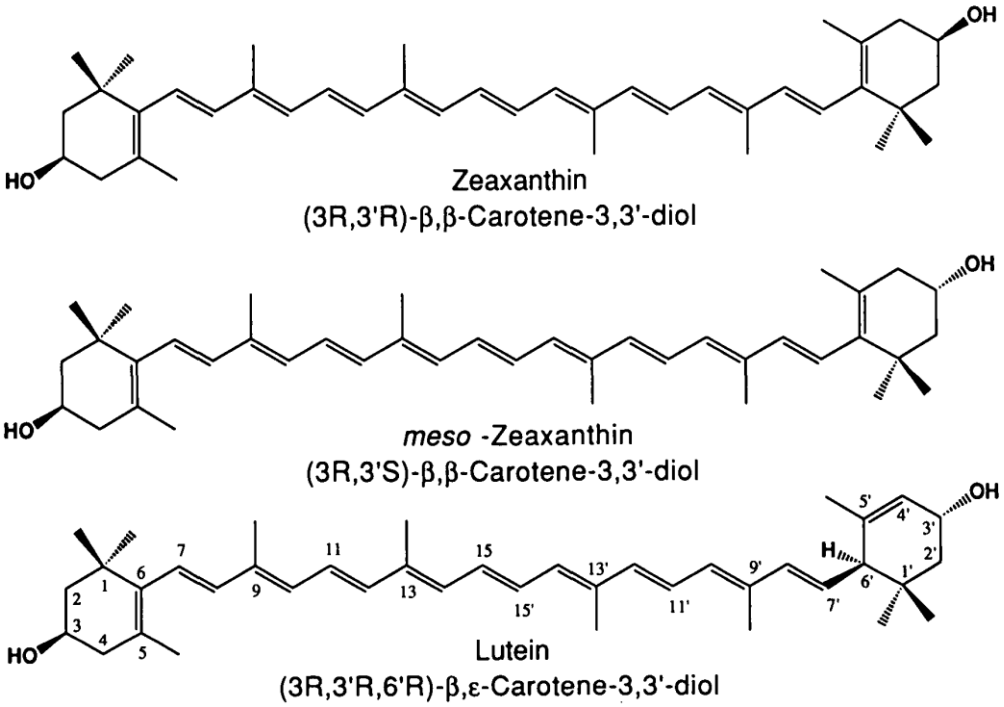

Figure 1.2 The structures of the three major components of MP [From Bone *et al.* 1993].[1]

1.1.5 MACULAR PIGMENT ABSORBANCE SPECTRUM

MP absorbance spectrum peaks at 458 nm (short wavelength light), thereby protecting the macula from photo-oxidative damage (Figure 1.3).[16;18] MP levels are maximal within the layer 'fibres of Henle' (which is anterior to the photoreceptors) in the foveola, which facilitates the absorption of short wavelength light at a pre-receptorial level, thereby altering the spectral distribution of light incident on photoreceptors in a favourable way (Figure

1.4). It has been estimated that, the quantity of short wavelength light incident on the fovea is substantially reduced as a result of the filtering properties of MP; this reduction is estimated at approximately 40%, but varies from 3-100% between individuals.[16;17]

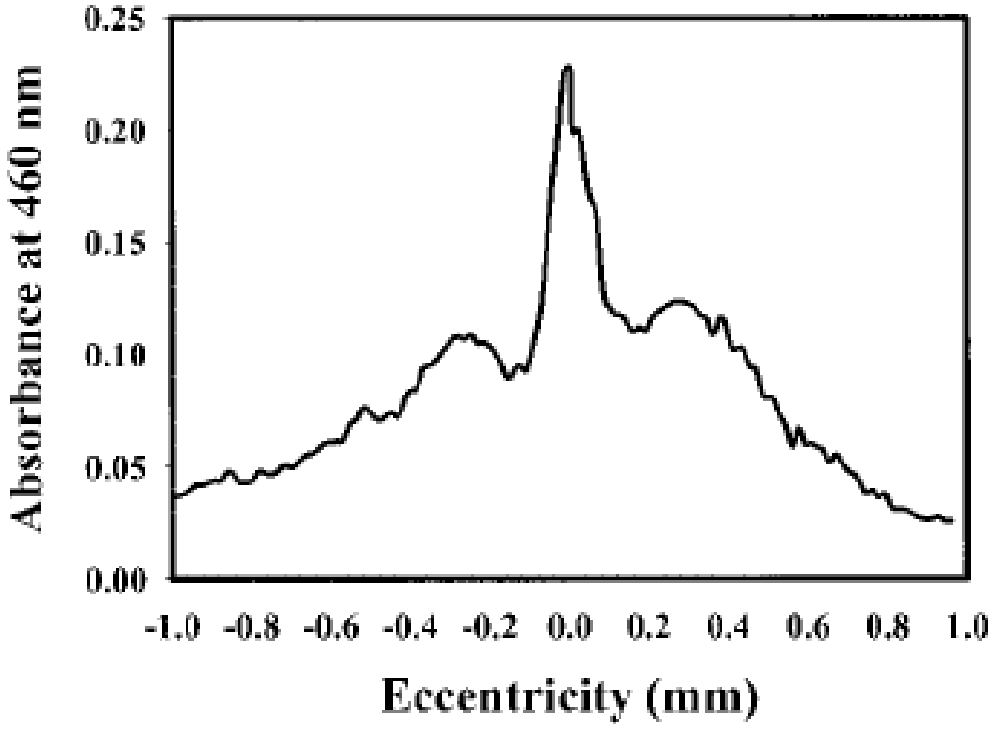

Figure 1.3 Graphical variation of the absorbance of MP with retinal eccentricity in the macaque monkey as measured by microspectrophotometric methods [From Snodderly *et al.* 1991].[5]

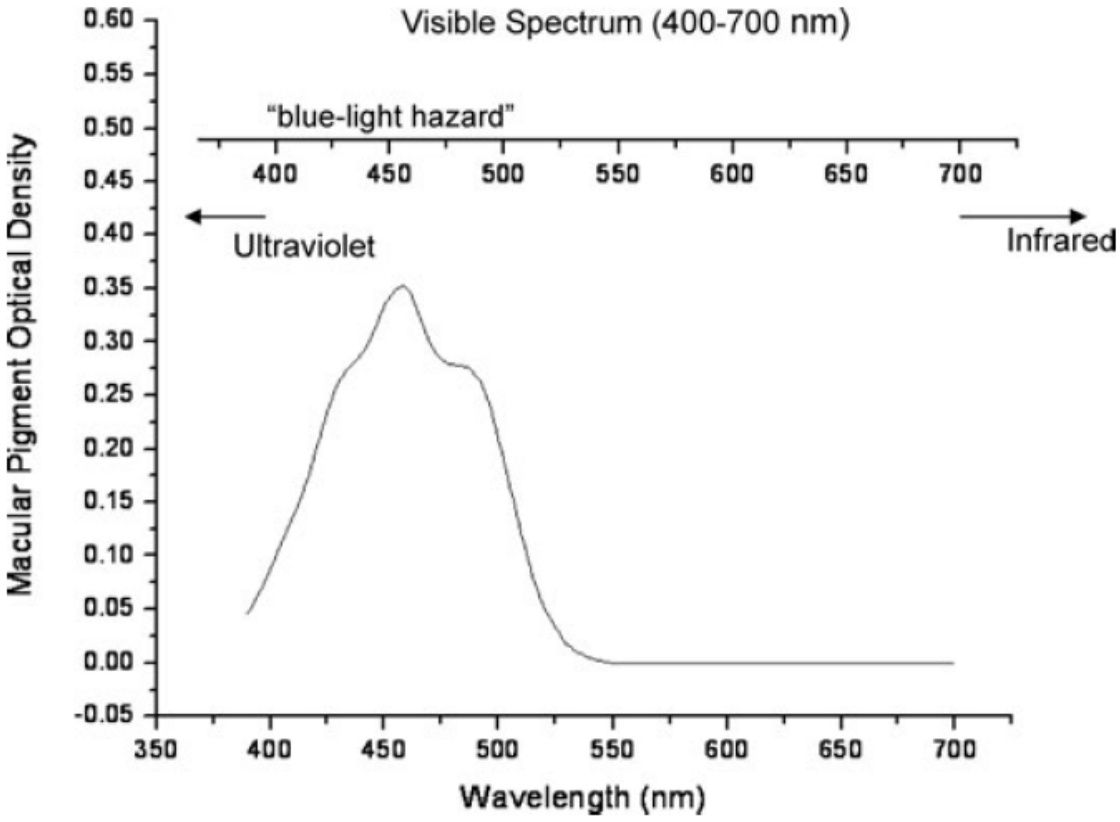

Figure 1.4 The absorbance spectrum of retinal L and Z compared to the visible spectrum [From Bone *et al.* 1992].[18]

1.1.6 OPTICAL AND ANATOMIC PROPERTIES OF MACULAR PIGMENT

MP's optical and anatomic properties have prompted the "optical" hypothesis of this pigment, which has been discussed in detail by Reading & Weale[19] and later by Nussbaum *et al*.[20] The optical effect of MP is somewhat evidenced by two entoptic phenomena known to exist which are specific to the macula, namely Maxwell's spot and Haidinger's brushes.[20] The former, first described in 1844, is attributed directly to the deposition of pigments at the macula and results in a dark red spot being visible around the fixation point if a brightly illuminated white surface is viewed alternately through purple and neutral filters. Magnussen *et al.*[21] have shown that the absence of short wave sensitive cones in the human foveola, which

normally goes unnoticed unless a subject's field of view is restricted to the foveola, producing the artificial colour vision defect of foveal tritanopia,[22;23] results in a blue scotoma which can be visualised as the negative afterimage of a short-wavelength adapting field on a larger white background. The afterimage has an annular shape with a lighter inner region that corresponds to Maxwell's spot, and a small bright spot in the centre, corresponding to the foveal blue scotoma. The MP distribution measured for the same observers closely corresponded to the lighter annular region of the afterimage.

Haidinger's brushes, first reported in 1844, refers to a propeller-shaped image which is seen most clearly through a rotating filter producing plane-polarised light. It is known that L has dichroic properties[24;25] and it has been shown that bovine L and Z bind to bovine retinal tubulin.[26] It is thus possible that dichroic MPs are laid down in a highly organised manner following the radial arrangement of Henle's fibres at the macula, thus explaining the shape and brush-like appearance of the propeller-like images.[27]

However, it should be noted that neither of these entoptic phenomena is visible in normal viewing conditions, probably because of adaptatory effects, particularly at the level of the visual cortex. It is uncertain whether the concentration of MP has any significant influence on vision under such conditions.

MP may be important for visual performance and/or experience however by at least one of the following mechanisms (summarised by Walls & Judd[28]): MP may enhance visual acuity by reducing chromatic aberration (effects); MP may reduce visual discomfort by attenuation of glare and dazzle; MP may facilitate enhancement of detail and visual contrast by the absorption of "blue haze". MP has the capacity to achieve the above optical effects because of its optical properties and because of its location within the retina. This traditional description of the "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual

performance, that are, perhaps, unrelated to the short wave filtration properties of MP. MP has been shown to exhibit dichroic properties[29] which may facilitate the reduction of glare disability through preferential absorption of polarised light. Higher MP optical density (MPOD) has also been observed to relate to a trend towards lower root-mean-square wavefront aberrations (in particular, higher order aberrations), thereby enhancing visual performance.[30]

There is one additional, and important, mechanism, whereby MP may have a beneficial effect on visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons.

The term macula lutea is actually attributable to the presence of the xanthophyll pigments, L, Z, and MZ at the central region of the retina, which give rise to the appearance of a yellow spot (macula lutea) when viewed under red-free light (Figure 1.5). The yellow colouration of MP is such that it selectively absorbs blue-green incident light, with maximum absorption at 458 nm and little or no absorption above 530 nm.[16;18] Given that (1) the peak retinal spectral sensitivity lies at 555 nm, (2) the proportion of blue (short wavelength sensitive) cones in the central macula is far lower than that of red (long wavelength sensitive) and green (medium wavelength sensitive) cones and (3) the region of maximal visual performance, the foveola, is essentially devoid of short wavelength sensitive cones, it would appear that the optical properties of MP are such that it attenuates the component of light that is least beneficial, and most deleterious, with respect to visual performance and experience. As Wald[31] summarised, the various adaptive mechanisms in the human eye serve to "withdraw vision from the blue" end of the spectrum.

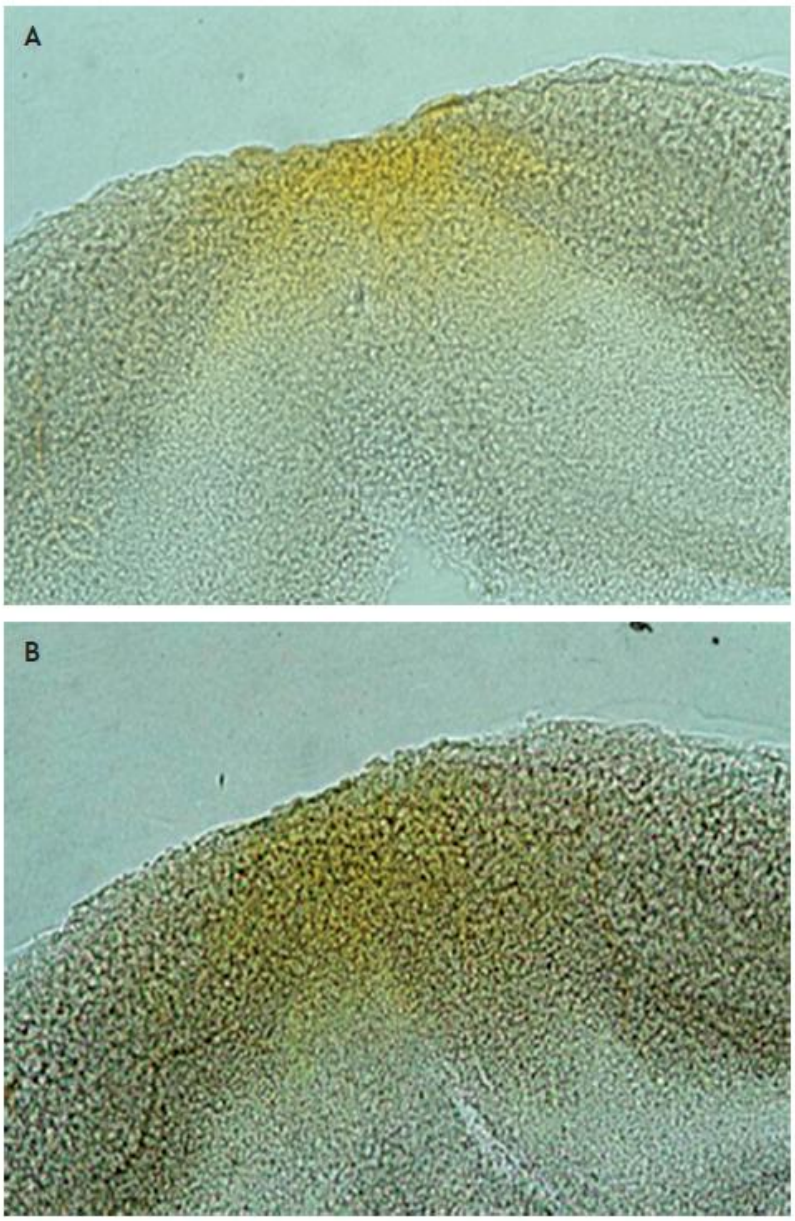

Figure 1.5 Histological section illustrating the spatial profile and pre-receptorial location of MP, the main location of MP is in the layer of fibres of Henle in the fovea (A) and in the inner nuclear layer at the parafoveal site (B). [From Trieschmann *et al.* 2008].[6]

Two aspects of MP's location within the retina are also central to the hypothesis that it has a role to play in visual performance. Firstly, although MP is found throughout the retina and other ocular structures,[32] it reaches its greatest concentration at the macula, and remains optically undetectable elsewhere. Secondly, and importantly, MP is located at a pre-receptorial

level, so that absorption of short wavelength light occurs prior to stimulation of the underlying photoreceptors, thereby altering the spectral distribution of light incident on such photoreceptors in a favourable way (Figure 1.5).

Short wavelength light absorption attenuates the more disadvantageous component of longitudinal chromatic aberration (LCA). Retinal image quality is thereby improved, and visual performance across the full contrast range is theoretically more refined. As MP absorption overlaps with that of rhodopsin, MP may reduce rod signal effectiveness in the mesopic range, and thus extend the usefulness of cone-mediated vision into the mesopic range.[30] In addition, short wavelength light absorption has the benefit of improving target contrast by selectively reducing the scattered short wavelength light in the background. Reduced LCA and reduced scatter effects, resulting from MP's absorptive characteristics, have the potential to improve visual acuity and target visibility, and perhaps in an interactively additive fashion.[33]

The higher energy and retinal irradiance associated with shorter wavelengths (International Commission on Non-Ionizing Radiation Protection, 1997[34]) also merits consideration. Bright light, which interferes with the quality of visual perception, is termed glare, of which there are numerous types. In high luminance or high contrast situations, where glare and dazzle are maximal, MP absorption of short wavelength light attenuates the highest energy light component, and reduces retinal irradiance, and therefore may minimise the impact of glare on performance, and increase the threshold for photophobia under normal viewing conditions. Because of their linear structure, L, Z, and MZ also exhibit dichroic properties,[29] which facilitate glare reduction by preferential absorption of polarised light. Glare symptoms remain a common and important clinical entity in optometric and ophthalmological practice, and very troublesome for those who experience it.[35] Furthermore, symptoms of glare remain difficult to quantify and treat. Interestingly, difficulty with glare is often one of the earliest manifestations of age-related macular degeneration (AMD). It should now be clear, because visual performance is a complex subject, which is difficult to

quantify, and dependent on numerous independent and overlapping variables, that to investigate the contribution of any one factor (such as MP) presents numerous challenges. It is with this thought in mind that currently available evidence on the impact of MP on visual performance and experience will now be explored.

1.2 VISUAL PERFORMANCE

1.2.1 CURRENT AND UNIFYING CONCEPTS

Snellen was the first to standardise the measurement of visual acuity with his letter chart in 1862, a chart design, which despite numerous limitations, remains the most widely used means of quantifying visual performance in the clinical setting.[119] There remains, however, a myriad of other independent and/or overlapping techniques by which one can measure visual performance and experience across a range of functional levels.

Vision includes the capacity to detect objects against a contrasting background, to detect gaps between objects, to perceive subtle vernier offsets (which provides one example of hyperacuity), to recognise and identify objects, to perceive colour, to detect movement, and to perceive depth, among other faculties. It is important to note that the capacity to recognise a small distant object bears little relation to the capacity to differentiate colours, or to detect a potential threat such as an oncoming vehicle in the peripheral field of view.

Visual performance is critically dependent on illumination, and the range of illumination we experience in the course of a typical day is vast. The visual system copes with such changes in illumination by adapting to the prevailing conditions, and can function through an approximate 8 log unit luminance range. Although adaptation facilitates performance over a wide range of ambient illumination levels, it does not follow that we see equally well at all levels. Under dim conditions, for example, the visual

system is very sensitive and can detect subtle changes in luminance, but acuity for pattern details and colour discrimination is poor.

Shlaer[36] has explored the relationship between illumination and visual acuity (Figure 1.6). Converting his findings to Snellen equivalent, daylight (photopic) performance of 6/3 reduces to 6/180 under dim conditions, a 60-fold reduction. Threshold visibility, colour appearance and visual acuity all vary dramatically with illumination, and these visual parameters change over the time-course of light and dark adaptation. Therefore, and by definition, no single test or testing condition can be used to investigate visual performance, and no single test can predict performance on other tests.

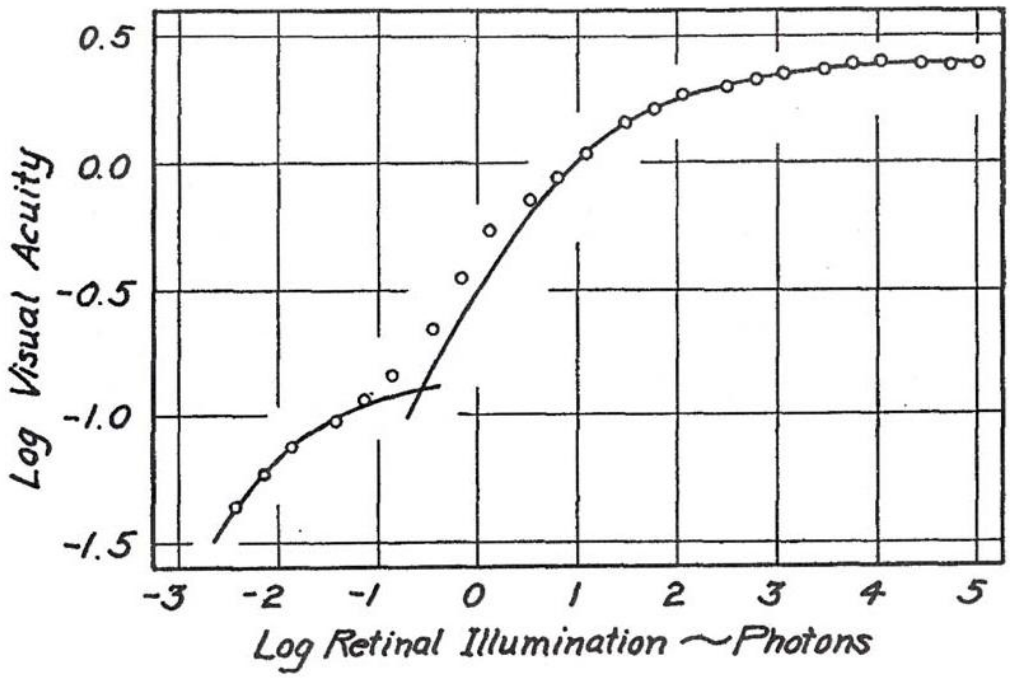

Figure 1.6 The relationship between visual performance (as log visual acuity) and retinal illuminance. As retinal illuminance increases, visual acuity increases by up to 2 log units (cone-mediated improvements accounts for the most significant improvements from approximately 6/60 to 6/3 Snellen equivalent-see upper portion of curve. [From Shlaer *et al.* 1937][36]

Further, any discussion of the visual processes must include those mechanisms contributing to perception. The visual system employs numerous anatomic and physiological strategies, including lateral interactions between cells, specific receptive field organisation, spatial retinotopic organisation in retinal and non-retinal areas of the pathway, colour opponency and parallel visual pathways, among others, in order to achieve an instantaneous, coherent and highly detailed perception of the outside world and our position within it. Such image processing is not exclusive to the brain, but extends throughout the visual pathway beginning at the retina.

The eyes and brain are thus inextricably linked with the visual universe. The eyes actively record the form, colour and movements of the world, and the brain moulds these raw perceptions into recognisable patterns. The retina essentially acts as a spatial, temporal and spectral filter of patterns of light striking its surface. Its anatomic structure and the functional properties of individual cells determine the type of information extracted from a visual scene and delivered to the brain.

1.2.2 SPECIALISATION OF THE MACULA

The macula, which comprises less than 4% of the total retinal area, subserves almost all of our useful photopic vision. Several distinctive anatomic and neural adaptations facilitate such a high level of visual performance. These include:

1. Cone density peaks at the centre of the macula (fovea), which intersects the line of sight. Cones here are smaller, more densely packed and more numerous than elsewhere in the retina, thus extending the limits of spatial acuity. Cone density exceeds rod density only at the lower part of the foveal slope, reaching a maximum at the base of the fovea (foveola) where cone density is over three times that observed at the foot of the foveal slope.[37] Rods, ganglion cells and all inner nuclear layer neurons are absent from the

foveola, so that only here light is directly incident on photoreceptors (elsewhere light must traverse the various retinal cells and layers to reach photoreceptors). It is also worth noting that short wavelength sensitive cones are absent at the foveola.

2. Midget pathways arising from these foveal cones dominate. Such parvocellular midget pathways are tuned to high spatial frequencies and also exhibit colour opponency.

3. Such midget pathways are distinctive because of the absence of convergence of photoreceptor signals onto bipolar and ganglion cells. Absent or reduced convergence of information preserves the data gathered at the fovea for delivery to the visual cortex. Such differences between foveal and extra-foveal pathways generate a hierarchy in the processing of information gathered by the retina.

1.2.3 RETINAL HEIRARCHY

Anatomic and physiological observations, such as the differential light sensitivity of photoreceptors, the variable density and distribution of photoreceptors and ganglion cells across the retina and the convergence of information from the extra-foveal retina, means that a hierarchy exists in the architecture of retinal processing, where foveal information is given higher priority. This hierarchy is preserved to the striate cortex, where a high percentage of cortical cells are dedicated to information of foveal origin. The central retinal pathways have by far the greatest proportion of representation (estimates range from 25% of the cortex devoted to the central 5°, 37% devoted to the central 15° and[38] 87% of the cortex devoted to the central 30° of visual field[39]).

Having outlined those anatomic and neural factors central to primates' capacity for high acuity vision, it is now important to consider the potential role of MP in visual performance. In order to do so, it is essential to

characterise (a) the optical limitations that might restrict visual performance (in particular chromatic aberration and light scatter) and (b) the properties of MP that might serve to lessen the effect of such limitations, and thereby facilitate optimal visual performance.

1.2.4 OPTICAL LIMITATIONS OF THE EYE

Monochromatic aberrations and diffraction limit the image quality produced by the eye, so that the image is not always a high quality representation of the object. While there is significant ocular and neural correction for, and adaptation to, such image defects, MP most likely has no role in altering their effects (although Kvansakul *et al.*[30] have noted some surprising observations of a trend towards lower root mean square wavefront aberrations in a small group of subjects following supplementation with L and Z, which, they postulate, may be as a result of the as yet unknown effects of carotenoid intake on crystalline lens function).

1.2.5 CHROMATIC ABERRATION

Chromatic aberration or colour fringing is a type of distortion in which there is a failure of lens to focus different wavelengths of lights, onto the same point (as the refractive index of the lens and image magnification varies with wavelength).[40]

1.2.5.1 TYPES OF CHROMATIC ABERRATIONS

1.2.5.1.1 CHROMATIC DIFFERENCE OF FOCUS

It is a type of chromatic aberration, where different wavelengths are focussed on different points on the optical axis. Chromatic difference of focus causes shorter wavelengths to focus in front of the retina and longer wavelengths to focus behind the retina.[40] It occurs because the refractive index of the given refracting medium (example: Lens) is inversely

proportional to the wavelength, resulting differences in focal lengths for different wavelengths. It is also termed as LCA [Figure 1.7]. The range of LCA is very consistent across individuals.[41]

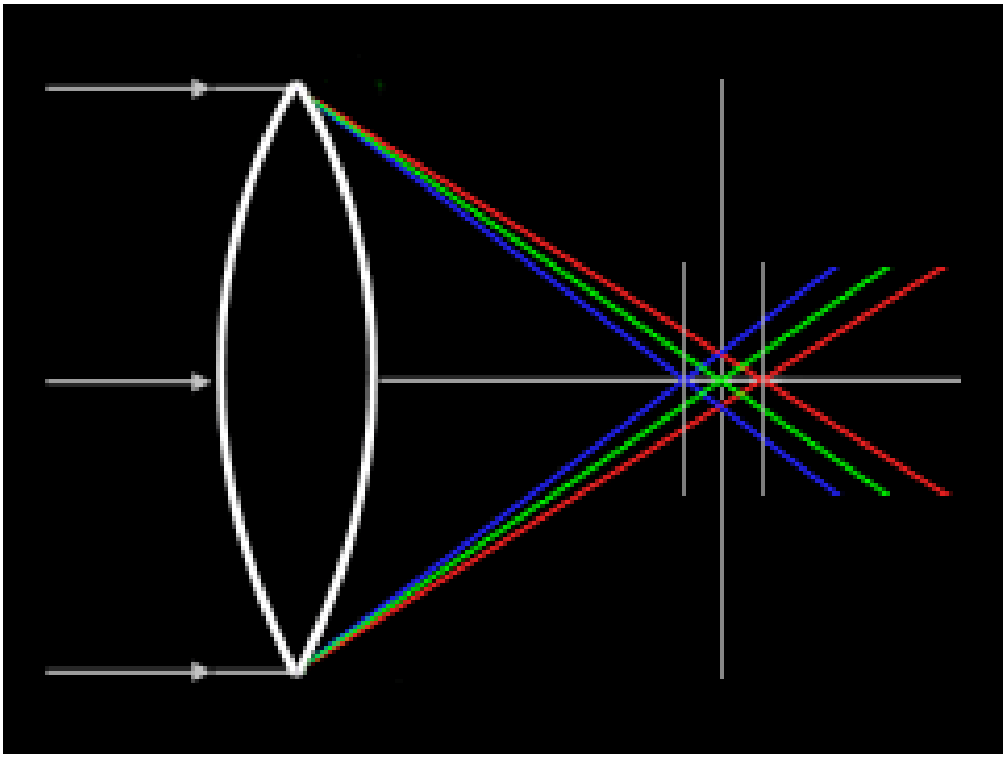

Figure 1.7 Illustration of longitudinal chromatic aberration.

1.2.5.1.2 CHROMATIC DIFFERENCE OF MAGNIFICATION

Chromatic difference of magnification is caused by axial pupil location [Figure 1.8].[40] For example, if the pupil is well in front of the nodal point, eccentric rays which are entering into pupil strike the refracting surface of the eye with greater angle of incidence. By Snell's law, these rays will be subjected to stronger chromatic dispersion, yielding a chromatic difference of magnification. On the other hand, if the pupil is centred on the nodal point of the eye, then rays would strike the refracting surface with normal incidence which spares the effects of chromatic dispersion.

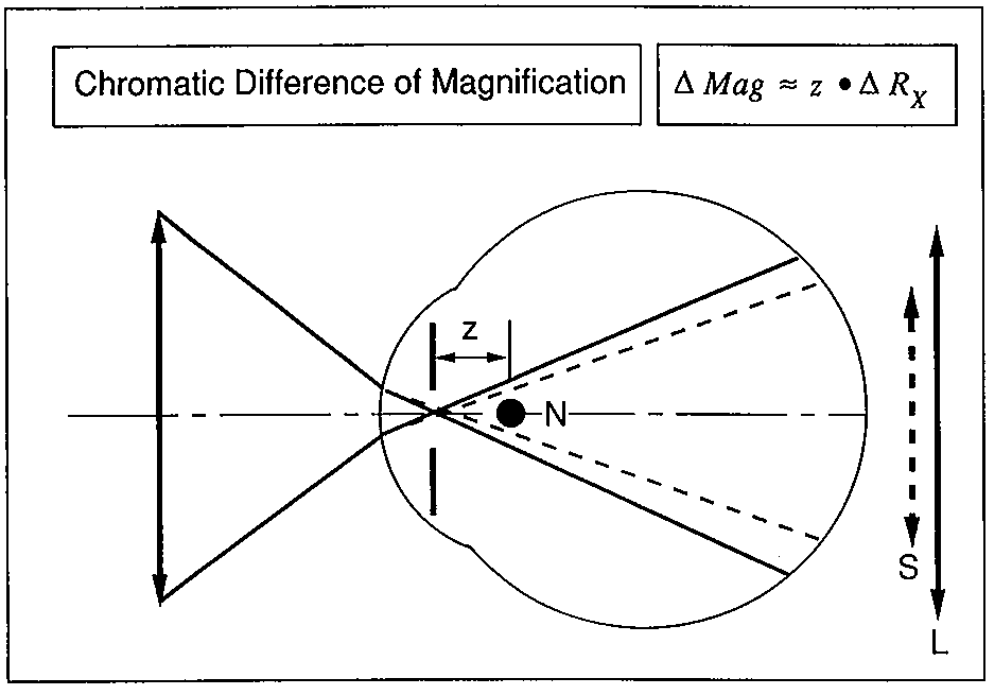

Figure 1.8 Illustration of chromatic difference of magnification. Axial distance z from entrance pupil to nodal point determines angle of incidence of rays admitted to the retina, hence the amount of chromatic dispersion. The result is differential image magnification which is directly proportional to the chromatic difference of refraction of the eye. Solid rays show path of long wavelengths; dashed rays show short wavelengths [Thibos *et al*. 1991].[40]

1.2.5.1.3 CHROMATIC DIFFERENCE OF POSITION

Chromatic difference of position is caused by pupil location and the path of the achromatic axis [Figure 1.9].[40] By definition, achromatic axis is an axis which joins nodal point to the centre of the pupil, which means it does not necessarily intersect the retina at the fovea. The angle between the visual axis (line joining nodal point and fovea) and achromatic axis constitutes chromatic difference of position, which is also termed as transverse

chromatic aberration (TCA). Simply, it is a type of chromatic aberration, where different wavelengths are focussed at different positions in the focal plane (perpendicular to the optical axis). It also occurs because of misalignment between cornea, lens and fovea.[42;43] The range of TCA varies across individuals.[44]

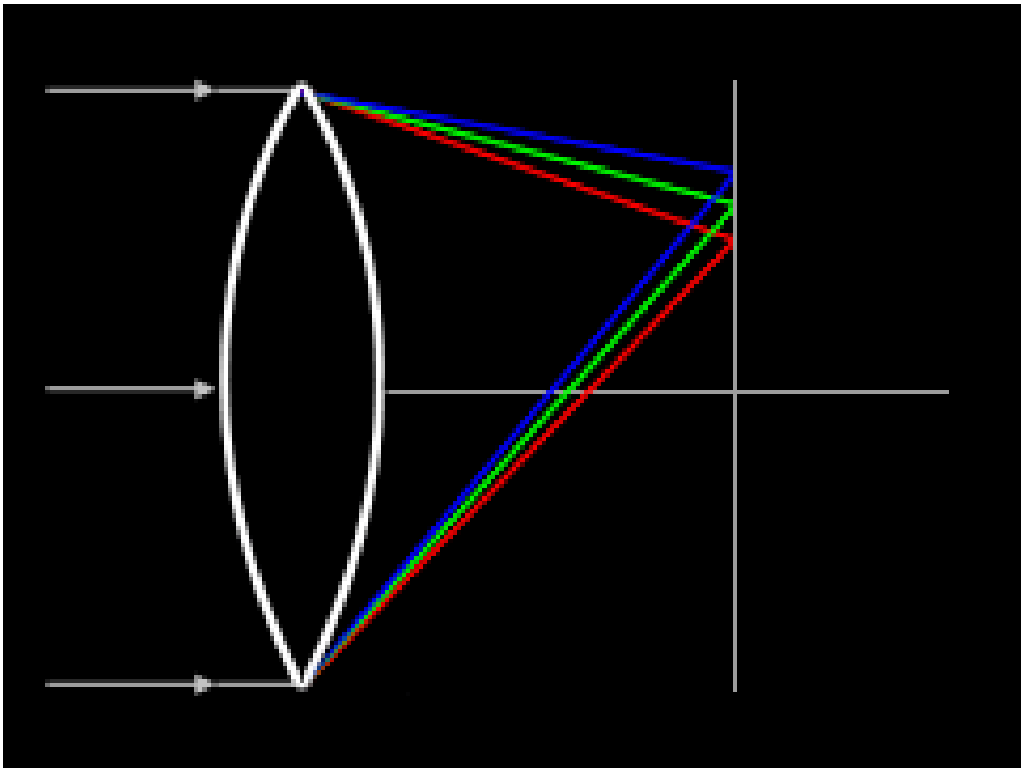

Figure 1.9 Illustration of transverse chromatic aberration.

1.2.5.2 EFFECT OF CHROMATIC ABERRATION ON VISUAL PERFORMANCE

Chromatic aberration, comprising both longitudinal (LCA) and transverse (TCA) components, has been cited as possibly the most significant aberration affecting visual quality.[41] Indeed, LCA creates up to 2 dioptres (D) of wavelength dependent optical defocus. Campbell and Gubbisch[45] have demonstrated improvements in contrast thresholds of up to 65% at intermediate spatial frequencies once monochromatic yellow light is employed in place of spectrally broadband white light. Although Bradley *et al*.[46] later modelled the effects of chromatic aberration, and concluded that the effect of chromatic aberration on the modulation transfer function (MTF) was small, and equivalent to approximately 0.15 D of defocus, upper resolution limits of the visual system however, are most likely defined by the effects of chromatic aberration.[40]

The effect of LCA across wavelength, in terms of blur, is non-linear, as shorter wavelengths are significantly more defocused than longer wavelengths. For example, an eye focussed at 550 nm, light at 460 nm suffers 1.2 D myopic defocus, while the equivalent long wavelength of 640 nm is only 0.50 D out of focus.[41] This serves to create a purple blur circle haze around the focussed "green" component.

Figure 1.10 demonstrates the non-linearity of defocus and the relative luminance profile across wavelength. As the spectral extremities have less luminosity, the effects of chromatic aberration on image focus are mitigated in terms of the effects on vision. Mitigation is potentially further aided by the fact that blue light is selectively absorbed by MP.

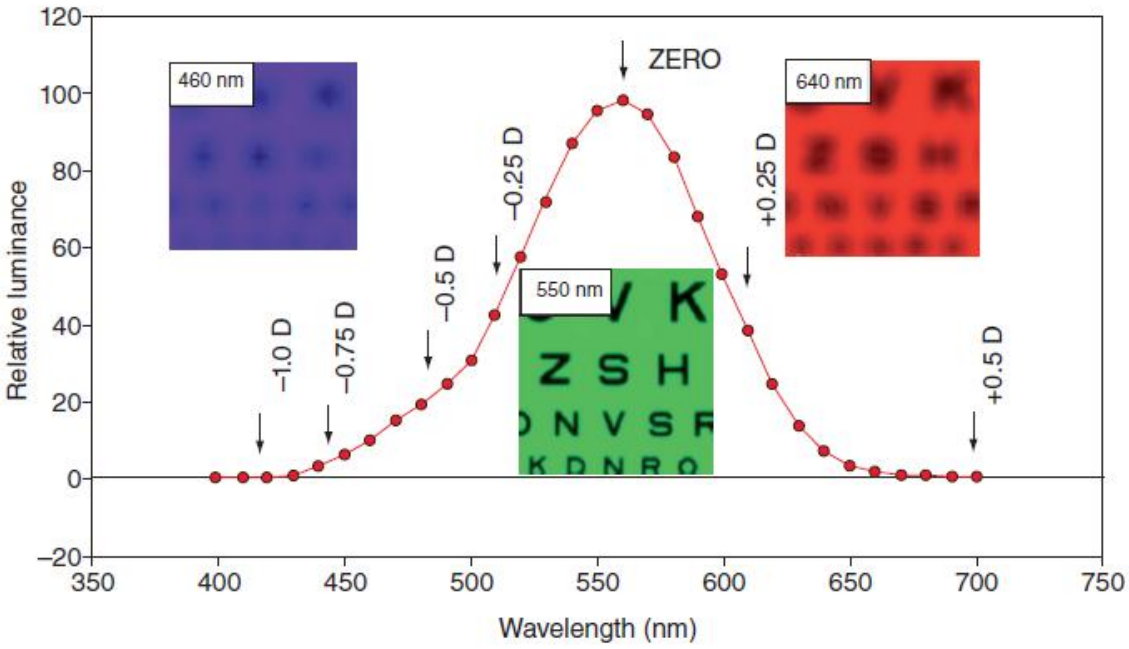

Figure 1.10 Illustration of the relative luminance profile and the effect of chromatic aberration across wavelengths. The relative blur is more pronounced at the blue end of the spectrum such that, for example, the short wave 460 nm text is significantly more difficult to recognise than the long wave 640 nm text for the above scenario where the optimal focus is between 540-560 nm. [From Thibos *et al*. 1991].[40]

1.2.6 LIGHT SCATTER

1.2.6.1 THE PHYSICS OF LIGHT SCATTER

A light ray can be diverted from a given path by several processes, e.g., absorption, reflection, refraction, diffraction. Among these processes the most important process related to our vision at the earth's surface is scattering.

Scattering is the process by which a particle in the path of an electromagnetic wave continuously (1) abstracts energy from the incident wave, and (2) reradiates that energy into the total solid angle centred at the particle. Scattering only occurs when the particle's refractive index differs from the surrounding medium (From McCartney, 1976).[47]

Scatter determines how far we can see and how well an object details can be resolved. The amount of scatter depends upon the concentration and the type of particles in the atmosphere.

There are two types of scattering which are very important and are concerned with the earth's atmosphere.

1.2.6.2 RAYLEIGH SCATTERING

According to Rayleigh's theory, small particles scatter light proportional to the inverse of the wavelength raised to the fourth power:

$$\beta_{sc} = c\lambda^{-4},$$

where β_{sc} refers to amount scattered, c is a constant, and λ is the wavelength.

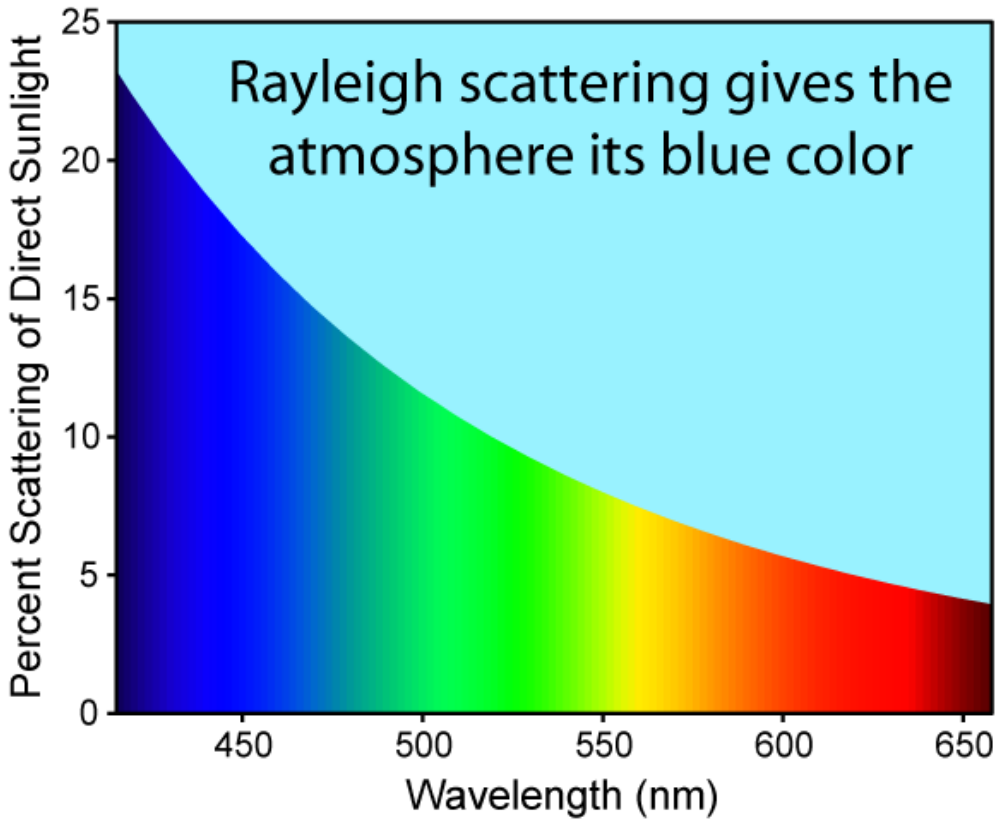

Figure 1.11 Illustration of Rayleigh scattering.

Rayleigh scattering strongly depends upon the size of the particle and wavelengths. It occurs when the particle size is smaller than about 0.1λ (much smaller than the wavelength of light). The intensity of Rayleigh scatter increases as the ratio of particle size to wavelength increases. Furthermore, Rayleigh scatter would give similar amounts of forward and backward light scatter.

As per equation, Rayleigh scattering is inversely proportional to the fourth power of the wavelength. For this reason, shorter wavelengths violet and blue light scatter more than the longer wavelengths of green and red lights. Though violet light is being scattered strongly in the atmosphere, due to the limitation of human eyes to shorter wavelengths violet light is detected weakly by our eyes. As a result, sky is seen as blue, despite the fact that it is violet (Figure 1.11).

Rayleigh scattering primarily occurs through light's interaction with the atmospheric gases (air molecules).

1.2.6.3 MIE SCATTERING

Mie theory explains scattering when the particle diameter is greater than about 0.1 λ. It represents light (electromagnetic radiation) scattering by a dielectric sphere. Dielectric spheres are known to scatter light, if the wavelength of light is similar to the size of the dielectric sphere.

Mie scattering occurs when the atmospheric particles are equal in size to the wavelengths of light being scattered. Mie scattering increases, as the particle size increases. It is independent of wavelength of light and also gives relatively more amount of forward light scatter compared to backward light scatter. It occurs mostly in the lower atmosphere where larger particles are abundant.

Scattering by atmospheric dust particles, smoke, pollen and water vapour which tends to affect longer wavelengths are explained by Mie scattering. The blue colour of the sky is due to Rayleigh scattering, as the size of the gas particles (air molecules) is much smaller than the wavelength of visible light. On the other hand, water droplets which make up clouds are of comparable size to the wavelengths in visible light, hence the light is scattered approximately and identically making the clouds to appear white or grey (Figure 1.12).

Figure 1.12 Illustration of Mie scattering.

1.2.6.4 HAZE AEROSOL

An aerosol is a dispersed system of particles suspended in a gas; the term haze aerosol emphasises the particle nature of haze. From the optical standpoint, haze is a condition wherein the scattering property of the atmosphere is greater than can be attributed to the gas molecules but is less than that of fog (from McCartney, 1976).[47] It also can be defined as an

aerial form of Tyndall effect. The Tyndall effect is a light scattering by particles in a colloid or particles in a fine suspension.

Haze aerosols are made by many particles such as dust, volcanic ash, sea salt, products of combustion and terpenes (aromatic hydrocarbon vapours exuded by plants). The size these particles vary from 0.01 to 10 μm. 'Blue haze' in the dense forests is formed by small particles that are generated by terpenes (plants exude) following reactions with sunlight and ozone.

Scattering through haze aerosols is explained by Mie theory. Unlike other atmospheric effects such as cloud and fog, scattering through haze aerosols is spectrally selective. The spectral energy distribution of scattered light in haze aerosols varies from blue to very blue.

In an atmospheric perspective, as the distance between target and viewer increases, it results in the reduction of contrast at all spatial frequencies between the target and its background, as well as a reduction in the ability to discriminate two targets side-by-side. The target's colour as well becomes less saturated, and shifts towards the background's colour, which is usually blue. (Except in some conditions such as sunrise or sunset distant colours may shift towards red).

Scattering through haze aerosols decreases visibility in the atmosphere (Figure 1.13). By definition, visibility is the clearness with which objects in the atmosphere stand out from their surroundings (Bennett, 1930, cited in Middleton, 1952).[48;49]

Figure 1.13 The ‘blue haze’ seen in an aerial perspective.

1.2.7 GLARE

Bright light, which interferes with the quality of visual perception, is termed as glare. Glare sources can be direct, such as the sun and lamps, or indirect, such as the surfaces that are too bright. The latter includes reflections of primary sources in glossy materials or off of water (i.e. veiling reflections).

There are three main types of glare: (1) disability glare, (2) discomfort glare, (3) light-adaptation glare.

1.2.7.1 DISABILITY GLARE

Disability glare is a term used to describe the degradation of visual performance typically caused by loss of retinal image contrast. Disability glare is often caused, for example, by surface light reflections, or bright

light sources such as car headlights, and typically is a consequence of increased forward light scatter within the eye.

Disability glare is totally due to the effects of stray light (at least for glare angles greater than 1°), and is independent of neural etiology.[50] It is the most commonly used clinical measure of glare.

1.2.7.1.1 RELEVANT PHYSIOLOGY

Light scatter occurs when the spacing between elements of different refractive index becomes comparable with or greater than the wavelength of light. Ocular media opacification is caused by light scattering.[51] Light scatter also occurs when the elements themselves becomes comparable in size with the wavelength of light.

1.2.7.1.2 FORWARD VERSUS BACKWARD LIGHT SCATTER

The light which is scattered forward onto retina is called 'forward light scatter'. This is the type of light scatter that causes reduced vision. The light which is scattered back from the eye toward the light source is called 'backward light scatter'. Forward and backward light scatter have been shown to be well correlated.[52;53] Glare tests aim to provide an indication of the amount of forward light scatter.

Light scatter is inversely proportional to the square of the glare angle.[54] Vision is reduced by scattered light in two ways. First, from the object itself light gets scattered, and reduces the contrast of its retinal image. This type of vision loss is very much asymptomatic. Second, a wide-angle intraocular light scatter from a peripheral glare source produces a veiling luminance on the retina and reduces the retinal image contrast. Intraocular light scatter can be determined by a Stray light meter.[55]

1.2.7.2 DISCOMFORT GLARE

Discomfort glare is the visual discomfort in bright-light situations. It could be the results of spasm of iris sphincter. It is more commonly seen in ocular inflammations such as 'iritis'. There are no commercially available tests to measure discomfort glare. In the laboratory discomfort glare can be measured by electromyography based on the eye squinting magnitude.[56;57] It can also be measured by subjective discomfort rating scales.

1.2.7.3 LIGHT ADAPTATION GLARE

Light adaptation glare is the reduction in visual function associated with central scotoma following exposure to a bright light. Unlike disability glare, light adaption glare has retinal aetiology.[58] The central scotoma, which causes a temporary state of retinal insensitivity, is produced by the bleaching of foveal cone photo pigments.

1.2.7.3.1 RELEVANT PHYSIOLOGY

Visual phototransduction is a process by which light is transformed into electrical signals in the retina of the eye. Photoreceptors contain a chromophore (11-*cis*-retinal, the aldehyde of Vitamin A1 and light-absorbing portion) bound to a cell membrane protein 'opsin'. Rods and cone photopigments differ only by the 'opsin' they contain. Rods contain a photo pigment rhodopsin, and cones contain three photopigments, cyanolable, chlorable and erythrolable. Each cone contains only one photopigment. Light absorption by visual pigment leads to the separation of chromophore from opsin. This process is called bleaching. Photochemical reactions of cone photopigments are similar to rod photopigments (rhodopsin) except the cone photopigments recover from bleaching at a faster rate than rhodopsin. It is assumed that, cone photopigment regeneration is similar to the rod photo pigment regeneration.[59] The regeneration cycle of rhodopsin is shown in the figure below (Figure 1.14).[59]

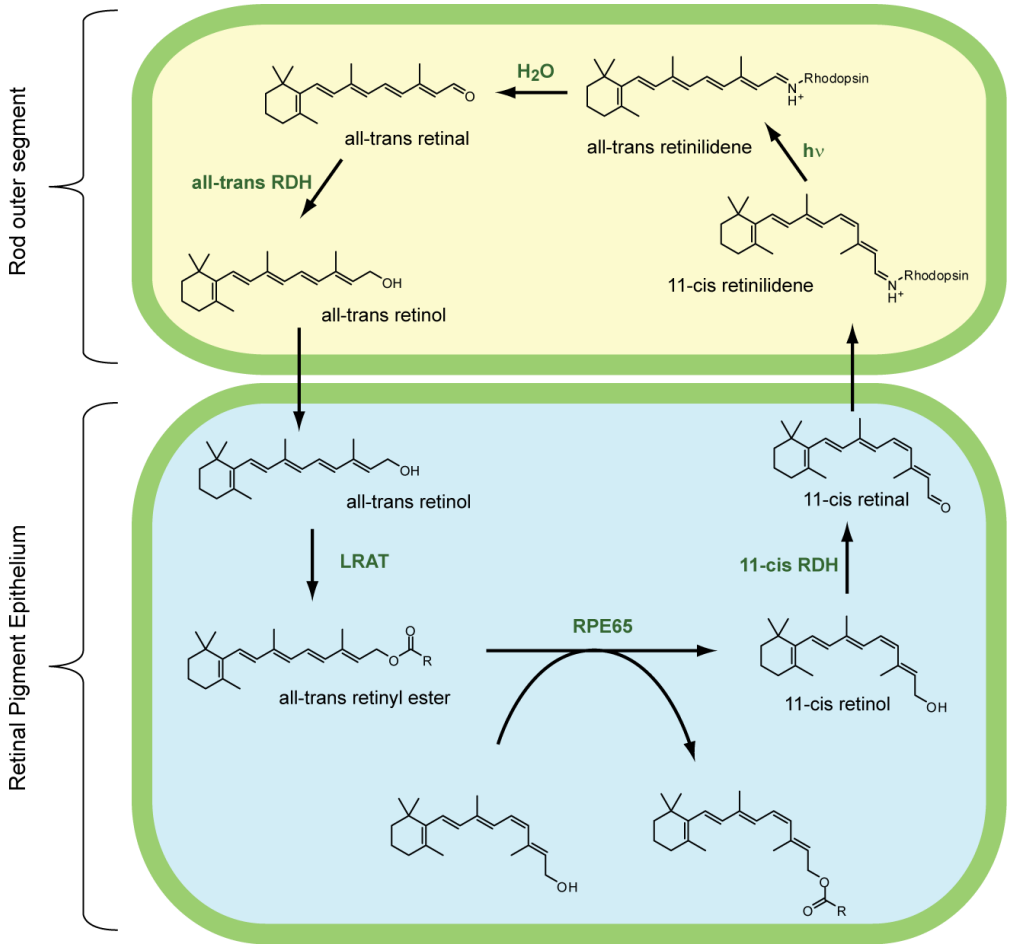

Figure 1.14 Illustration of the visual cycle.

There are several causes of prolonged light adaptation or recovery time. The RPE plays a major role in the 'vitamin A cycle' and also involved in the phagocytosis of the outer segment of photoreceptor cells. Dysfunction of RPE-receptor complex (e.g. AMD, angioid streaks, retinitis pigmentosa (RP)) affects these processes and slows the regeneration of photopigments.[60;61;62]

Light adaptation glare is clinically measured as a photostress recovery time (PRT). Macular automated photostress (MAP) is one of the tests to measure PRT.[63;64] PRT testing is a most useful clinical tool for discriminating optic nerve dysfunction from macular disease. Because optic

nerve disorders (e.g. optic neuritis, ischemic optic neuropathy) do not affect visual cycle, so PRT remains normal.[65] Prolonged PRT suggests macular disease.

1.3 MACULAR PIGMENT WITH RESPECT TO VISUAL PERFOMANCE

Beyond its "protective" hypothesis, MP's optical and anatomic properties have prompted the "optical" hypotheses of this pigment. The "optical" hypotheses of MP have been previously discussed by Reading *et al*.[19] and later by Nussbaum *et al*.[20] and include MP's putative ability to enhance visual performance and/or comfort by attenuation of the effects of chromatic aberration and light scatter, via its short wave light-filtering properties.[28] This traditional description of the "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual performance, that are, perhaps, unrelated to the short wave filtration properties of MP. MP has been shown to exhibit dichroic properties[29] which may facilitate the reduction of glare disability through preferential absorption of polarised light. Higher MPOD has also been observed to relate to a trend towards lower root-mean-square wavefront aberrations (in particular, higher order aberrations), thereby enhancing visual performance.[30]

There is one additional, and important, mechanism, whereby MP may have a beneficial effect on visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons.

1.3.1 MACULAR PIGMENT – THE ACUITY HYPOTHESIS

Light must pass through MP before being processed by the photoreceptors. MP's spectral absorbance profile is shown in Figure 1.4. MP's absorbance is quite specific and significant. MP absorbs 1/3rd of the short wavelength portion of the visible spectrum. MP's peak absorbance varies as high as 1.39 optical density units (meaning only about 5% of the "blue" or short wavelength light is transmitted onto the photoreceptors).[33]

Chromatic aberration, comprising both longitudinal (LCA) and transverse (TCA) components, has been cited as possibly the most significant aberration affecting visual quality.[41] Indeed, LCA creates up to 2 D of wavelength dependent optical defocus. Campbell and Gubbisch[45] have demonstrated improvements in contrast thresholds of up to 65% at intermediate spatial frequencies once monochromatic yellow light is employed in place of spectrally broadband white light. Although Bradley *et al.*[46] later modelled the effects of chromatic aberration, and concluded that the effect of chromatic aberration on the MTF was small, and equivalent to approximately 0.15 D of defocus, upper resolution limits of the visual system however, are most likely defined by the effects of chromatic aberration.[40]

The effect of LCA across wavelength, in terms of blur, is non-linear, as shorter wavelengths are significantly more defocused than longer wavelengths. For example, an eye focussed at 550 nm, light at 460 nm suffers 1.2 D myopic defocus, while the equivalent long wavelength of 640 nm is only 0.50 D out of focus.[41] This serves to create a purple blur circle haze around the focussed "green" component.

Schultz in 1866 postulated that the MP which works as an optical filter reduces chromatic aberration by absorbing blue end of the visible

spectrum.[66] This hypothesis was supported by Nobel laureate George Wald.[31]

Reading and Wale in 1974 quantitatively evaluated the "acuity hypothesis". They calculated the size of the blur circle due to chromatic aberration in daylight, computed the spectral transmittance necessary to reduce the resultant violet penumbra to a threshold level and proposed that the characteristics of such an ideal filter are similar to the transmittance of the MP. According to these calculations, they proposed that an average amount of MP is sufficient to do this function; any additional amount of MP would be superfluous.[19]

McLellan *et al*. has shown that when other monochromatic aberrations (mainly wavefront) are considered, short wavelength light defocus is not as blurred as previously thought, that the potential image quality for short wavelength cones is comparable to that for long and medium wavelength cones, and that MP has no significant function in improving visual acuity.[67]

The limitations of study done by McLellan *et al.* are as follows:
McLellan *et al*. dilated their subject's pupil to 7 mm, which increased the optical blurring caused by spherical aberration and increased the number of wavefront aberrations. A normal pupil diameter, in typical daylight, is approximately 3 mm would yield considerably less spherical aberration and fewer wavefront defects but a similar degree of chromatic aberration (approximately 1D).

Engles *et al.*[68] have investigated the "acuity hypothesis", exploring the relationship between MPOD and gap acuity and vernier acuity under photopic conditions. They report that neither gap acuity nor vernier acuity were significantly related with MPOD, and concluded that their "data suggest that the predictions of the acuity hypothesis do not hold". While the authors qualify their findings as appropriate to their specific testing conditions alone, several study limitations (other than those recognised by

the authors) warrant brief discussion and is explained later in section 1.3.6.3.2.

1.3.2 MACULAR PIGMENT – THE VISIBILITY HYPOTHESIS

Wooten and Hammond proposed a model where they quantitatively explained how MP determines our visibility and discriminability through haze aerosols (blue haze).[33]

In an atmospheric perspective, 'blue haze' acts as a background or veiling luminance with respect to the targets seen through it. Furthermore, as the viewing distance increases, the background luminance increases and becomes increasingly shortwave dominant (blue haze). On the other hand, as the viewing distance increases, the luminance of the target decreases and becomes increasingly shortwave deficient.[33]

Since MP absorption primarily is in the range of 420-500nm, it has a quantitatively different effect on the background luminance compared to target luminance. MP improves contrast for a target in a 'blue haze' by attenuating background luminance more than the target luminance (Figure 1.15).

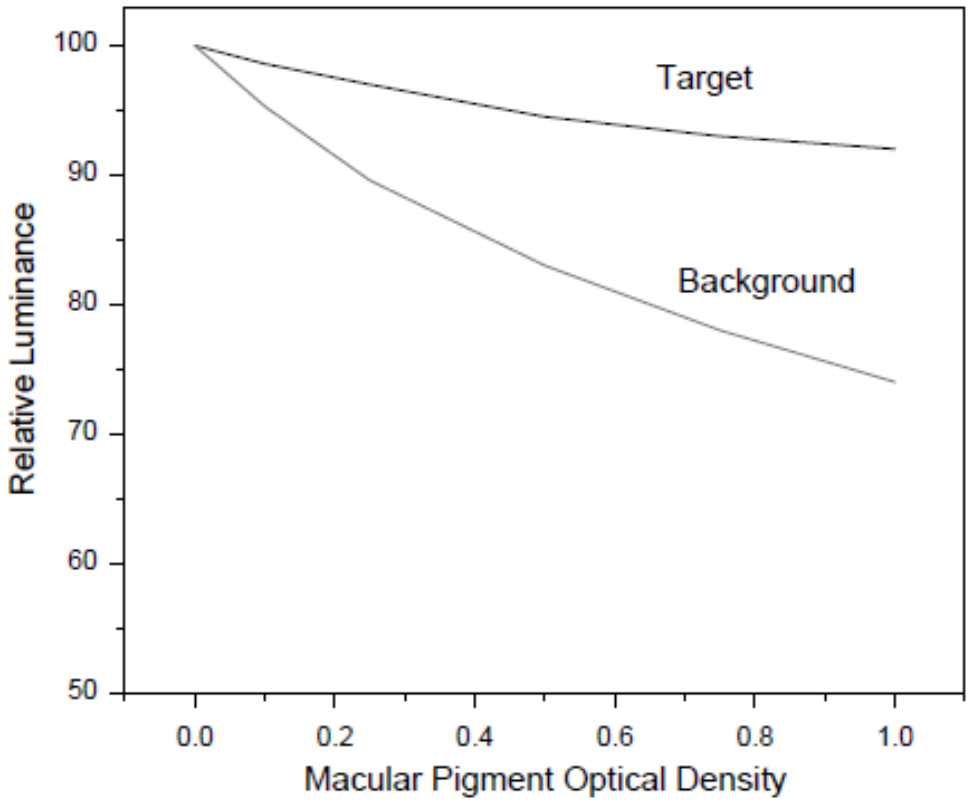

Figure 1.15 Illustration of the relative luminance of the target and background as a function of MPOD. Target curve is relatively shallow with an attenuation of 8% compared to background curve which has an attenuation of 26% for a 1.0 peak MPOD [From Wooten *et al.* 2002].[33]

1.3.2.1 IMPLICATIONS OF THE VISIBILITY HYPOTHESIS

Theoretically, a 1 log unit increase in MPOD attenuates the veiling luminance of the short-wave dominant background by 26% (or 17% for a more practical 0.5 log unit increase in MPOD), while having minimal effect on the short wave deficient distant target.[33] The attenuation of the effects of light scatter is thereby observed to enhance target detection and discrimination capacity, and extend the visual range by up to 18.6%.

1.3.3 MACULAR PIGMENT – THE GLARE HYPOTHESIS

Intraocular light scatter produces a veiling luminance over the retina which reduces the retinal image contrast. It is believed that intraocular light scatter includes a relatively large amount of blue scatter (Rayleigh scatter). MP as a short wavelength light filter could reduce the veiling luminance over the retina, and improve the retinal image contrast, therefore decreasing the disability glare. MP could also reduce light adaptation glare (i.e. decreasing the photostress recovery time (PRT) by preventing the bleaching of photopigment.[69;70]

Short wavelength light is a stronger contributor to discomfort glare, than mid or long wavelength light. It is possible that MP could reduce visual discomfort associated with glare/dazzle.[56;57]

1.3.4 MACULAR PIGMENT – CONTRAST SENSITIVITY

Contrast sensitivity is an important aspect of spatial vision. MP might influence contrast sensitivity. For photopic conditions, this function might be attributable to the attenuation of the effects of chromatic aberration and light scatter, whereby image refinement potentially causes lateral inhibitory surround responses to be dampened, and the resultant ganglion cell response optimised.[71;72]

For mesopic conditions, it is more likely that enhanced contrast sensitivity is a consequence of the selective diminution of rod mediated signals. While rod and cone photoreceptors operate interactively in the high mesopic conditions,[71] rods remain optimally sensitive to shorter wavelengths than cones (explaining the Purkinje shift in peak retinal spectral sensitivity towards blue under mesopic conditions). The pre-receptorial absorption of short wavelength light by MP might, therefore, serve to attenuate rod activity and allow cone mediated vision which

typically exhibits better contrast sensitivity[73] to dominate further into the mesopic range.

1.3.5 MACULAR PIGMENT – COLOUR VISION

Hue discrimination and colour vision in general are most acute at the fovea[74] corresponding to increased cone density, specialised anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good colour vision is possible beyond the fovea).[75] It is plausible that colour discrimination at a small angular subtense would be influenced by the optical density of MP at the fovea. Indeed it has long been speculated that inter-observer differences in colour matching by colour-normal observers are at least partially due to differences in macular pigmentation.[76;77] Also it is known that even subjects with ophthalmoscopically-normal fundi exhibit substantial variations in MPOD, contributing to a range of pre-receptorial light absorption at 460 nm from 3% to almost 100%.[78]

Since the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 458 nm,[16;18] it would seem likely that these pigments influence colour vision through selective absorption of short wavelengths, thereby influencing the short wave sensitive cones and the blue-yellow opponent-colour channel.

1.3.6 EVIDENCE THAT MACULAR PIGMENT PLAYS A ROLE IN VISUAL PERFORMANCE AND EXPERIENCE

1.3.6.1 BACKGROUND

The evidence in relation to a role for MP in visual performance is sparse and is largely associative. To our knowledge, there are limited studies which have satisfactorily investigated the hypothesis that MP influences visual performance and experience.[68;70] However there are numerous and conflicting reports on the effect of yellow filters on visual performance,[79] but none of these have included measures of MPOD. Failure to do so confounds any reasonable interpretation of short wavelength light absorption effects on visual performance, as variations in MPOD between and within study populations could account for the reported observations.

There are thus two strategies to investigate the impact of MP on visual performance. The first is to quantify performance using a range of functional tests, and to correlate the results with measures of MPOD. Given the other variables involved in vision, the true effect of MP would, in our opinion, prove difficult to isolate with such a paradigm. The alternative and most appropriate means to investigate the effect of MP is to measure baseline visual performance, as above, and to record baseline MPOD, and then repeat functional vision tests during an extended period of supplementation with MP xanthophylls.

If MP influences visual performance it must do so either as (1) a short wavelength light filter or (2) through some biological mechanism. With respect to the former (1), any effects on visual performance should follow the known absorbance characteristics of the pigments. Hence, the visual stimuli to be used to investigate the role of MP should have significant amounts of short wave energy, in order to replicate the effects of ecologically valid stimuli (e.g. the sun) which have lots of short wave

energy. This traditional description of the "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual performance, that are, perhaps, unrelated to the short wave filtration properties of MP. MP has been shown to exhibit dichroic properties[29] which may facilitate the reduction of glare disability through preferential absorption of polarised light. Higher MPOD has also been observed to relate to a trend towards lower root-mean-square wavefront aberrations (in particular, higher order aberrations), thereby enhancing visual performance.[30] Biological effects (2) would likely be based on either enhanced protection (healthier retinas and crystalline lenses would lead to better vision, especially in the elderly) or effects throughout the visual system.[20;80] The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons.

If MP has a role, and its contribution is related to either, its optical density and spectral absorbance characteristics, or to possible biological effects on retinal, crystalline lens and visual system health, then increasing MPOD through supplementation should result in improved visual performance and experience. The key then is to accurately detect and quantify any such changes through a comprehensive battery of appropriate tests that analyse vision on a number of functional levels, including basic acuity, contrast sensitivity across illumination levels, colour perception, and glare sensitivity, among others.

Those studies that have addressed visual performance are largely confined to populations with established eye disease (summarised in Table 1.1), and therefore the results should be interpreted with full appreciation of the fact that the findings do not necessarily hold true for subjects without retinal pathology. Studies involving normal subjects will therefore be reviewed separately here (summarised in Table 1.2).

Table 1.1 Publications exploring the relationship between MP and visual performance and experience in subjects with ocular disease.

Study (Author, Year)	Subjects (n)	Supplement (Dose per/day & Time)	Outcome Measure	Findings
CROSS SECTIONAL STUDIES				
Hammond *et al.* 1997	Cataract	None	Crystalline lens transparency versus MPOD†	Higher MPOD correlated with a more transparent crystalline lens
Brown *et al.* 1999	Cataract	None	Incidence of cataract versus MPOD	Higher MPOD correlates with decreased cataract formation
Chasan-Taber *et al.* 1999	Cataract	None	Incidence of cataract versus MPOD	Higher MPOD correlates with decreased cataract formation
Schupp *et al.* 2004	CF (10)	None	Contrast sensitivity, colour discrimination & ERG amplitude	No statistical difference between CF patients and normals although normals had marginally better performance
INTERVENTION (SUPPLEMENTATION) STUDIES				
Andreani & Volpi, 1956*	RP (8)	L‡ dipalmitate	Dark adaptation	Primary & secondary portions of dark adaptation curve improved
Mosci, 1956*	RP	L dipalmitate	Light sensitivity	Sensitivity improved
Cuccagna, 1956*	Myopia & RP	L dipalmitate	Dark adaptation	Dark adaptation improved
Pfeiffer, 1957*	Abnormal dark adaptation (13)	L dipalmitate	Dark adaptation	Only marginal improvements observed, but used smaller doses than others
Hayano, 1959*	RP	L dipalmitate	Dark adaptation	Dark adaptation improved proportional to the increase in blood L
Muller-Limroth & Kuper, 1961*	RP (18)	L dipalmitate	ERG potentials	No change
Asciano & Bellizzi, 1974*	Progressive myopia with chorio-retinal atrophy (50)	L dipalmitate	Light & chromatic sensitivity	Sensitivity on both measures improved

Richer, 1999	AMD§ (14)	10mg L (5 ounces spinach 4 times per week)	Contrast sensitivity	92% shown improvements in contrast sensitivity
Dagnelie *et al.* 2000	RP (16)	40mg L (2 months) followed by 20mg (4 months)	Visual acuity and visual field	Visual acuity improved 0.7 dB, visual field area increased by 0.35 dB, largest gains in blue eyes
Aleman *et al.* 2001	RP (47) & Usher syndrome (11)	20mg L (6 months)	Foveal sensitivity	No improvement – lower dose than Dagnelie study, MP density may be affected by stage of retinal disease
Duncan *et al.* 2002	Choroideremia (13)	20mg L (6 months)	Foveal sensitivity (dark adapted)	No improvement
Falsini *et al.* 2003	AMD (30)	17 subjects took 15mg L + 20mg vitamin E + 18mg nicotinamide (6 months); 13 subjects had no supplementation	Focal ERG amplitude	Significant improvement, MPOD not recorded
Olmedilla *et al.* 2003	Cataract (17)	15mg L or 100mg α-tocopherol or P 3 times per week	Visual acuity & glare sensitivity	Improvements in both measures, No change in P group or α-tocopherol group
Richer *et al.* 2004	AMD (90)	10mg L or 10mg L + antioxidants or P (1 year)	Visual acuity, CSF & Amsler	Significant improvement in both groups L = 5.4 letter increase, L + antioxidants = 3.5 letter increase; no effect on contrast sensitivity; improved performance on amsler grid
Bartlett & Eperjesi, 2007	ARM‖ & AMD (25)	6mg L + vitamins A, C + E, + zinc + copper	Contrast sensitivity	No improvement in performance
Aleman *et al.* 2007	Stargardts' disease or cone-rod dystrophy (17)	20mg L (6 months)	Visual acuity and foveal sensitivity	No improvement with increased L, MPOD was inversely related to stage of disease
Parisi *et al.* 2008	Early AMD	Vitamin C & E, Zinc, Copper, 10mg L, 1mg Z, 4mg Astaxanthin (12 months)	Multifocal ERG RAD	Central (5º) RAD reduced at baseline in AMD compared with healthy controls, central (5º) RAD improved significantly in the supplemented group, MPOD not recorded

* Data from Nussbaum *et al.* 1981[20]; † MPOD: macular pigment optical density; ‡ L: Lutein; § AMD: age-related macular degeneration; ‖ ARM: age-related maculopathy

Table 1.2 Publications exploring the relationship between MP and visual performance and experience in normal subjects.

Study (Author, Year)	Subjects (n)	Supplement (Dose & Time)	Outcome Measure	Findings
CROSS SECTIONAL STUDIES				
Hammond *et al.* 1998	Normals	None	Scotopic sensitivity & short wave sensitivity	Higher MPOD† associated sensitivities equivalent to younger observers
Stringham *et al.* 2003	Normals	None	Photophobia	Higher MPOD correlated with less photophobia
Stringham *et al.* 2003	Young normals (16)	None	Short wave increment thresholds	No correlation with MPOD
Stringham & Hammond, 2007	Normals (36)	None	Photostress recovery and grating visibility	Higher MPOD relates to shorter recovery times and improved sensitivity
Engles *et al.* 2007	Normals (80)	None	Gap acuity and vernier acuity	No correlation with MPOD
INTERVENTION (SUPPLEMENTATION) STUDIES				
Monje, 1948*	Normals (14)	L‡ dipalmitate (2-6 months)	Dark adaptation & scotopic visual acuity	Both dark adaptation and scotopic visual acuity shown transient improvements
Wustenberg, 1951*	Normals (7)	L dipalmitate	Dark adaptation	No improvement but experimental error has been suggested
Klaes & Riegel, 1951*	Normals	L dipalmitate	Dark adaptation	Dark adaptation improvement lasting up to 4 months
Andreani & Volpi, 1956	Normals (10)	L dipalmitate	Dark adaptation	Primary & secondary portions of dark adaptation curve improved
Mosci, 1956*	Normals	L dipalmitate	Light sensitivity	Sensitivity improved

Hayano, 1959*	Normals	L dipalmitate	Dark adaptation	Dark adaptation improvements proportional to blood L increase
Wenzel *et al.* 2006	Normals: No supplement (6); supplement (4)	30mg L + 2.7mg Z§ (12 weeks)	Photophobia	MPOD correlated with baseline sensitivity and improved with supplementation
Rodriguez-Carmona *et al.* 2006	Normal trichromats (24)	10mg (6 months) + 20mg (6 months) of L or Z, 10mg L+ 10mg Z or P	Blue/yellow colour discrimination	No effect of supplementation on colour discrimination
Kvansakul *et al*. 2006	Normals (34)	10mg L, 10mg Z, 10+10mg combination or P (6 months)	Mesopic contrast acuity	Supplementation improved performance with L, Z or combination, no improvement with P
Bartlett & Eperjesi, 2008	Normals (46)	6mg L + vitamins A, C, E + zinc + copper	Visual acuity (near + distance), contrast sensitivity and photostress recovery	No performance improvement over 9 months or 18 months
Stringham & Hammond, 2008	Normals (40)	10mg L+ 2mg Z (6 months)	Photostress recovery and grating visibility	Increased MPOD led to improved performance and faster recovery

* Data from Nussbaum *et al.* 1981[20]

† MPOD: macular pigment optical density

‡ L: Lutein

§ Z: Zeaxanthin

1.3.6.2 STUDIES IN SUBJECTS WITH RETINAL PATHOLOGY

1.3.6.2.1 HEREDITARY RETINAL DEGENERATIONS

Abnormal light sensitivity, difficulty associated with glare, loss of contrast and slow dark adaptation are symptoms commonly reported by patients with hereditary retinal degenerations. It is possible that such symptoms could be attributable, at least in part, to the failure of MP to absorb scattered light, resulting in reduced contrast and definition along with excessive photoreceptor pigment bleaching by short wavelength light components.

The antioxidant and absorptive properties of MP would therefore suggest a potentially useful role for the macular carotenoids in retinal degenerations, where the clinical aim includes optimisation of current visual status in the short term and preservation of macular vision in the long term. Indeed, it is noteworthy that there have been reports (some dating back >50 years) suggesting that patients with RP demonstrated improvements in visual performance following supplementation with L-containing compounds (reviewed elsewhere[20]).

Dagnelie *et al.*[81] assessed the effect of L supplementation in patients with RP, and reported moderate visual improvements following short-term supplementation with L. Mean visual acuity improved by 0.7 decibel (dB) and mean visual-field area by 0.35 dB, although the largest gains were observed in blue-eyed participants. Aleman *et al.*[82] explored the relationship between visual function and L supplementation in RP patients over a six month period, and despite increases in MPOD, could find no significant improvement in visual performance (measured as absolute foveal sensitivity). The dosage used in this latter study was lower than that in the Dagnelie report, which may explain the discrepancy in the findings of these two studies. Neither study, however, analysed visual function in sufficient detail or followed patients for sufficient time to make meaningful comment

on whether the natural history of RP is modified following supplementation with L.

Duncan *et al.*[83] analysed MP levels and macular function in choroideremia (a progressive degeneration of photoreceptors, RPE and choroid). Once again, and in spite of augmented MPOD following supplementation, no improvement in retinal sensitivity was observed.

Aleman *et al.*[84] measured MPOD in patients with Stargardt's disease or cone-rod dystrophy with known or suspected disease-causing mutations in the *ABCA4* gene, and investigated response to supplemental L in terms of changes in MPOD and central visual function. They reported that MPOD is inversely related to the stage of *ABCA4* disease at baseline, and could be augmented by supplemental L in about two thirds of patients. However, measures of visual function, including visual acuity and foveal sensitivity, exhibited no discernable improvement after 6 months of supplementation. They concluded that the long-term influences of L supplementation on the natural history of such macular degenerations require further study.

1.3.6.2.2 Age-related macular degeneration

AMD, as the leading cause of blindness in the western world, is the most commonly investigated retinal condition with respect to the potential benefits of supplemental L, Z, or MZ. Observations, including relative preservation of short wave sensitive cones centrally when compared to the perifoveal region[85] and the initiation of geographic atrophy in the perifovea, where MPOD is lowest, are consistent with the view that MP protects against AMD and against psychophysical changes known to precede this condition. Since publication of the findings of the Eye Disease Case-Control Study Group, where a 60% risk reduction for AMD in association with a high dietary intake of L and Z was reported,[86] numerous investigators have further explored the relationship between dietary and serum levels of MP's constituent carotenoids and risk for AMD.[87] With a couple of exceptions

(outlined below), studies investigating serum levels of, dietary intake of, or supplementation with, L and/or Z with respect to risk for AMD and/or its progression have (understandably) considered preservation, rather than enhancement, of visual performance, to represent the most appropriate outcome measure (reviewed elsewhere[88]).

Richer *et al*.[89] evaluated the effect of dietary modification on visual performance for patients with atrophic AMD. Fourteen male patients (70 ± 9 years), receiving 0.73 ± 0.45 portions of dark-green, leafy vegetables/day base intake, were placed on an additional portion of 5 ounces sautéed spinach 4 to 7 times per week or L based antioxidant (3 subjects). Patients demonstrated short-term enhancement of visual function in one or both eyes in terms of Amsler grid testing, Snellen acuity, contrast sensitivity, glare recovery, and subjectively on the Activities of Daily Vision Subscale. The authors concluded that the effect of dietary modification on the natural course of atrophic AMD warranted investigation in the context of a randomised, controlled trial.

Such an evaluation was conducted in the Lutein Antioxidant Supplementation Trial (LAST) study. Richer *et al*.[90] evaluated the effect of supplementation on visual performance in atrophic AMD on 90 subjects in a double blind, placebo (P)-controlled trial. Average MPOD increased by 0.09 log units (or 50%) after 12 months, in the L and L plus antioxidant groups. The investigators observed concurrent and statistically significant improvements in contrast sensitivity, visual acuity and subjective measures of glare recovery in both treatment groups, but not in the control group. Snellen-equivalent acuity improved by 5.4 letters in patients supplemented with L, and by 3.5 letters in patients supplemented with L plus antioxidants, whereas improvements in contrast sensitivity were significantly better in the L plus antioxidant group than in the L group.

Falsini *et al*.[91] studied the effect of supplemental L on central retinal function, assessed electrophysiologically, in patients with early AMD, and reported a significant improvement in focal electroretinogram (ERG)

amplitude after six months of supplementation, and this was followed by regression back to baseline values following discontinuation of the supplement. Unfortunately, the investigators did not measure MPOD, and therefore conclusions must be interpreted with full appreciation of this limitation.

Bartlett and Eperjesi *et al.*[92] undertook a prospective, 9 month, double-masked randomised controlled trial (RCT) of the effect of supplementation with L combined with vitamins and minerals on contrast sensitivity among participants with age-related maculopathy (ARM) and atrophic AMD. Contrast sensitivity was assessed using a Pelli-Robson chart and participants were randomised into active (A) and P treatment groups. The authors report no significant improvement in contrast sensitivity among either group and suggest that supplementation with 6 mg/L and other antioxidant vitamins and minerals has no tangible benefit to this group (although one could argue that preservation rather than enhancement of performance might be a more suitable outcome measure for AMD patients) and further, that determination of optimum dosage levels requires further work. Their findings are naturally confined to the somewhat limited measure of contrast sensitivity with a Pelli-Robson chart that may not be best equipped to detect subtle changes in performance. Failure to record MPOD at baseline, and the low dosage of supplemental L, represent design flaws in that study, and limit the scope for meaningful comment. Parisi *et al.*[93] have also recently explored the influence of short-term carotenoid and antioxidant supplementation on electrophysiologically assessed retinal function in early AMD. Of the 27 early AMD patients enrolled in their study, 15 had daily oral supplementation of vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), L (10 mg), Z (1 mg), and astaxanthin (4 mg) for 12 months, while the remaining 12 had no dietary supplementation during the same period. Fifteen age-similar healthy controls were also assessed at baseline and followed-up for the duration of the study period without supplementation. Multifocal ERGs, in response to 61 M-stimuli presented to the central 20° of the visual field (averaged across 5 retinal eccentricity areas between the fovea and mid-periphery: 0° to 2.5°,

2.5° to 5°, 5° to 10°, 10° to 15°, and 15° to 20°) were assessed at baseline in controls and in early AMD patients, and again at 6 months and 12 months. At baseline, they observed highly significant reductions of N1-P1 response amplitude densities (RADs) for the central 5° surrounding the fovea in AMD patients when compared with healthy controls. For more peripheral retinal eccentricities, RADs were not significantly different from controls. After 6 and 12 months of treatment, the treated group shown highly significant increases in N1-P1 RADs for the two most central retinal areas, but not for more peripheral eccentricities beyond 5°. The non-treated control group exhibited no significant RAD changes at any retinal eccentricity. These findings suggest that in early AMD eyes, central retinal function (0° – 5°) can be improved by supplementation with carotenoids and co-antioxidants. The study design, however, does not clarify whether such improvements in retinal function have a measurable impact on visual performance and experience, and the failure to measure and record MPOD somewhat limits the interpretation of these potentially important findings.

1.3.6.2.3 CATARACT

Olmedilla *et al.*[94] investigated whether supplemental L influences visual function in patients with age-related cataract, where visual performance was evaluated by measures of visual acuity and glare sensitivity. This randomised, P-controlled trial revealed significant improvements in visual acuity and glare sensitivity following supplemental L, and the observed improvements were related to changes in serum levels of L, whereas no such improvements were observed in patients supplemented with P or with α-tocopherol. While contrast sensitivity was not recorded at baseline or during the supplementation phase, it is interesting to note that in cataract patients supplemented with L, contrast sensitivity at the end of the supplementation period was similar to or even better than that expected for control subjects of a similar age. The authors postulated that the observed improvements in the outcome measures were not the result of any change in

the crystalline lens, but more likely to be the result of improved retinal function.

1.3.6.3 STUDIES IN NORMAL POPULATIONS

1.3.6.3.1 PHOTOPHOBIA AND GLARE

Photophobia is a phenomenon experienced by all persons when illumination is suddenly and dramatically changed from dark to light, and is typified by the experience of switching on a bedroom light at night time. However, under normal daylight conditions, the experience of photophobia is somewhat more variable. Numerous clinical conditions (e.g. RP & AMD) are associated with photophobia, and, even in the absence of detectable disease, clinicians are often presented with patients whose primary complaint is of periodic or persistent sensitivity to bright light (but at levels which do not similarly affect colleagues/friends/family). Given its absorption characteristics, the optical density of MP may be important in determining an individual's threshold for the subjective complaint of photophobia.

Stringham *et al.*[56] explored the effect of the spectral composition of a target on visual discomfort, using electromyography and a rating scale to determine photophobia thresholds. They shown that, while there was a positive relationship between wavelength and the energy needed to produce photophobia for wavelengths between 520 and 640 nm, at shorter wavelengths there was a notch centred at 460 nm, the trough and shape of which resembled the log transmittance spectrum of MP. Their findings led the authors to suggest that MP may attenuate photophobia or visual discomfort induced by short wavelength sources.

These observations prompted a subsequent study investigating the relationship, if any; between MP and photophobia.[95] This two-part experiment explored the relationship between baseline MPOD levels and photophobia thresholds, as well as the effect of augmenting MPOD on such

thresholds. Four subjects were supplemented with 30mg L and 2.7 mg Z daily for 12 weeks. Peak MPOD was observed to increase from 0.452 (±0.11) at baseline to 0.536 (±0.11) at the end of the period of supplementation. A significant and inverse relationship between baseline MPOD and threshold for photophobia was observed, such that individuals with higher MPOD had higher tolerance for short wavelength light. Furthermore, increasing MPOD over a 12-week period appeared to increase the threshold for photophobia for all subjects for short wavelength sources.

Recently, Stringham & Hammond have explored the influence of glare on visual performance, and how MPOD might influence any observed relationships. They first looked at baseline visual performance under glare conditions by evaluating photostress recovery (a sensitive indicator of macular function) and grating visibility.[69] The effect of veiling glare on grating visibility was explored using a five cycles per degree (cpd) contrast grating stimulus, surrounded by a concentric annulus of adjustable intensity. For the photostress recovery test, the same stimulus was viewed following photostress with a 5° xenon white disc providing 5.5 log trolands of retinal illuminance over 5 seconds duration. MPOD was a significant determinant of the deleterious effects of glare, with visual thresholds and photostress recovery times significantly and inversely related to MPOD. Further, high MPOD was associated with better visual performance in a way that was consistent with its spectral absorbance and spatial profile.

These observations prompted the same investigators to design and execute a trial of supplemental L (10 mg per day) and Z (2 mg per day), using the same testing conditions, but on this occasion looking for changes in performance associated with augmentation of MPOD. In this instance, they found that, following six months of supplementation, and an average increase in MPOD from 0.41 to 0.57, most subjects exhibited improved photostress recovery and glare tolerance in association with an increase in MPOD. More specifically, a 39% increase in MPOD enhanced tolerance of intense glaring light by up to 58% and reduced photostress recovery times by 14%.[70]

Although the authors wisely suggest a cautionary approach to the interpretation of their data and the wider implications of such findings, their conclusion that the results are "both large enough and sufficiently general to be meaningful in real life", and that "supplementing L and Z could indeed be palliative for those suffering the consequences of glare", is important and warrants further investigation in the form of a randomised clinical trial.

1.3.6.3.2 SPATIAL VISION

Engles *et al.*[68] have investigated the "acuity hypothesis", exploring the relationship between MPOD and gap acuity and vernier acuity under "photopic" conditions. They report that neither gap acuity nor vernier acuity were significantly related with MPOD, and concluded that their "data suggest that the predictions of the acuity hypothesis do not hold". While the authors qualify their findings as appropriate to their specific testing conditions alone, several study limitations (other than those recognised by the authors) warrant brief discussion.

Firstly, although the authors report that their conclusions are relevant for photopic conditions, their adopted background luminance levels are in the low photopic range at best (17 candela per square metre (cd m^{-2}) for the achromatic condition, and 15.7 cd m^{-2} for the chromatic condition), and certainly not appropriate for evaluation of photopic visual function. Indeed, given the subtle nature of any performance changes likely to be facilitated by MP, the background luminance difference (≈8%) between the two testing conditions is also a potentially confounding factor.

Secondly, while all subjects were corrected to 6/6, it is plausible, indeed probable, that the actual acuity limits of their study population ranged widely between the 6/6 level employed up to a likely 6/3 limit for a young healthy subject. This potential two-fold range in acuity, subserved by individual optical, anatomic and neural architectures, would have a strong

influence on both gap and vernier acuity tasks, almost certainly more powerful than MPOD. Also, by adopting a 6/6 limit, the investigators most likely failed to correct for potentially significant amounts of uncorrected axial astigmatism in some subjects, which could significantly influence performance on both of the chosen tasks (testing of vernier and gap acuity limits). While the authors could argue that any such variables remained consistent between testing conditions, we believe it would be more appropriate to eliminate sources of variability such as residual refractive error, so that all subjects operate at their limits of acuity.

The adoption of a single spatial frequency and contrast setting further limits the conclusions that can be drawn from this paper. The effect of MP, for example, might differ significantly under different spatial frequency and or contrast ranges. Assessment of visual performance across the full contrast sensitivity function (CSF) might represent a more thorough and rigorous assessment of MP's capacity to affect visual performance through attenuation of the effects of chromatic aberration and light scatter.

Finally, the subjects employed in the Engles *et al*. study typically exhibited average to high MP levels, with few subjects exhibiting MP levels below 0.20. Reading and Weale[19] previously modelled the potential effect on MP in terms of attenuation of the effects of chromatic aberration, and suggested that, due to the non-linear nature of the effect, MPOD levels above 0.30 were probably superfluous. Based on the assumptions of this model, a study on the effect of MP on visual performance might require the inclusion of relatively more subjects that exhibit low MPOD levels in order to demonstrate an effect.

These limitations of the cited study serve to emphasise the challenges inherent in investigating the role of MP in visual performance and experience, which rest on the need (insofar as is possible) to disentangle the influence of MP from the often unquantifiable and variable influences of individual optical and neural architectures.

Bartlett and Eperjesi *et al*.[96] set out to explore the effect of L supplementation on visual performance among healthy observers. Similar to their AMD trial,[92] the authors report no effect of supplementation on performance measures ranging from distance and near visual acuity, contrast sensitivity and photostress recovery. The results are somewhat unsurprising however given (a) the low dose, 6 mg/L supplement used, (b) the basic nature of the series of tests employed to evaluate visual performance, and (c) the small number of subjects tracked over 9 months (n = 46) and 18 months (n = 29) across such a broad age range (22-73 years). Once again, their failure to record MPOD or serum L and Z levels means that only qualified comment can be made as to the significance of the reported findings.

Armstrong *et al*. in a pilot study (involving only one subject) presented at a recent conference (Association for Research in Vision and Ophthalmology (ARVO) 2008, Poster # 4964/D984), evaluated macular function on a serial basis throughout a 4-month period of supplementation with L and Z. Looking at a series of psychophysical and electrophysiological outcome measures, they evaluated the effect of supplementation on dark-adapted thresholds and recovery kinetics, pattern visual evoked potentials (PVEP) [before and after photostress], and pattern ERG (PERG) amplitude. An MPOD increase of approximately 33% was accompanied by a 23% improvement in 650nm dark adapted thresholds (from 30 dB to 37 dB) and by an increase in PERG amplitude, but not by a change in cone recovery kinetics or photostress PVEP recovery. Although these findings should be interpreted with caution, particularly given that only one subject was tested, they are again suggestive of an improvement in macular function following augmentation of MPOD in young healthy subjects.

The inconsistencies in spatial vision data with respect to MPOD reflect the difficulty inherent in isolating performance tasks which may be influenced by MP. Furthermore, the wide inter-individual variability of MPOD[8] renders the interpretation of such studies all the more challenging,

particularly where such investigations depend on cross-sectional rather than longitudinal data. It would however seem to be the case that, as far as spatial vision is concerned, the effect, if any, of MP on performance appears small, at least for individuals with average to high MPOD.

1.3.6.3.3 COLOUR VISION

Moreland and Dain[97] reported that hue discrimination, measured using the Farnsworth-Munsell 100 Hue test (FM100), is indeed adversely affected primarily for short wavelengths by simulation of high MPOD using liquid filters containing carotene in a benzene solution. Comparing the results with those obtained with a neutral filter, they concluded that this effect was not simply the result of reduced retinal illuminance. However, to our knowledge there are no published studies on the effects of actual (rather than simulated) MPOD on conventional measurements of hue discrimination thresholds. Further evidence supporting an effect of MPOD on short wavelength vision has been obtained from studies of short-wave sensitive cone sensitivity.[98;99] Finally, it has been shown that colour discrimination measured by a colour matching technique is influenced by MPOD.[100;101]

However, two recent studies using alternative methods, produced conclusions differing from those of the above mentioned studies. Firstly, a study of the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP (measured by heterochromatic flicker photometry (HFP)) and red-green (RG) or yellow-blue (YB) colour discrimination thresholds, though it was reported that RG vision tends to improve with augmentation of MP.[102] Secondly, RG cancellation profiles have been reported to be highly correlated with MPOD, while profiles for YB were independent of both retinal eccentricity and MPOD.[78] However, changes in spectral sensitivity across the fovea, macula and paramacula are accompanied by relatively little change in colour appearance, depending on whether corrections are made for MP absorption.[103;104]

Thus there is no consensus in the literature on the relationships, if any, between MPOD and colour vision parameters on the one hand, and mechanisms on the other hand. This may or may not simply reflect the innate differences between, for example, spectral sensitivity measurements of the isolated short-wave sensitive cone mechanism and the overarching hue discrimination function at short wavelengths. It is also necessary to distinguish between the effects on colour vision (mechanisms, sensitivity or appearance) of (1) distribution of MP across the retina, and (2) variation of MPOD between subjects at a given retinal locus.

1.3.6.3.4 PRESERVATION OF 'YOUTHFUL' VISION INTO OLD AGE

In the elderly, pre-retinal image degradation and slower encoding results in featurally compromised representation of spatially-extended search arrays. Even with appropriate optical correction, older adults therefore do not possess the spatial resolving power of the young adult. Such losses are not confined to high spatial frequencies, but contrast sensitivity losses are observed across a range of intermediate frequencies.[105] Indeed, many changes in both structure and function of the visual system, such as pupillary miosis and loss of crystalline lens transparency,[106] accompany the aging process (summarised elsewhere[107]). The consequence of such changes is a reduction in retinal illuminance, such that equiluminant stimuli do not result in equal retinal illuminance for different age groups. Human visual performance therefore tends to decrease with age (Figure 1.16). Such effects are to some extent unavoidable, and a natural consequence of aging.

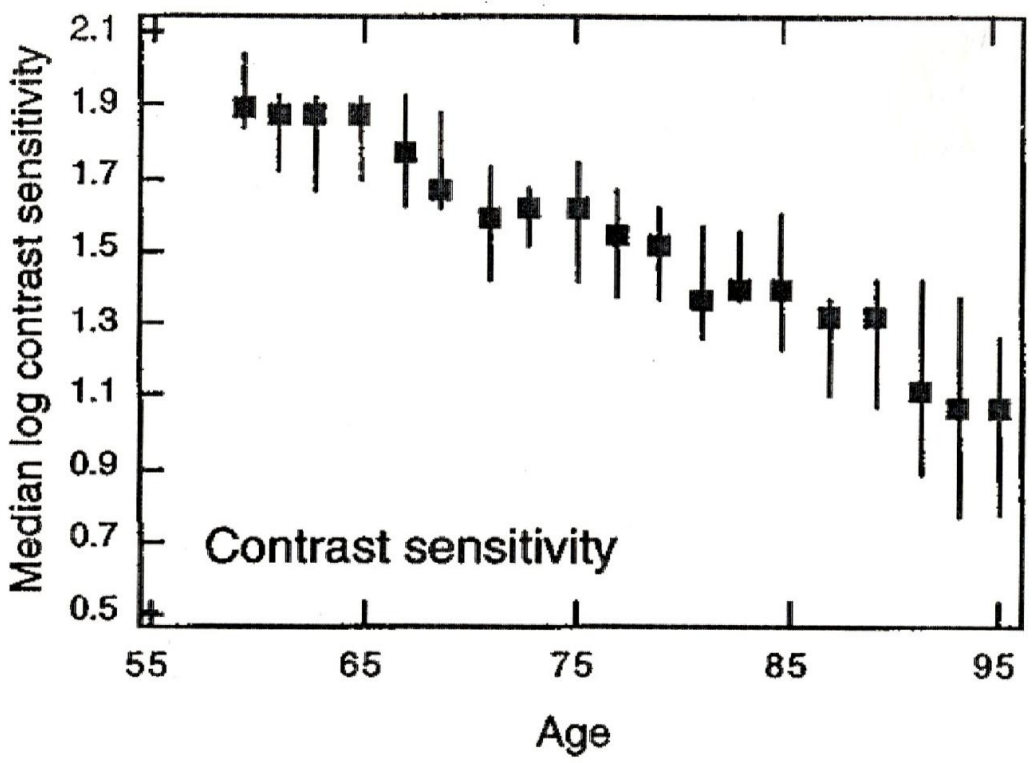

Figure 1.16 Effect of normal aging on contrast sensitivity. Experimental data show a 1-log unit sensitivity decrease from age 60 to 95. [From Haegerstrom-portnoy *et al*. 2007][108]

The most significant role of MP in vision, however, may rest on the potential of L, Z and MZ to retard the aging process through their antioxidant properties. It is important to note that MP acts, uniquely, as an antioxidant, both passively and actively, the former mechanisms being dependent on its ability to limit photo-oxidative damage by filtering short wavelength light at a pre-receptorial level and the latter mechanism attributable to its capacity to quench reactive oxygen intermediates (ROI).

The inter-individual variability in MPOD, consistently observed in cross-sectional studies, may have important implications for the long term health and viability of the central retina. In subjects with little MP, the cumulative and chronic effects of increased exposure of photoreceptors to short wavelength light, coupled with a weaker local capacity to quench free

radicals, could, in theory at least, accelerate the onset of physiological and pathological aging of the retina.

In support of such a notion, Hammond *et al*.[98] have shown that high MPOD was associated with the retention of youthful scotopic and short wave sensitivity and suggested that MP may retard an age-related visual decline. The potential benefits of increased MPOD appear not to be confined to the retina. Hammond *et al*.[109] reported a positive and significant association between crystalline lens transparency and MPOD, and speculated that high concentrations of the macular carotenoids in the lens probably accompany high concentrations at the macula, and protect against the effects of oxidation in the lens (thereby maintaining transparency). Indeed, other studies have shown an association between a high dietary intake of L and Z with decreased incidence of cataract formation.[110;111]

Werner and Steele[112] demonstrated age-related sensitivity losses of foveal colour mechanisms across all three cone types, although the sensitivity loss for short wavelength sensitive cones was lower (at 0.08 log units per decade), when compared to 0.11 log units loss per decade for both medium and long wave cones. Werner *et al*.[99] later explored the senescence of foveal and parafoveal cone sensitivities and their relation to MPOD. Again, they report age-related decline of foveal and parafoveal increment thresholds. Interestingly however, and consistent with the hypothesis that the MP protects the photoreceptors from senescent losses in sensitivity, a significant and positive correlation was found between foveal MPOD and differential short wavelength cone log sensitivity losses at the fovea and at the parafovea, but not with differential medium and long wavelength cone log sensitivity losses at the retinal loci. This finding, however, was independent of age, prompting the authors to postulate that it was due to local gain changes, resulting from differential filtering of incident light by the MP between the fovea and the parafovea.

Haegerstrom-Portnoy[113] also examined short wavelength cone versus long wavelength cone sensitivity in a group of young and older adults to

determine whether MP protects the human fovea from retinal neural damage caused by visible-light exposure over a lifetime. While there was no difference observed for long wavelength cone sensitivity between groups, the older group shown a significant differential loss of short wavelength cone sensitivity across the retina compared with the younger group, with greater loss of sensitivity at non-foveal locations than at the fovea. This observation is again suggestive of a protective effect of MP on foveal function.

Schupp *et al.*[114] endeavoured to explore the hypothesis from a different perspective, postulating that if high levels of MP might forestall the effects of normal aging, then low levels of MP might accelerate the normal aging process. Cystic fibrosis (CF) is a condition associated with defective gastrointestinal absorption of carotenoids as a result of pancreatic insufficiency. Low serum concentrations of carotenoids, including the constituents of MP, are invariably reported in CF patients. Given the repeatedly observed positive and significant relationship between MPOD and serum concentrations of its constituent carotenoids (reviewed elsewhere[115]), it can be reasoned that patients with CF would have low MPOD. Schupp *et al.*[114] assessed visual performance in ten CF patients, in whom serum concentrations of L and Z and MPOD were predictably and significantly lower than control subjects, and typically less than 50% of the values observed among control subjects. However, visual performance (contrast sensitivity, colour discrimination and multifocal ERG amplitudes) were statistically similar for CF patients and control subjects.

While the basic rationale of this study is provocative, there are however a number of concerns with the methodology. With six of the ten CF subjects aged between 21-27 years, it is unlikely that such a youthful population sample would demonstrate accelerated aging effects on visual function (even in the presence of chronically low MPOD levels). In any case, given the theoretical possibility that higher levels of MP might be associated with enhanced visual performance, it is unclear from this publication as to how functional differences, which might have been

observed between the CF and control groups, could be attributable to age effects rather than simply to differences in MPOD. The authors conceded that a longitudinal assessment of an older CF population is required to address the hypothesis more appropriately.

Hammond and Wooten[116] investigated the relationship between MP, critical flicker fusion frequency (CFF) and age, citing CFF as a general measure of visual health. They found a significant decline in CFF values with age. There was a significant and positive relationship however between MPOD and CFF values that was independent of age. The authors conclude that these results are consistent with a protective effect of MP on visual health across the lifespan. While such investigations appear to be at a very early stage, preliminary results suggest a role for MP in temporal vision and, specifically, that high MPOD may protect the retina and defer some typical age-related changes in temporal vision.

1.4 CONCLUSIONS

Visual performance in the normal human is less than ideal, and it has been shown that visual performance improves once chromatic and monochromatic aberrations are removed.[117] As a consequence, numerous interventions which attenuate these aberrations have been developed in an attempt to optimise and/or enhance visual performance, wavefront-guided laser refractive eye surgery, wavefront–guided spectacle lenses, short wavelength-filtering intra-ocular lens (IOL) implants, short wavelength filtering contact lenses and short wavelength filtering spectacle lenses are all directed towards improving or optimising visual performance. These techniques, however, are primarily intended for persons with pre-existing ocular abnormality or disease, and there has been a conspicuous lack of concerted effort to improve (or maintain) visual performance in subjects without demonstrable ocular pathology. Augmentation of MPOD by means of supplementation remains a plausible and realistic means (in theory at

least) of optimising and/or enhancing visual performance in a normal population.

Future studies should address the issue of whether variations in MPOD relate to visual performance, and whether high MP levels can preserve or prolong optimal central visual function into old age. Indeed, some studies have reported that high levels of MP are associated with preservation of retinal sensitivity in the elderly.

MP has ideal properties, in terms of location and spectral absorbance, to be beneficial for visual performance and experience. Longer life expectancy, increased exposure to short wavelength light (ancestors had little or no short wavelength light exposure after dark), increased effects of scatter from expanding smog and haze, modern visual requirements and the ever increasing incidence of AMD heightens the importance of both optimising (and possibly enhancing) visual performance in the working population, and preserving such performance into old age. Robust evidence, in support of the psychophysically plausible rationale, that MP contributes to visual performance and experience in a favourable way is, however, still lacking. The findings of the studies cited above, whether demonstrating a benefit of MP to visual performance and experience or not, should be interpreted with full appreciation of their design limitations, and it should be understood that a cross sectional study represents an inappropriate design to investigate fully any contribution that MP makes to visual performance. It is unwise to assume that the role of MP in visual performance, if any, can be easily studied, given the multitude of typically individual and occasionally enigmatic factors that influence our visual experience.

Given the numerous optical and neural factors that influence and dictate visual performance, and the consequential and associated difficulties in isolating improvements in visual performance, any study designed to investigate the influence of MP in this regard should include questionnaire-based analyses of subject perceptions of personal visual experience. Such an approach will facilitate investigation of the potential role of MP in visual

performance in the real world, in a natural and ever-changing environment, which is often poorly reflected in our current and limited arsenal of testing modalities. None of the studies which reported a beneficial effect of MP augmentation adequately address the question of (1) whether such increases in MPOD and the observed psychophysical functional improvements translate into tangible improvements in visual experience outside the laboratory or (2) whether such improvements can be longitudinally maintained to preserve functional performance and experience into old age.

Because of the inter-individual variability in MPOD and psychophysical visual function, a study designed to investigate the contribution of MP to visual performance and experience should be able to study the relationship between changes in these parameters within subjects over time, and only a study where MP is augmented by supplementation and/or dietary modification can meet this essential criterion. Interestingly, of the studies cited in this review, there appears to be one reasonably consistent finding, despite varied design limitations, studies involving supplementation among normal and diseased eyes typically report measurable benefits in terms of visual performance, in terms of photophobia thresholds, glare sensitivity, dark adapted thresholds, PERG amplitudes and mesopic contrast sensitivity among others.

Thus far, there appears to be little or no evidence of any adverse effect of higher levels of MP on visual performance. In a study designed to determine the influence of MP absorption on blue-on-yellow perimetry, Wild and Hudson[118] found that the net effect of ocular media and MP absorption relative to 460 nm was to attenuate the blue-on-yellow visual field at the fovea by approximately 0.80 log units and elsewhere by 0.40 log units, the difference being attributable to MP. The possibility of an adverse effect of MP augmentation on colour vision, short wavelength sensitivity and other functional measures does merit future investigation.

The optical, physiological and neurological interactions that contribute to vision suggest that the optimal level of MPOD, from a performance

perspective, may be personal to an individual eye. In other words, and for example, even if MP is found to be important for visual performance and experience, exceeding a particular optical density of the pigment may yield no further measurable or appreciable advantage, and this level may vary substantially from one individual to the next. It is also important to note that testing conditions are often incapable of reflecting more natural environments, and any observed absence or presence of MP's contribution to visual performance and experience may not necessarily hold true in a natural environment (for example, against the background of a bright blue sky).

Although it remains difficult to draw firm conclusions regarding the relationship between MP and visual performance, certain patterns do appear to exist. In normal observers, the effect on spatial and colour vision appears small in comparison to the observed effects on photophobia and glare sensitivity, while, in subjects with established eye disease, there appears a relatively consistent beneficial effect of MP supplementation on visual performance. Any effects observed, whether through optical or biological mechanisms, may also be magnified when increased emphasis is afforded to those with chronically low MPOD levels. We need and should support an appropriately powered, randomised, controlled trial, which is designed to further evaluate whether visual performance and experience can be optimised or enhanced, or indeed adversely affected, with supplemental macular carotenoids.

1.5 METHOD OF LITERATURE SEARCH

A comprehensive literature search was performed using the PubMed, ScienceDirect and Google scholar databases. Articles, abstracts and text book references were generated from review of the original bibliographies. The following is a list of key words, and combinations thereof: *macular pigment; lutein; zeaxanthin; visual performance; visual acuity; disability*

glare; contrast sensitivity function; photostress recovery time; randomised clinical trial.

1.6 PURPOSE OF THIS MASTER OF SCIENCE (BY RESEARCH) THESIS

This M.Sc. (by Research) thesis has four primary objectives:

1. To present and critically appraise the current literature germane to the contribution of MP, if any, to visual performance and experience (Introduction).
2. To assess whether MPOD is associated with visual performance (Study One).
3. To investigate whether augmentation of MPOD enhances visual performance in normal subjects (Study Two).
4. To assess whether MPOD is associated with Colour discrimination and matching (Study Three).

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-CHAPTER TWO-

STUDY ONE

The Relationship between Macular Pigment and Visual Performance: Cross-Sectional Investigation

2.1 INTRODUCTION

As discussed above, MP's optical and anatomic properties have prompted the ''optical" hypotheses of this pigment. The ''optical" hypotheses of MP were originally discussed by Reading *et al.*[1] and later by Nussbaum *et al.*[2] and include MP's putative ability to enhance visual performance and/or comfort by attenuation of the effects of chromatic aberration and light scatter, via its light-filtering properties.[3]

The objective of the present study was to evaluate, in a cross sectional manner, the relationship between MPOD and visual performance and comfort across a broad and refined range of functional tests.

2.2 METHODS

2.2.1 SUBJECTS AND STUDY SITES

One hundred and forty-two healthy subjects volunteered to participate in this study, which was approved by the research ethics committees at both Waterford Institute of Technology (WIT) and Dublin Institute of Technology (DIT). Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki (Appendix 7.2 and 7.3). The study was conducted at WIT and DIT vision science laboratories, located in the southeast and east of the Republic of Ireland, respectively. Self-selected recruitment of subjects (WIT: n = 61 and DIT: n = 81) was facilitated by poster (Appendix 7.1) and newsletter advertisement, and also by word of mouth, in the respective local communities.

All subjects were aged between 18 and 41 years, in perfect general (self report) and ocular health, and with visual acuity of at least 6/9 in the study eye, refractive error outside -6 D to +6 D. The study eye was selected on the basis of ocular dominance, determined using the Miles Test[4] with the

dominant eye chosen as the study eye, except in cases of observed equidominance, in which case the right eye was selected.

A typical study visit lasted approximately four hours. Those aspects of visual performance assessed, and their sequence, are presented in Appendix 7.5. All subjects recruited into the study could be classed as naïve observers to the tests carried out (with the exception of the visual acuity test, with which all subjects were familiar). All tests were conducted with the subject's optimal subjective refraction in place. To optimise performance, and also to minimise any potential learning effects on performance, all subjects underwent a defined period of pre-test training. This training consisted of careful explanation of the nature of each test, pictorial and/or video demonstration of the test requirements and procedure, and was followed by a defined session of pre-test practice. One subject (CWIT 2553) was excluded from analysis due to inability to use the Densitometer to obtain reliable MPOD data.

2.2.2 DEMOGRAPHIC, MEDICAL, LIFESTYLE AND VISION CASE HISTORY QUESTIONNAIRES

The following details were recorded for each volunteer by questionnaire: demographics; general health status; medication; age; sex; smoking habits (never, current or past); alcohol consumption (average unit weekly intake); exercise (minutes per week); body mass index (BMI, kg/m^2); blood pressure; ethnicity; marital status; education; occupation.

Vision case history included: time since last eye examination; spectacles or contact lens use; history of ocular treatment or surgery; history of occlusion therapy or visual training in childhood; family history of eye disease; current problems with vision; asthenopia associated with computer use; history of headaches.

2.2.3 SPECTACLE REFRACTION, VISUAL ACUITY AND OCULAR DOMINANCE

Each subject underwent precise spectacle refraction by an experienced optometrist to determine refractive error and best corrected visual acuity (BCVA) for each eye. A computer generated LogMAR (Logarithm of the Minimum Angle of Resolution) test chart (Test Chart 2000 Pro; Thomson Software Solutions) was used to determine BCVA at a viewing distance of 4 metres, using a Sloan early treatment diabetic retinopathy study (ETDRS) letterset [Figure 2.1]. BCVA was determined as the average of three measurements, with letter and line changes facilitated by the software pseudo-randomisation feature. BCVA was recorded using a letter-scoring visual acuity rating, with 6/6 visual acuity assigned a value of 100. BCVA was scored relative to this value, with each letter correctly identified assigned a nominal value of one, so that, for example, a BCVA of $6/6^{+1}$ equated to a score of 101, and $6/6^{-1}$ to 99. The study eye was selected on the basis of ocular dominance, determined using the Miles Test[4] with the dominant eye chosen as the study eye, except in cases of observed equidominance, in which case the right eye was selected. All subsequent tests were conducted with the subject's optimal subjective refraction in place.

Figure 2.1 The Test Chart 2000 Pro.

2.2.4 GLARE DISABILITY

Glare disability is a term used to describe the degradation of visual performance typically caused by loss of retinal image contrast. Glare disability is often caused, for example, by surface light reflections, or bright light sources such as car headlights, and typically is a consequence of increased forward light scatter within the eye. Glare disability was assessed using the Functional Acuity Contrast Test™ (FACT) [Figure 2.2],[5;6] displayed using the Functional Vision Analyzer™ (Stereo Optical Co., Inc., Chicago, Illinois) [Figure 2.3], which is a desktop device that allows the measurement of contrast sensitivity, and includes a customised internal glare source for assessing the impact of glare on this measure of visual performance.[7] The test comprised linear, vertically oriented, sine wave gratings presented at five different spatial frequencies including 1.5, 3, 6, 12 and 18 cpd. Nine circular patches were presented at each spatial frequency,

the contrast of each patch decreasing by 0.15-log units from the previous. Gratings were tilted -15°, 0° or +15° with respect to the vertical, to keep them within the orientation bandwidth of the visual channel. The background was tapered into a grey field in order to keep retinal illumination constant and avoid ghost imaging. Baseline contrast sensitivity was determined on the basis of the lowest contrast compatible with accurate determination of patch orientation across all five spatial frequencies for mesopic (3 cd m^{-2}) instrument background conditions, initially in the absence of a glare source. Subjects were asked to identify grating orientation, starting with the patch at highest contrast, and continuing until identification was no longer possible due to reducing contrast. Subjects were instructed not to guess, but to respond "don't know" if patch orientation could not be correctly identified. As this procedure represented a non-standard psychophysical method of threshold detection, each subject was required to re-identify the orientation of certain gratings in a pseudo-random fashion in order to confirm the validity of the subject responses at each spatial frequency. Glare disability was assessed using a radial glare source consisting of 12 white light emitting diodes (LEDs) arranged circumferentially in an oval pattern surrounding the grating charts (ranging from 4.5° to 6° from central fixation). These LEDs have a colour temperature of 6500K, and the spectral emission profile demonstrated a single large peak at 453nm (close to MP peak absorption), where the spectral irradiance was approximately double that of the peak emissions in the flatter emission spectrum across mid to long wavelengths [Figure 2.4]. Two customised intensity settings were used to determine the effect of different levels of glare on contrast sensitivity. Glare source settings were set at a medium intensity of 42 Lux and a higher intensity of 84 Lux. All correct responses were entered into the Eyeview software provided, and contrast sensitivity scores for no glare, medium and high glare conditions were determined for the respective spatial frequencies.

The Functional Vision Analyzer™ test reports showing typical sets of contrast sensitivity values recorded are presented in Figure 2.5 & 2.6.

Detailed procedure for operation of the Functional Vision Analyzer™ is given in the SOP for this instrument, provided in Appendix 7.10.

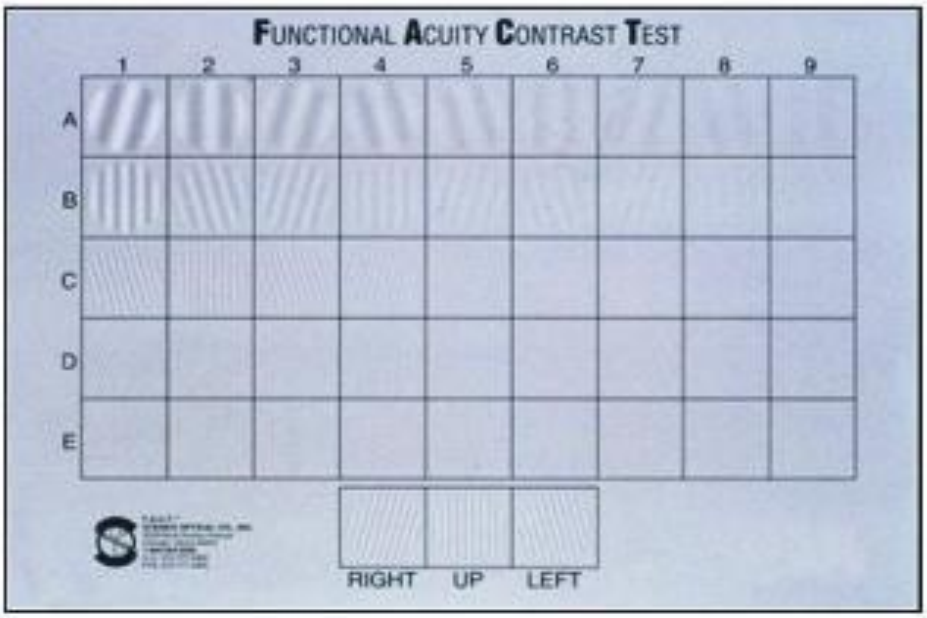


Figure 2.2 The Functional Acuity Contrast Test™.

Figure 2.3 The Functional Vision Analyzer™.

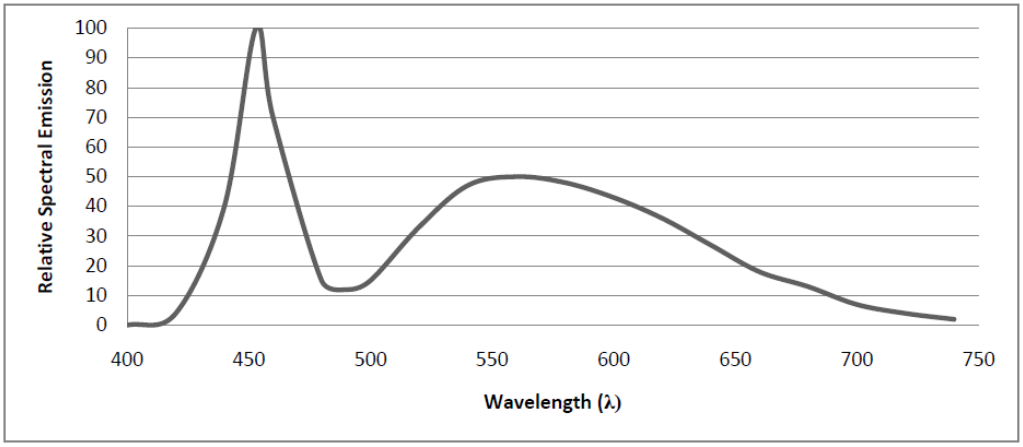


Figure 2.4 Spectral irradiance profile of the Functional Vision Analyzer™ LEDs, measured with a Bentham DMc 150 Double Monochromator Scanning Spectroradiometer (measured at the plane of the subject's pupil).

Vision Sciences Research Corporation
San Ramon, CA 94583
(800) 426-6872

Patient:
ID Number:
Test Date:

Session Summary Score Report

FACT Scores:

Eye	Correction	Illumination	Glare	Examiner
OS	Yes	Mesopic 3 cd/M2	No Glare	

	Patch Scores	Contrast Scores
Row A (1.5)	8	71
Row B (3)	8	114
Row C (6)	7	90
Row D (12)	6	43
Row E (18)	5	17

Eye	Correction	Illumination	Glare	Examiner
OS	Yes	Mesopic 3 cd/M2	Level 1	

	Patch Scores	Contrast Scores
Row A (1.5)	6	36
Row B (3)	7	80
Row C (6)	6	64
Row D (12)	4	22
Row E (18)	4	12

Eye	Correction	Illumination	Glare	Examiner
OS	No	Mesopic 3 cd/M2	Level 2	

	Patch Scores	Contrast Scores
Row A (1.5)	6	36
Row B (3)	6	57
Row C (6)	5	45
Row D (12)	3	15
Row E (18)	1	4

Figure 2.5 The Functional Vision Analyzer™ test report showing a typical set of contrast sensitivity values recorded for a subject.

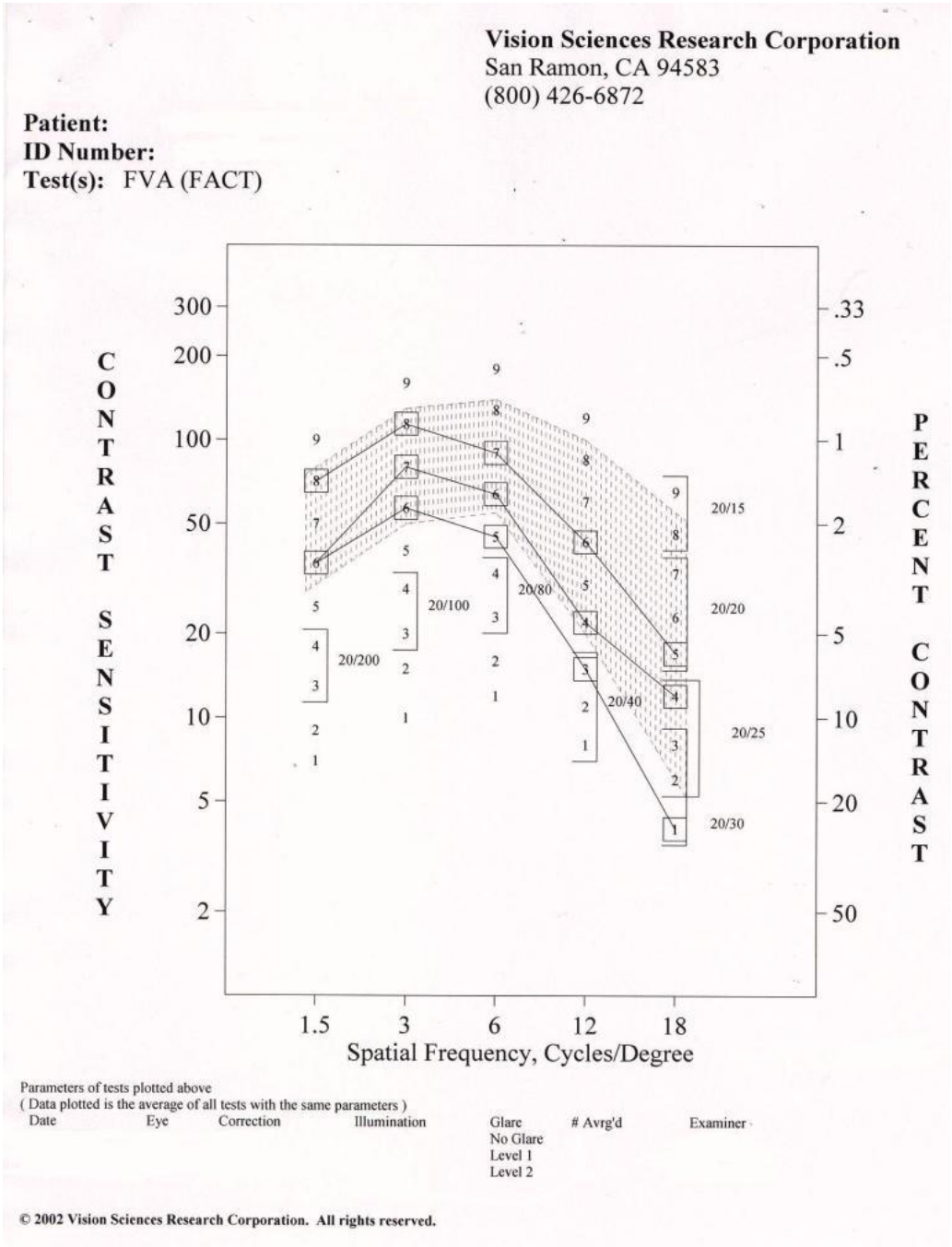


Figure 2.6 A typical test report of the Functional Vision Analyzer™.

2.2.5 CONTRAST SENSITIVITY FUNCTION

2.2.5.1 INTRODUCTION

"Contrast threshold is the smallest amount of contrast required to be able to see a target. Contrast sensitivity is the reciprocal value of the contrast threshold." Contrast sensitivity is measured with *sine wave gratings* or letter charts. "*Sine wave gratings* are repetitive light and dark bar stimuli with luminance profiles that have the shape of the simple mathematical function sine." Here the transition from bright to dark bar is gradual (sinusoidal) transition, not an abrupt transition. "One adjacent pair of light and dark bars makes up one cycle". (From 'Clinical refraction' by Borish).[8]

Michelson contrast is generally used when calculating gratings. It is defined as,

$$(L_{max} - L_{min})/(L_{max} + L_{min})$$

L_{max} and L_{min} are the luminances of the lightest and darkest points of the grating, respectively. It is a unit less quantity, which varies from 0 to 1or 0% to 100%.

Spatial frequency of a grating specifies the number of cpd of a visual angle (e.g., 30 cpd). A plot of contrast sensitivity over a range of spatial frequencies gives the CSF (Figure 2.7). A normal CSF peaks at intermediate spatial frequencies, between 2 and 6 cpd, gradual fall-off in sensitivity at lower spatial frequencies and a rapid fall-off in sensitivity at higher spatial frequencies. The shape of the CSF is called 'bandpass'.

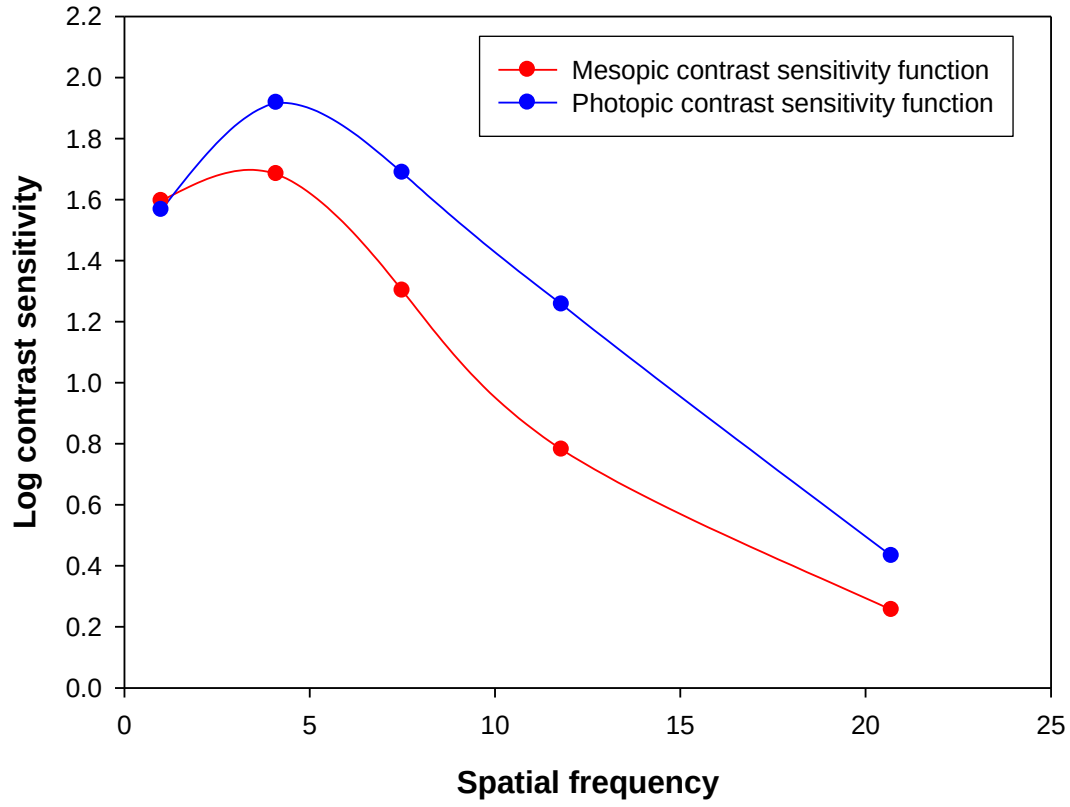


Figure 2.7 Illustration of the typical CSF.

2.2.5.2 INSTRUMENTATION

A Dell Dimension 9200 computer and a Metropsis Visual Stimulus Generation device (VSG (ViSaGe S/N: 81020197), Cambridge Research Systems Ltd., Cambridge, UK) were used to generate and control the stimuli. The VSG provided 14-bit output resolution per phosphor. The stimuli were displayed on a 19" ViewSonic professional series p227f colour CRT flat screen monitor with a frame rate of 119.98Hz. The resolution of the monitor was set to 1024 x 769 pixels. Non-linearities in the screen luminance output were eliminated by gamma correction prior to testing using a photometer system (Opti-Cal; Minolta, Japan). The Metropsis software calculated the inverse curves required to correct for the monitor's non-linearities.

The Metropsis contrast sensitivity system [Figure 2.8] generated luminance modulated sine gratings (Gabor patches). The orientation of the stimuli was vertical. The Gabor patches were presented on the CRT monitor and subtended a visual angle of 4.2°. The mean luminance was used as the background luminance. The Gabor had a two-dimensional spatial Gaussian envelope and was radially symmetrical with equal standard deviations (SD), δx and δy.

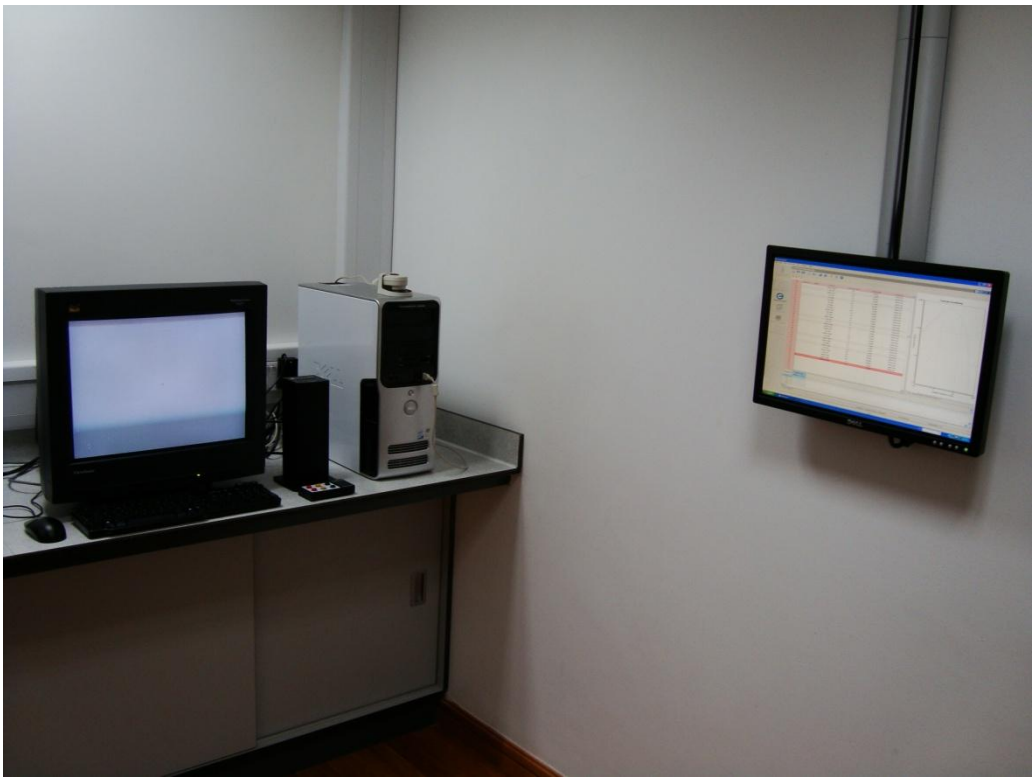


Figure 2.8 The Metropsis contrast sensitivity system.

2.2.5.3 TESTING PROCEDURE

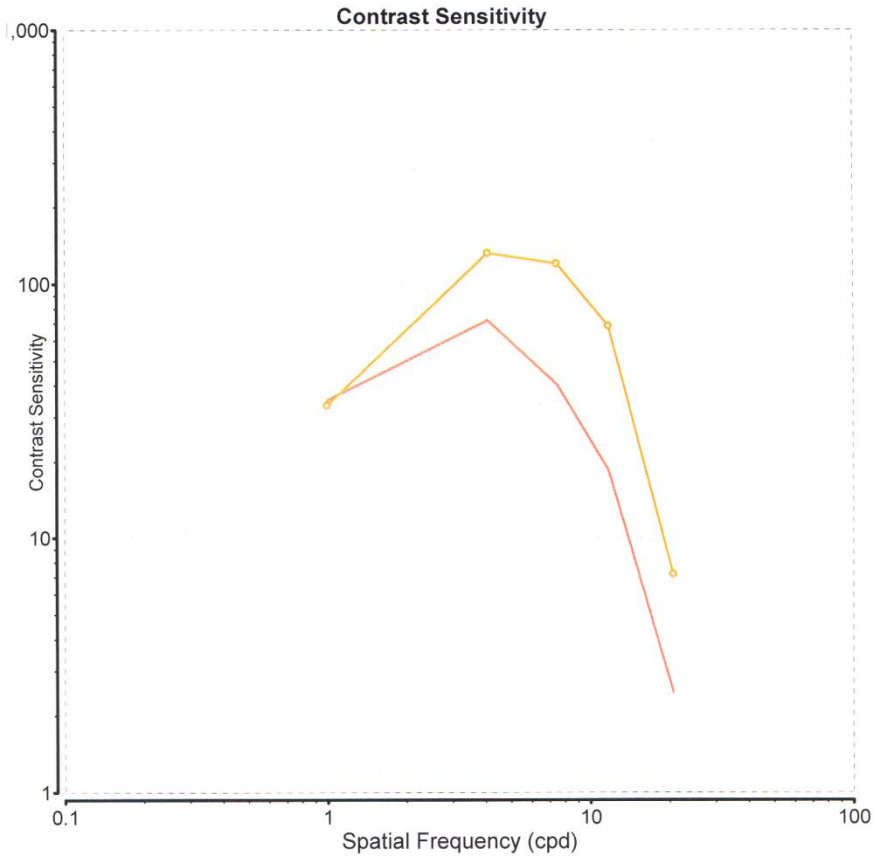
CSFs were determined under both mesopic and photopic conditions. Each subject was seated at a fixed viewing distance of 1.5m from the cathode ray tube (CRT) monitor. Natural pupils were used throughout the experiment. The non-dominant eye was occluded. Testing was carried out in a light free (other than CRT background mesopic and photopic light) environment. The subject was dark adapted for 5 minutes and a five-minute training session

was given prior to testing under mesopic conditions. Subject responses were recorded using a handheld responder (CR6, Cambridge Research Systems Ltd., Cambridge, UK), which communicated with the VSG device via an infra red link. A four alternate forced choice testing system was used, with four possible target locations. The stimuli were randomly presented at 2° spatial offset from the central cross target. The subject indicated the location of the target in relation to the fixation cross using the appropriate button on the responder box. The subject's contrast sensitivity was determined for five different spatial frequencies (1.0, 4.1, 7.5, 11.8 and 20.7 cpd) under both mesopic and photopic conditions, all at a mean luminance of 3 cd m^{-2} (mesopic) and 100 cd m^{-2} (photopic).

A linear staircase method was used to determine the contrast threshold. The first Gabor at a particular location was presented at an initial contrast level where it was anticipated that the observer would be able to detect the Gabor patch for that particular spatial frequency (initial contrast settings were informed by a brief pilot study involving five young healthy subjects). Subsequently, the contrast of the Gabor patch was varied using an adaptive staircase procedure, which was computer controlled and depended upon the subject's responses. The stimulus contrast was reduced in steps of 0.3 log units until the subject did not detect the Gabor patch (first reversal). The contrast was subsequently increased by 0.15 log unit steps until the subject saw the Gabor patch and responded correctly (second reversal). The Metropsis software calculated the contrast threshold for each location and spatial frequency by taking the mid-point between the mean for peaks and troughs for 12 reversal points. The SD was calculated by taking the deviations of the peak reversals from their peak means and using the average square of these deviations to calculate a peak variance. This method was repeated for the troughs. The square root of both variances were then calculated and averaged to provide the threshold SD.

For each subject, the Metropsis software plotted the inverse of the contrast threshold against the range of spatial frequencies tested to provide a

CSF under both mesopic and photopic conditions [Figure 2.7]. The Metropsis test report showing a typical set of contrast (%) values recorded for a subject is shown in Figure 2.9. Detailed procedures for CSF testing using the Metropsis are given in the SOPs for this instrument, provided in Appendix 7.11 and 7.12.



Test Date:
Test Time:
Subject:
Age:
Eye:
Examiner:
Comments:

Protocol: mesopic
Mean Luminance: 3
Stimulus: Gabor
Duration (ms): 5000
Envelope: Square
Spatial Offset: 2
Fixation Target: On
Test Method: 4AFC
Adaptive Procedure: Linear Staircase
Control Trials: Off

Spatial Frequency (cpd)	Contrast (%)	Standard Deviation (%)
1.0	2.837	0.179
4.1	1.375	0.516
7.5	2.450	0.259
11.8	5.338	0.746
20.7	40.013	1.485

Test Date:
Test Time:
Subject:
Age:
Eye:
Examiner:
Comments:

Protocol: photopic
Mean Luminance: 100
Stimulus: Gabor
Duration (ms): 5000
Envelope: Square
Spatial Offset: 2
Fixation Target: On
Test Method: 4AFC
Adaptive Procedure: Linear Staircase
Control Trials: Off

Spatial Frequency (cpd)	Contrast (%)	Standard Deviation (%)
1.0	2.975	0.542
4.1	0.750	0.312
7.5	0.825	0.260
11.8	1.450	0.187
20.7	13.775	1.528

Figure 2.9 A typical test report of the Metropsis.

2.2.6 PHOTOSTRESS RECOVERY

PRT was calculated using a MAP test.[9;10] MAP is a novel photostress method for the evaluation of macular function using the Humphrey® field analyzer (Model 745*i* Carl Zeiss Meditec Inc. Dublin, CA, USA) [Figure 2.10]. The foveal threshold feature of the field analyzer was used to establish baseline foveal sensitivity as the average of three consecutive foveal sensitivity measurements recorded in dB, with each dB representing a 0.1 log unit sensitivity variation.

Following baseline foveal sensitivity calculation, the subject was exposed to a photostress stimulus, which consisted of a 5-second exposure to a 300-watt, 230 volt tungsten lamp head from a viewing distance of one metre. The spectral irradiance in the wavelength range, 300-800 nm, was measured using a Bentham DMc 150 double monochromator scanning Spectroradiometer [Figure 2.11]. The input optic consisted of a very high precision cosine response diffuser (f_2 error <1%) and the measurements were performed in 1 nm intervals. Calibration was carried out with reference to a quartz-halogen lamp traceable to the United Kingdom National Physical Laboratory. The illuminance at 1 metre was obtained by using the photopic weighting function.

Immediately post-photostress, a continuous and timed cycle of foveal sensitivity measurements were conducted and recorded for each subject. The reduction in foveal sensitivity from baseline, along with the time taken to recover to baseline foveal sensitivity, was recorded. Detailed procedure for photostress recovery testing using the Humphrey® field analyzer is given in the SOP for this test, provided in Appendix 7.15.

Figure 2.10 The Humphrey® field analyzer with Arri 300 photostress lamp.

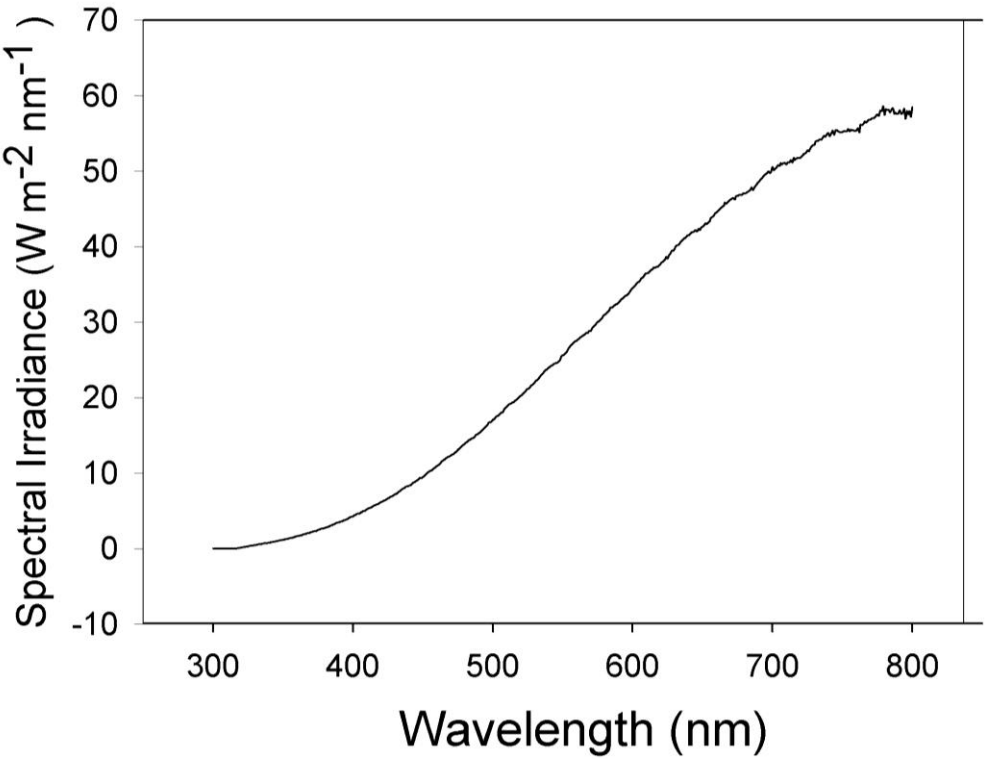


Figure 2.11 Spectral Irradiance at 1 metre fixation distance from Arri 300 photostress lamp.

2.2.7 FUNDUS PHOTOGRAPHY

Fundus photographs were obtained in both eyes using a NIDEK non-mydriatic fundus camera (AFC-210) [Figure 2.12]. Fundus photographs were assessed by an expert eyecare professional to exclude fundoscopically evident retinal pathology. Detailed procedure of fundus photography using a NIDEK non-mydriatic fundus camera is given in the SOP, provided in Appendix 7.19.

Figure 2.12 The NIDEK non-mydriatic fundus camera (AFC-210).

2.2.8 VISUAL FUNCTION IN NORMALS QUESTIONNAIRE

A 30-part, non-validated, Visual Function in Normals questionnaire (VFNq30) was designed specifically for the study (Appendix 7.6). The design was based loosely on a previously validated visual activities questionnaire,[11] but adapted to suit a normal, young and healthy population sample. This questionnaire allowed the subject to quantify their visual performance using three separate metrics: situational analysis (SA) which required the subject to rate their visual performance in specified daily life situations; comparative analysis (CA) which required the subject to compare their perceived visual performance to that of their peers/family/friends; subject satisfaction score (SSS) which required the subject to provide an overall estimate of their perceived quality of vision. Each of the three metrics above was computed to give a performance score for five different functional aspects of their vision: acuity/spatial vision; glare disability; light/dark adaptation; daily visual tasks; colour discrimination.

2.2.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Blood samples (6–8 mL) were collected from all patients on the same day as MPOD assessment. Serum was separated from blood by centrifugation (DESAGA Starstedt–Gruppe, GC12) 2500 RPM for 10 min, and then aliquoted into two amber light-sensitive microcentrifuge tubes and stored at minus 70°C until time of analysis. A 400 μL aliquot of serum was pipetted into an amber light-sensitive microcentrifuge tube (1.5 mL total capacity). Ethanol (300 μL) containing 0.25 g/L butylated hydroxytoluene (BHT) and 200 μL internal standard (α-tocopherol acetate [0.25 g/L]) were added to each tube. Heptane (500 μL) was then added and samples were vortexed vigorously for 2 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 amber light-sensitive microcentrifuge 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 200 μL methanol (containing 0.25 g/L BHT), and 100 μL was injected for high performance liquid chromatography (HPLC) analysis. We used an Agilent 1200 series (Agilent Technologies Ltd., Dublin, Ireland) system with photodiode array detection at a wavelength of 450 nm [Figure 2.13]. A 5 μm analytical/ preparative 4.6 × 250 mm 201TP specialty reverse phase column (Vydac, Hesperia, California, USA) 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. DSM Nutritional Products (Basel, Switzerland) provided L and Z standards to generate response factors that were used to calculate serum concentrations of L and Z. An internal standard, α-tocopherol acetate, made up in ethanol (0.25 mg/L) was used to correct for recovery of extractions for HPLC analysis and assist quantification. All chromatograms were integrated manually by drawing a baseline and dropping perpendicular lines to quantify the peaks of interest. All carotenoid peaks were integrated and

quantified using Agilent ChemStation software. Figure 2.14 shows a typical chromatogram generated from the above described assay. Detailed procedures of HPLC analysis are given in the SOPs for this test, provided in Appendix 7.21, 7.22, 7.23 and 7.24.

Figure 2.13 The Agilent 1200 series HPLC system used for analysing L and Z.

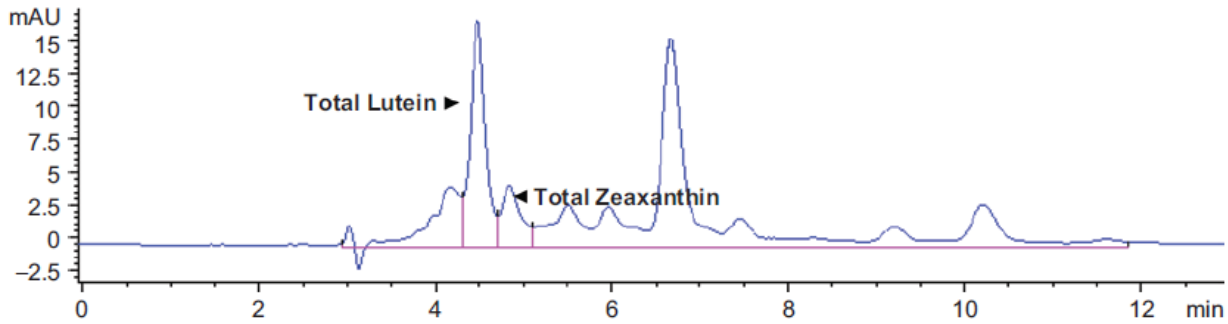


Figure 2.14 HPLC chromatogram showing L and Z peaks.

2.2.10 FOOD FREQUENCY QUESTIONNAIRE

Dietary intake of L and Z was assessed by a self-administered, semi quantitative food frequency questionnaire (FFQ) developed by the Scottish Collaborative Group (SCG) at the University of Aberdeen, Scotland, UK. This semi-quantitative FFQ (see Appendix 7.7, 7.8 and 7.9) is the primary method used in epidemiological studies to assess dietary intake of various nutrients and foods most commonly consumed in a Western diet.[12] This FFQ is a development of FFQs used in the Scottish Heart Health Study, and it has been validated against weighed food records and biomarkers, and its validity, in terms of quantifying dietary intake of L and Z (separately), has been confirmed in a study by O'Connell *et al*.[13-15]

The questionnaire is designed to assess a subject's dietary intake over the preceding two to three months. Although subjects were assessed at different time-points throughout the year, it has been shown that month-to-month serum concentrations of L and Z and MPOD are relatively stable over a 24 month period.[16] The FFQ consists of 170 foods and drinks, grouped into 21 sections. A standard portion or measure for each type of food or drink is specified and subjects are required to indicate how many portions they consumed per day and how often they consumed each type of food, ranging in frequency from "rarely or never" to "7 days per week". The questionnaire includes a colour photograph depicting examples of standard food measures [Figure 2.15] and an example of how to fill out the questionnaire. The questionnaire was completed by the subject in the presence of the investigator, following detailed instructions for the required task. This questionnaire took between 20 and 30 minutes to complete.

Completed FFQs were inputted into a Microsoft™ Access® spreadsheet developed for analysis purposes by the SCG, and then emailed back to the SCG for analysis. Nutrient analysis was performed using a Visual Basic for Microsoft™ Access® program using food composition data based on *McCance and Widdowson's The Composition of Foods*.[17] Dietary intake of L and Z was calculated using food composition data from the UK,

European and US data sources using standard principles or criteria for the matching of food items, and standardised recipes or manufacturer's ingredient information where necessary.[18-21]

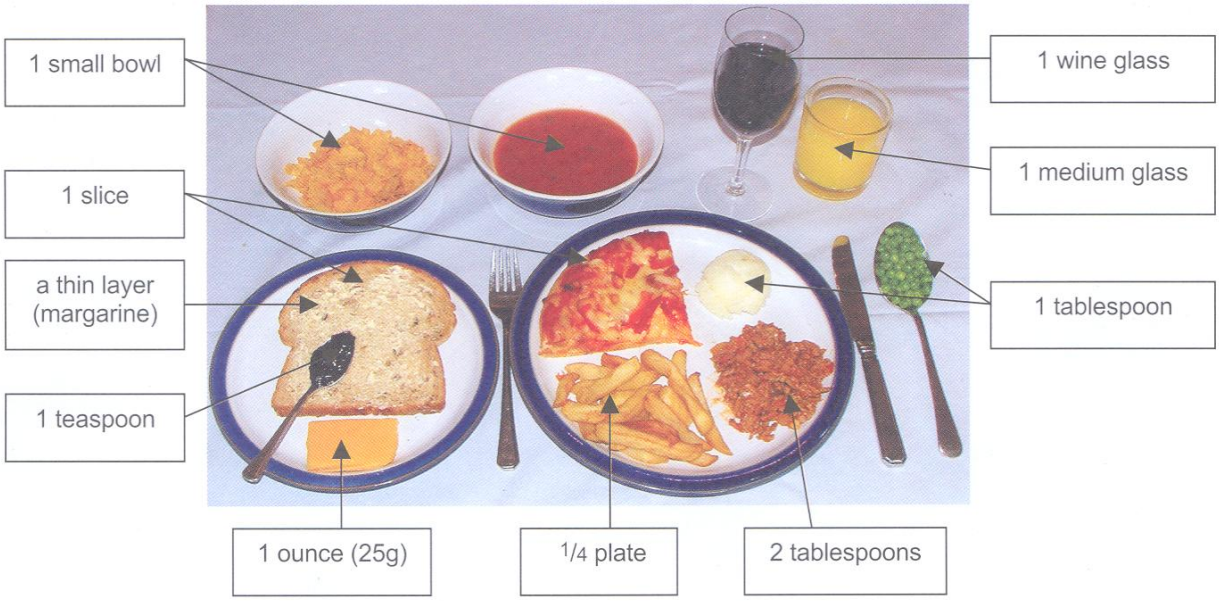


Figure 2.15 FFQ photograph, illustrating sample standard food and drink portions.

2.2.11 MEASURMENT OF MACULAR PIGMENT OPTICAL DENSITY USING HETEROCHROMATIC FLICKER PHOTOMETRY

2.2.11.1 HETEROCHROMATIC FLICKER PHOTOMETRY

HFP was the first technique, and remains one of the most widely used techniques, for measuring MPOD *in vivo* [Figure 2.16]. HFP is a subjective psychophysical method, which requires the subject to make iso-luminance matches between green and blue flickering lights. The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus (the "reference point," where MPOD is assumed to be zero), gives a measure of the individual's MPOD. This method has been validated against the absorption spectrum of MP *in vitro*.[22;23]

Measurement of MPOD by HFP also correlates well with results obtained by reflectometry and autofluorescence, both of which are objective techniques for measuring MPOD *in vivo*.[24] However, in this study by Delori *et al*. despite good correlation between the methods, they found that reflectometry systematically underestimated MPOD and that autofluorescence systematically overestimated MPOD when compared with measurement by HFP.[24]

Of note, it has been reported that MP is detectable at up to 8º retinal eccentricity and, in theory, therefore, HFP instrument should use a reference point close to, or at this location.[25-28] We assume that flicker perception is dominated by the edges of the disc-shaped stimuli presented in each HFP instrument used in our studies,[29] although other research has suggested that this may not be the case.[30]

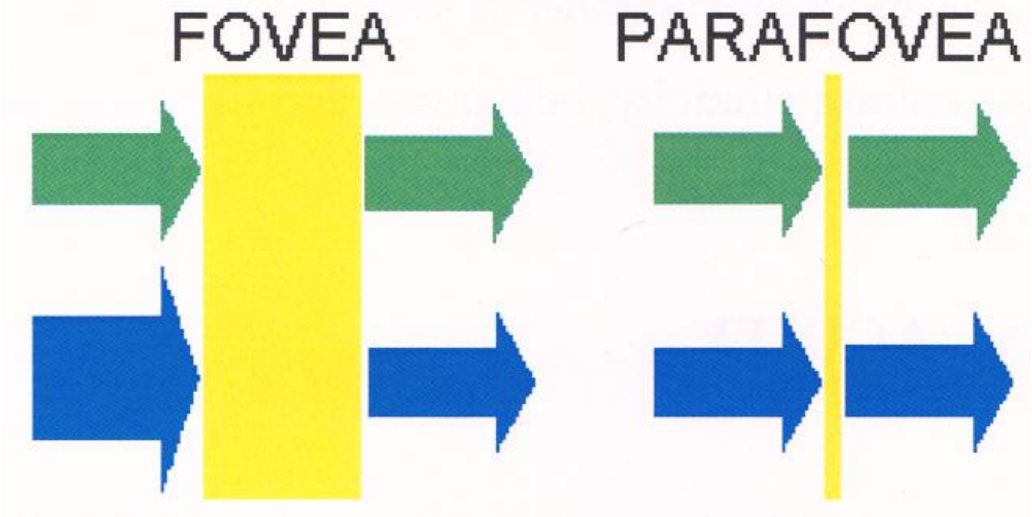


Figure 2.16 Illustration of the principle of HFP. At the fovea, more blue light (indicated by the large blue arrow) is required to match the luminance of the green light, as the MP (indicated in yellow) is concentrated at the fovea, and is optically undetectable at the parafoveal reference point. At the fovea, the MP absorbs a proportion of incident blue light (indicated by the smaller blue arrow following passage through the MP).

2.2.11.2 THE MACULAR DENSITOMETER™

The Macular Densitometer™ [Figure 2.17] was developed by Professor Billy Wooten of Brown University, Providence, Rhode Island, USA (for a more detailed description of this instrument and its methods of use, please refer to Wooten et al., 1999).[31] The Macular Densitometer™ uses HFP to obtain a valid measure of MPOD at a given retinal location.[32] This method has recently been refined and is now referred to as customised HFP (cHFP). For a detailed description of this protocol please see recent publications by our research group and others.[28;33;34] The reproducibility and test-retest variability of the spatial profile of MP, measured by Macular Densitometer™ was investigated by Kirby *et al.*[35] A schematic of the optical system used to measure MPOD in free view in the Macular Densitometer™ is shown in Figure 2.18.

Figure 2.17 The Macular Densitometer™.

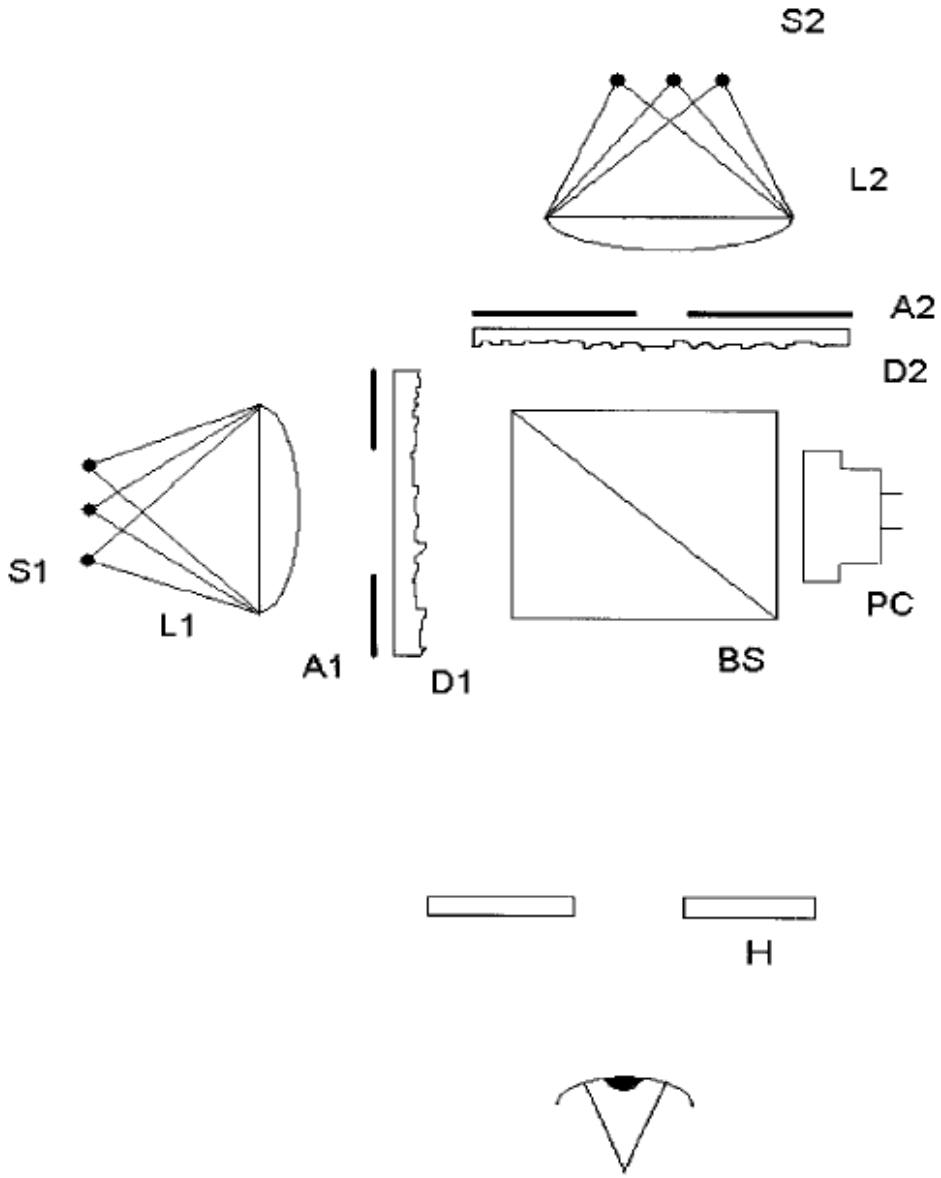


Figure 2.18 A schematic of the optical system used to measure MPOD in free view in the Macular Densitometer™. A1 and A2: apertures 1 and 2; BS: beam splitter; L1 and L2: planoconvex achromatic lenses; PC: photocell; H: hot mirror used to reduce heat transmission; S1and S2: light sources; D1 and D2: optical diffusers. [From Wooten *et al*. 1999][31]

2.2.11.2.1 LIGHT SOURCES

The Macular Densitometer uses LEDs as light sources [Figure 2.19]. The major advantages of LEDs are that they are small, inexpensive, easily driven from simple power supplies, and emit near-monochromatic light. In the Macular Densitometer the luminance of both the green (564 nm wavelength) and the blue (460 nm wavelength) LEDs are varied in a yoked manner. This means that, as the luminance of the green LED increases, the luminance of the blue LED decreases, and vice versa. This is an important feature of this instrument as it eliminates any changes in the overall illuminance of the target being viewed, which may be confusing for some subjects during the test. The illumination of the blue and green LEDs is alternated in square-wave counterphase. The emitted blue light is maximally absorbed by MP, whereas the emitted green light is not absorbed by this pigment.

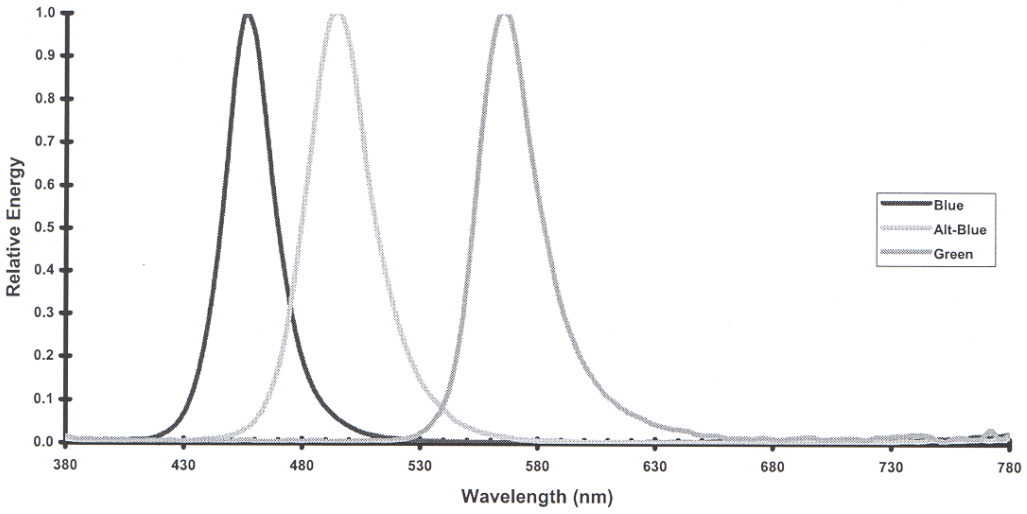


Figure 2.19 The relative spectral energy curve of the LEDs that were used in the Macular Densitometer™.

2.2.11.2.2 TEST FIELDS

The Macular Densitometer has a reference point at a 7º retinal eccentricity and, by presenting the test field at various retinal eccentricities, it can be used to map a subject's MP spatial profile across the macula [Figure 2.20]. We measured MPOD at 0.25º, 0.50º, 1º, 1.75º, 3º retinal eccentricities.

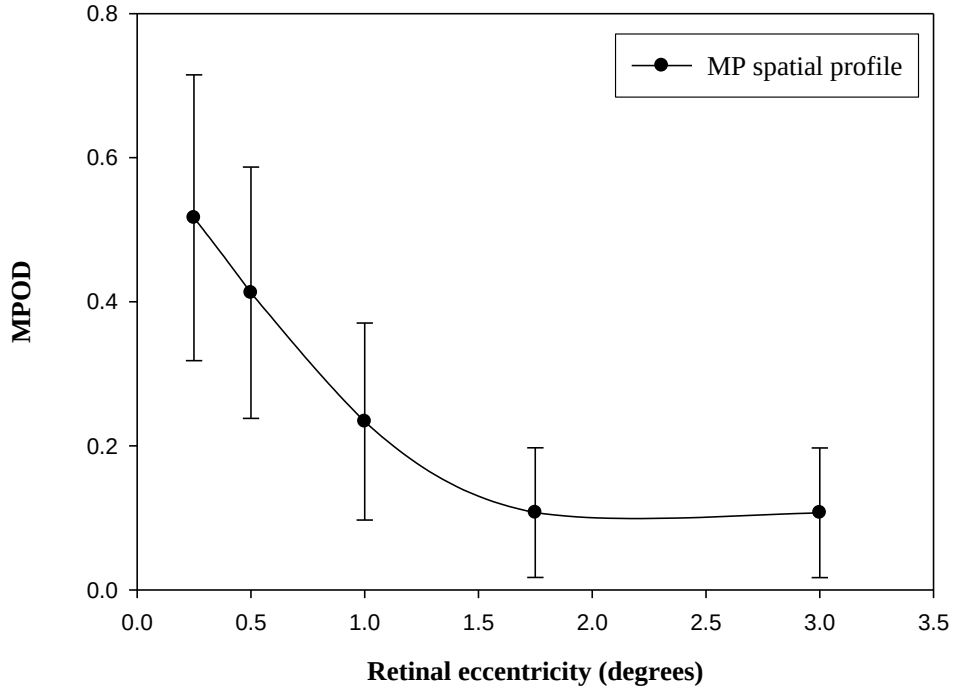


Figure 2.20 Typical MPOD spatial profile.

The target used to measure MPOD at 0.25º of retinal eccentricity is a centrally located circular stimulus of 1º diameter, with a central fixation spot, at which the subject fixates [Figure 2.21]. The target used to measure MPOD at 0.50º of retinal eccentricity is a centrally located circular stimulus of 1º diameter, with a central fixation spot, at which the subject fixates. The targets used to measure MPOD at 1 and 1.75º of retinal eccentricity are both ring-shaped targets, with a central fixation spot, at which the subject fixates during testing. The 7º reference target, on the other hand, uses an eccentrically located red LED, 5 minutes in diameter, as the fixation spot. This is presented to the left-hand side of a blue/green flickering circular disc, which has a diameter of 2º and is centred at an eccentricity of 7º from

the red fixation LED. Both the central and reference targets are presented on a blue background test field, provided by a source consisting of three LEDs with a peak wavelength at 468 nm and a luminance of 2.6 cd m^{-2}. This saturates short wavelength cones, ensuring that they play no part in the determination of the null flicker end point of the test.

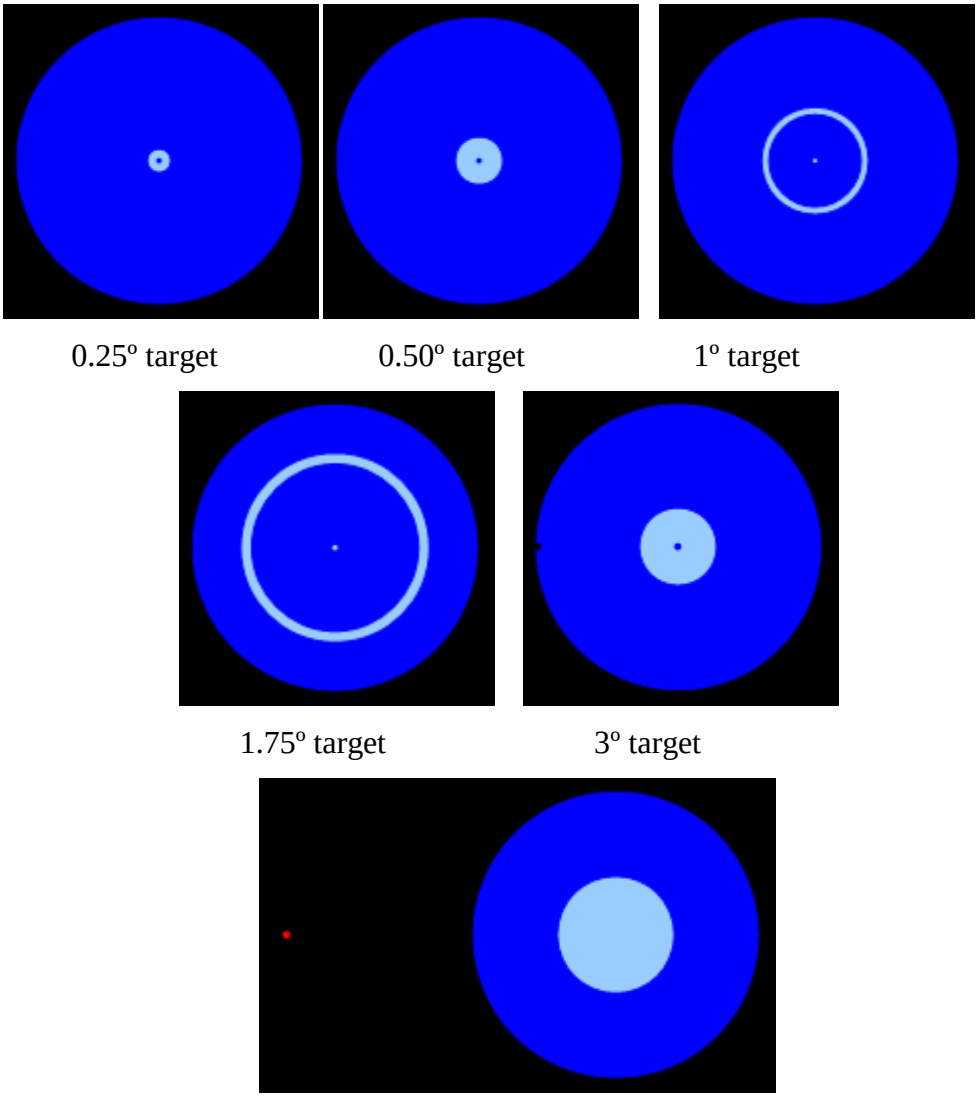


Figure 2.21 Targets used in the Macular Densitometer™.

2.2.11.2.3 FLICKER FREQUENCY

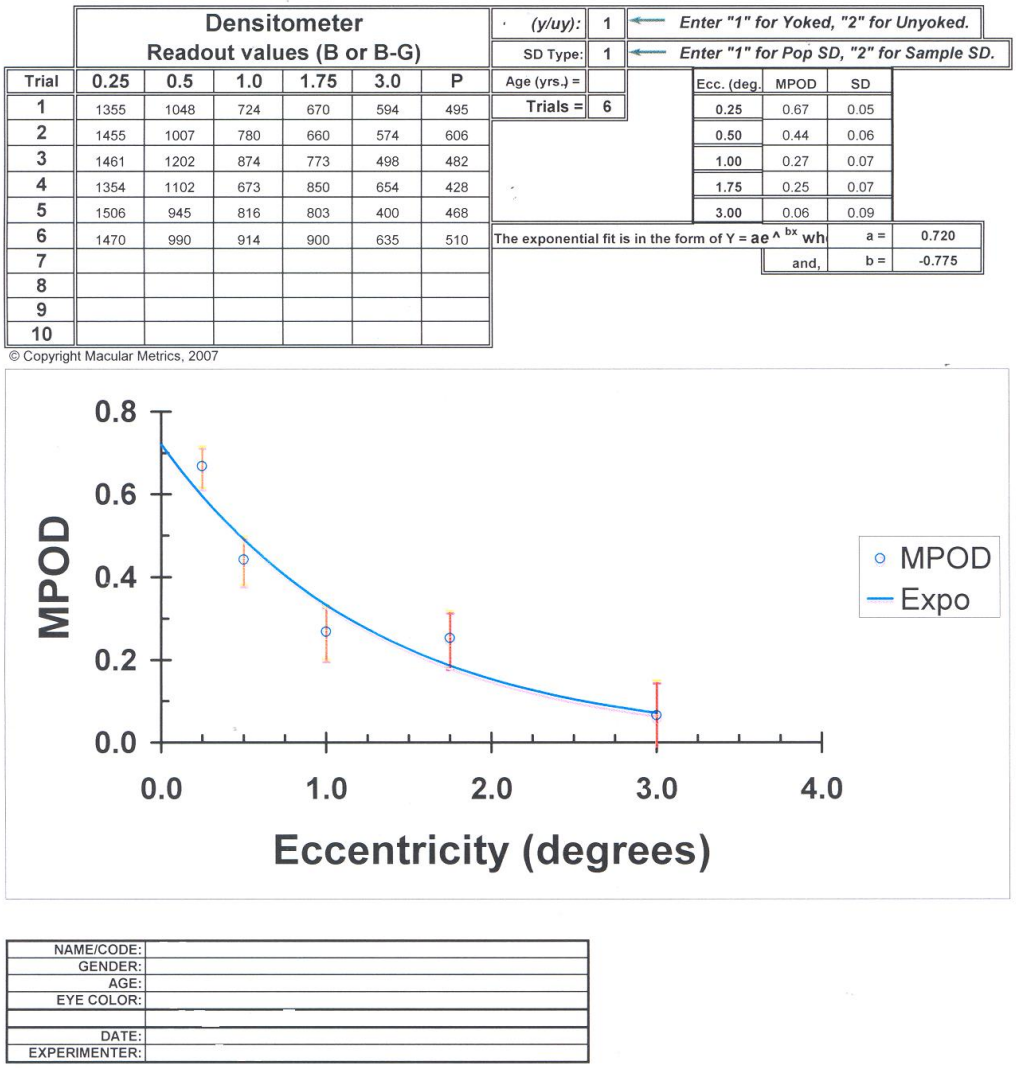
One of the most important features of the Macular Densitometer™ is that it has the option to adjust the flicker frequency. This enables the investigator to customise the optimal flicker fusion frequency (OFF) for each subject, which results in a more discrete end point for the test, thus minimising the variance between readings, as each subject will have a different CFF, which is the frequency of flicker above which flicker is no longer perceived. The desired endpoint when using the Macular Densitometer is a point of zero, or null flicker, i.e. a point where the perceived luminance of the green and blue flickering LEDs is the same.

2.2.11.2.4 MEASURING MACULAR PIGMENT OPTICAL DENSITY WITH THE MACULAR DENSITOMETER™

Prior to using the Densitometer, all subjects were shown an explanatory video describing the method for recording null flicker matches. The investigator then recorded the subject's CFF and OFF using an algorithm developed by Dr. Nolan and Dr. Stringham at Prof. Max Snodderly's Vision Laboratory, Medical College of Georgia, Augusta, Georgia, USA. If a subject could not reach null flicker, the investigator increased the flicker frequency in increments of 1 Hz, until null flicker was perceived. Alternatively, if a subject exhibited a wide variation in null flicker readings (>10% of mean radiance at null flicker), the flicker frequency was decreased in increments of 1 Hz, until an acceptable null flicker range was achieved. An acceptable null flicker range was defined as one where the null flicker radiance values achieved by the subject were within 10% of the mean null flicker radiance at that test locus. Subjects were required to perform at least five null flicker matches per target (foveal and parafoveal targets), as recommended in the standard operating procedure (SOP) for the Densitometer. Further readings were taken if the variance of the first five

readings was >10%, and outliers were then removed, such that the variance of the remaining readings was <10%. All recordings were made under conditions of dimmed light, as detailed previously. Radiance values were then entered into a Microsoft Excel spreadsheet, which calculated the MPOD values at each locus. [Figure 2.22]

Detailed procedures for operation of the Macular Densitometer™ are given in the SOPs for this instrument, provided in Appendix 7.17 and 7.18.



Densitometer
Readout values (B or B-G)

Trial	0.25	0.5	1.0	1.75	3.0	P
1	1355	1048	724	670	594	495
2	1455	1007	780	660	574	606
3	1461	1202	874	773	498	482
4	1354	1102	673	850	654	428
5	1506	945	816	803	400	468
6	1470	990	914	900	635	510
7						
8						
9						
10						

(y/uy): 1 ← Enter "1" for Yoked, "2" for Unyoked.
SD Type: 1 ← Enter "1" for Pop SD, "2" for Sample SD.
Age (yrs.) =
Trials = 6

Ecc. (deg.	MPOD	SD
0.25	0.67	0.05
0.50	0.44	0.06
1.00	0.27	0.07
1.75	0.25	0.07
3.00	0.06	0.09

The exponential fit is in the form of Y = ae ^ bx wh
and, a = 0.720
b = -0.775

NAME/CODE:	
GENDER:	
AGE:	
EYE COLOR:	
DATE:	
EXPERIMENTER:	

Figure 2.22 A typical test report of the Macular Densitometer™.

2.2.11.3 TROXLER'S FADING AND AFTER-IMAGES

Troxler's fading is a phenomenon of visual perception, originally described in 1804. When fixating a central target, stimuli in the periphery may appear to fade and can disappear completely with sufficiently intent central fixation. The effect is enhanced if the stimulus is small, and also with increasing distance from the fixation point. This phenomenon is understood to be due to neural adaptation in the visual system, resulting in fading or complete failure to perceive an unvarying visual stimulus. Normally this

effect is prevented by micro-saccadic eye movements, which continually result in excitation of adjacent photoreceptors, allowing sustained perception of an image. The effect is greater in the periphery because of the larger receptive fields of the peripheral retinal neurons.

After-images are often revealed when viewing any self-illuminated or bright object against a dark background for prolonged periods of time. After images may be quite obtrusive and may even obscure the target of fixation itself.

Both Troxler's fading and the effect of after-images may interfere with MPOD testing using HFP. To minimise the appearance and impact of these effects, the subject under test should be advised to make null flicker matches reasonably quickly, to avoid being over pernickety, to blink normally throughout testing, and to look away from the test target in between null flicker matches.

2.2.12 RELIABILITY TESTING OF METHODS

Given that all subjects recruited into the study were classed as ''naïve" to the tests carried out (with the exception of the visual acuity test), we conducted a pilot reliability study prior to the study commencing. Following pre-test training (see above section 2.2.1), repeat testing on 10 subjects at three separate study visits (over a 10 day period) was conducted. The intraclass correlations (ICC) obtained for all methods were high and are presented in Table 2.1. In addition, repeat testing of radiance values obtained to compute MPOD values had previously been conducted by our research group. The data from this investigation concluded that the radiance values obtained using the Densitometer were very high (i.e. ICC in the range of 0.93–0.96; see recent publication by (Kirby *et al*. 2009).[35] In addition, we conducted Bland-Altman analyses[36] of differences in MPOD at eccentricities 0.25°, 0.5°, 1° and 1.75°, measured at two separate study visits. The limits of agreement, at all eccentricities, were in the range 0.06–

0.07 units away from the mean difference, which seems satisfactory. The coefficient of repeatability ranged from about 6% at the central eccentricities (0.25°, 0.5°), to 19.4% at 1.75°.

Mean differences in MPOD between study visits were 0.02, -0.01, 0.02, and 0.0 at eccentricities 0.25°, 0.5°, 1° and 1.75°, respectively. The first two of these differences were statistically significant, at the 5% level, using the paired t-test, suggesting bias; clinically, however, a bias of this very small magnitude is of no practical importance.

Table 2.1 Reproducibility of visual performance tests used in studies one and two, assessed using ICC.

Test	Visit 1	Visit 2	Visit 3	ICC*
Mesopic CSF† by Functional Vision Analyzer™				
with no glare				
1.5 cpd‡	1.55 (±0.21)	1.68 (±0.23)	1.62 (±0.20)	0.683
3 cpd	1.67 (±0.27)	1.74 (±0.24)	1.77 (±0.23)	0.852
6 cpd	1.51 (±0.58)	1.64 (±0.27)	1.61 (±0.25)	0.682
12 cpd	0.78 (±0.61)	0.88 (±0.52)	0.97 (±0.57)	0.867
18 cpd	0.56 (±0.45)	0.43 (±0.53)	0.39 (±0.46)	0.843
with medium glare lights				
1.5 cpd	1.47 (±0.20)	1.55 (±0.22)	1.45 (±0.21)	0.626
3 cpd	1.31 (±0.54)	1.52 (±0.34)	1.43 (±0.57)	0.533
6 cpd	1.03 (±0.77)	1.16 (±0.69)	1.18 (±0.68)	0.893
12 cpd	0.49 (±0.59)	0.60 (±0.58)	0.51 (±0.62)	0.770
18 cpd	0.19 (±0.37)	0.25 (±0.39)	0.33 (±0.41)	0.767
with high glare lights				
1.5 cpd	1.25 (±0.52)	1.34 (±0.32)	1.28 (±0.52)	0.829
3 cpd	1.26 (±0.55)	1.33 (±0.56)	1.30 (±0.51)	0.942
6 cpd	1.01 (±0.77)	0.94 (± 0.71)	0.98 (±0.74)	0.978
12 cpd	0.48 (±0.57)	0.33 (±0.50)	0.36 (±0.55)	0.485
18 cpd	0.19 (±0.37)	0.07 (±0.20)	0.13 (±0.27)	0.707
Mesopic CSF by Metropsis				
1.0 cpd	1.54 (±0.10)	1.55 (±0.15)	1.60 (±0.11)	0.432
4.1 cpd	1.73 (±0.15)	1.77 (±0.13)	1.77 (±0.17)	0.399
7.5 cpd	1.32 (±0.09)	1.31 (±0.15)	1.34 (±0.18)	0.683
11.8 cpd	0.83 (±0.14)	0.84 (±0.18)	0.82 (±0.23)	0.732
20.7 cpd	0.22 (±0.07)	0.24 (±0.09)	0.25 (±0.09)	0.746
Photopic CSF by Metropsis				
1.0 cpd	1.60 (±0.17)	1.58 (±0.15)	1.59 (±0.15)	0.645
4.1 cpd	1.95 (±0.13)	1.98 (±0.13)	1.97 (±0.13)	0.462
7.5 cpd	1.75 (±0.13)	1.75 (±0.17)	1.78 (±0.18)	0.632
11.8 cpd	1.29 (±0.21)	1.34 (±0.25)	1.39 (±0.25)	0.727
20.7 cpd	0.43 (±0.24)	0.43 (±0.19)	0.41 (±0.20)	0.857

Photostress Recovery Test				
Baseline Sensitivity	37.41 (±1.30)	38.41 (±1.52)	38.08 (±1.68)	0.560

* Intraclass correlation coefficient

† Contrast sensitivity function

‡ Cycles per degree

2.2.13 STATISTICAL ANALYSIS

The statistical software package SPSS (version 17) was used for analysis. All variables investigated exhibited a typical normal distribution. Mean ± SD's are presented in the text. Pearson correlation coefficients were calculated to investigate bivariate relationships and partial correlation coefficients when controlling for confounding variables. We used the 5% level of significance throughout our analysis. A statistical power analysis determined a minimum sample size of 91 subjects in order to achieve 99% power with a one-tailed 5% test, with an affect size of ρ (rho) = 0.4. The 142 subjects recruited exceed these stringent statistical requirements, but more importantly, allowed for continued follow up (and standard drop-out) as part of the Collaborative Optical Macular Pigment ASsessment Study (COMPASS) L interventional study (International Standard Randomised Controlled Trial Number (ISRCTN) = 35481392), which was designed to investigate whether MPOD augmentation, following L supplementation, improves visual performance.

2.3 RESULTS

2.3.1 DEMOGRAPHIC, MEDICAL, LIFESTYLE, ANTHROPOMETRIC, AND OCULAR RELATED DATA

The demographic, medical, lifestyle, anthropometric, and vision- related data of the 142 subjects recruited into the study are summarised in Table 2.2. No subject was excluded from the study on the basis of fundus findings. The mean (±SD) age of the sample was 29 (±6) and ranged from 18 to 41 years. The mean (±SD) BMI was 25 (±4) and ranged from 19 to 43.

Table 2.2 Demographic, medical, lifestyle, anthropometric, and ocular related data for the entire study group.

Characteristic	**n***
Sex	
Male	74
Female	68
Medical history	
Diabetes	1
High blood pressure	4
High cholesterol	6
Angina	0
Stroke	0
Family history of eye diseases	
Unknown	3
AMD	22
Cataract	12
Glaucoma	28
Retinal problem	4
None	82
Smoking habits†	
Never smoked	86
Ex-smoker	25
Current smoker	31
Exposed second-hand smoke	17
BMI	
Desirable weight (BMI <25)	82
Overweight (BMI 25-30)	43
Obese (BMI >30)	17
Ocular dominance	
Right	86
Left	53
Equidominant	3
BCVA	
<100	1
100-105	3
>105-110	42
>110-115	79
>115-120	17

* n = sample size

† Smoking habits: ex-smoker = smoked ≥100 cigarettes in lifetime but none in last 12 months; current smoker = smoked ≥100 cigarettes in lifetime and at least 1 cigarette per week in last 12 months; exposed second-hand smoke = commonly exposed to second-hand smoke at home or in the work place

2.3.2 MACULAR PIGMENT OPTICAL DENSITY

The mean (±SD) MPOD, at all degrees of retinal eccentricity measured, is summarised in Table 2.3. MPOD at peak (0.25° of retinal eccentricity) was positively and significantly correlated with MPOD at all other degrees of retinal eccentricity (r = 0.472–0.919, $P < 0.01$ for all).

Table 2.3 Mean (±SD) MPOD at all measured degrees of retinal eccentricity, for the entire study group.

Retinal eccentricity*	MPOD†
0.25°	0.48 (±0.19)
0.5°	0.39 (±0.17)
1°	0.21 (±0.12)
1.75°	0.09 (±0.09)
3°	0.09 (±0.07)
Average	0.25 (±0.12)

n = 141 (One subject was excluded from analysis due to inability to use the Densitometer to obtain reliable MPOD data)

* Degrees retinal eccentricity
† Mean (± SD) macular pigment optical density

2.3.3 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH BEST CORRECTED VISUAL ACUITY

The mean (±SD) BCVA of the study group was 112 (±3). There was a positive and statistically significant relationship between MPOD at each retinal eccentricity measured and BCVA (r = 0.237–0.308, $P < 0.01$ for all). The relationship between MPOD at 0.25° of retinal eccentricity and BCVA is presented in Figure 2.23.

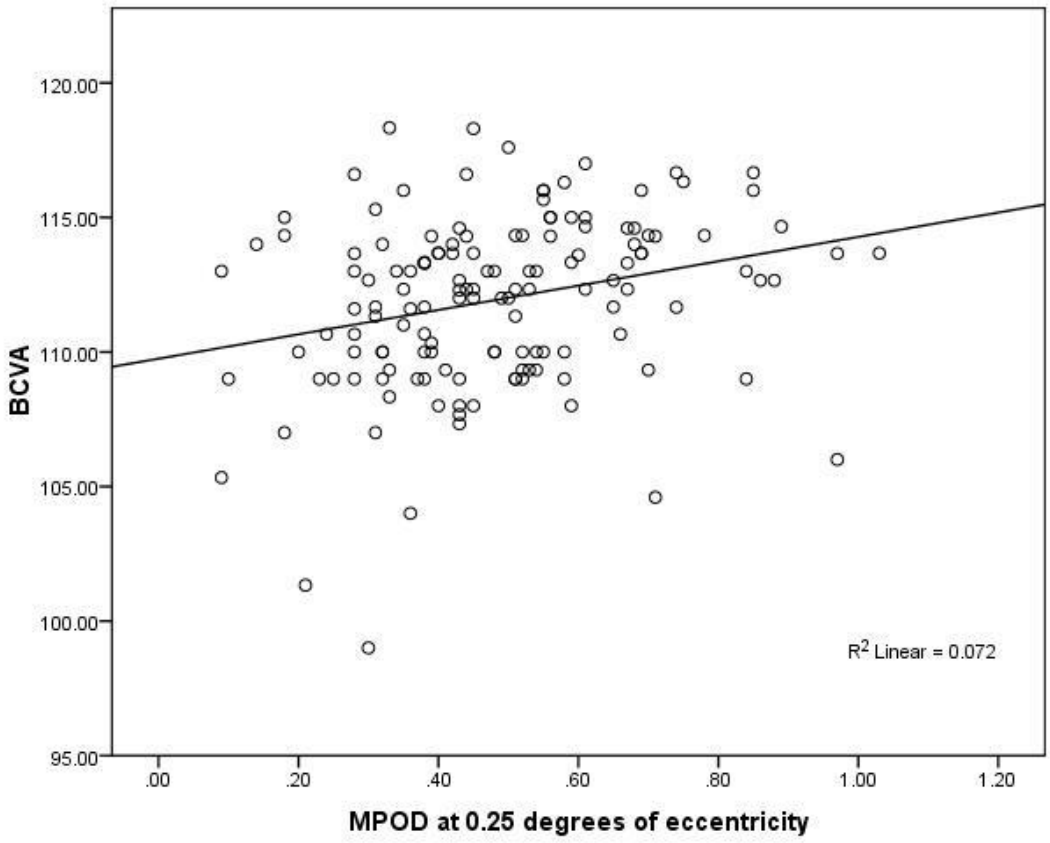


Figure 2.23 The relationship between MPOD at 0.25º and BCVA.

2.3.4 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH CONTRAST SENSITIVITY FUNCTION

The relationships between MPOD at each retinal eccentricity measured and log mesopic and photopic contrast sensitivity at different spatial frequencies are presented in Table 2.4. The strongest relationship was seen between MPOD at 0.25° and log contrast sensitivity at 7.5 cpd for mesopic conditions ($r = 0.22$, $P < 0.01$) (Figure 2.24).

Table 2.4 The relationships between MPOD and mesopic and photopic contrast sensitivity at different spatial frequencies.

MESOPIC Spatial frequency	**MPOD 0.25º**	**MPOD 0.50º**	**MPOD 1.0º**	**MPOD 1.75º**	**MPOD 3.0º**
1.0 cpd‡	-0.019	-0.034	-0.120	-0.200*	-0.097
4.1 cpd	0.065	0.016	-0.046	-0.080	-0.093
7.5 cpd	0.220†	0.192*	0.138	0.102	0.111
11.8 cpd	0.184*	0.183*	0.122	0.084	0.031
20.7 cpd	0.139	0.113	0.028	0.089	0.024
PHOTOPIC Spatial frequency	**MPOD 0.25º**	**MPOD 0.50 º**	**MPOD 1.0 º**	**MPOD 1.75º**	**MPOD 3.0º**
1.0 cpd	0.210*	0.159	0.108	0.160	0.081
4.1 cpd	0.124	0.100	0.007	0.067	0.053
7.5 cpd	0.176*	0.167*	0.115	0.133	0.101
11.8 cpd	0.193*	0.187*	0.135	0.131	0.114
20.7 cpd	0.153	0.153	0.082	0.132	0.117

* Correlation is significant at the 0.05 level

† Correlation is significant at the 0.01 level

‡ Cycles per degree

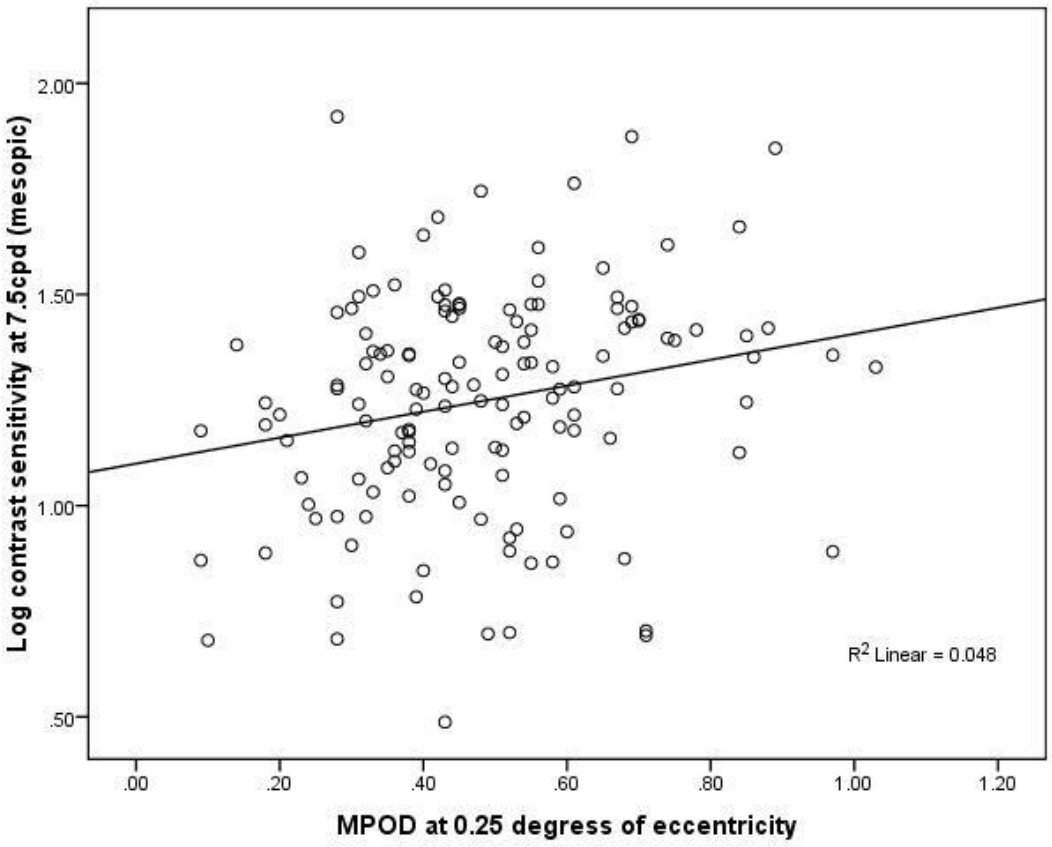


Figure 2.24 The relationship between MPOD at 0.25º and log contrast sensitivity at 7.5cpd for mesopic conditions.

2.3.5 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH GLARE DISABILITY

There was no statistically significant relationship between MPOD, at any of the eccentricities measured, and mesopic contrast sensitivity observed under medium or high glare conditions for any spatial frequency ($P > 0.05$, for all), with the exception of the negative and statistically significant relationship between peripheral MPOD (at 1.0°, 1.75° and 3.0°) and mesopic contrast sensitivity under medium glare conditions (r = -0.178 to -0.213, $P < 0.05$).

2.3.6 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH PHOTO STRESS RECOVERY TIME

The mean (±SD) foveal sensitivity of the study group was 38.1 (±1.4) dB. The mean (±SD) sensitivity post-photostress was 27.7 (±2.9) dB, representing a mean sensitivity reduction of 27.3% from baseline, across the entire study group. The mean (±SD) PRT (recorded as the time taken for foveal sensitivity to recover to 95%, or typically to within 2 dB, of the baseline value) was 135.8 (±63.9) s. There was no statistical relationship between MPOD at any of the eccentricities measured and either foveal sensitivity reduction (%) caused by photostress ($P > 0.05$, for all), or PRT ($P > 0.05$, for all).

2.4 DISCUSSION

Demographic, medical, lifestyle and anthropometric data were collected since studies done by Nolan *et al.* have shown significant associations between these variables and MPOD.[37;38]

Given the central and pre-receptorial location[27;39] and the optical properties of MP,[23] it is reasonable to hypothesise that MP would impact on visual performance, via its potential to attenuate chromatic aberration and light scatter.[1;2;40;41] In this study, we investigated the relationship between MPOD at various degrees of retinal eccentricity (i.e. at 0.25°, 0.5°, 1.0°, 1.75° and 3° of retinal eccentricity) and clinically important parameters of central visual performance including BCVA, contrast sensitivity, glare disability, and photostress recovery.

We report that MP (at each degree of retinal eccentricity) is positively associated with BCVA in our study population, which suggests that MP may play a role in the optimisation of visual acuity under photopic conditions; however, it is important to note that the ‘r’ values ranged from

0.237 to 0.308 and the observed relationships can therefore only explain 5.6 (r^2 = 0.056) – 9.5% (r^2 = 0.095) of the variability. This finding is all the more provocative given that subjects in the current study were young, free from ocular pathology, and uniformly demonstrated high visual acuity. Indeed, it is somewhat intriguing to note that this statistically significant relationship was detected in a population sample where the majority of participants exhibited average to high levels of MP (at 0.25° of retinal eccentricity). Indeed, only a very small number of subjects (~13.4%) had central MPOD of less than 0.3 in the current study. It has been previously suggested that levels above 0.3 might be superfluous to visual performance, due to the non linear nature of the effect of MP on vision.[1]

It is important to point out that extensive efforts were made by the COMPASS study investigators to probe the limits of visual acuity, so that even the most subtle contributions of MP to visual performance might be detected. This was facilitated by customisation of the vision test charts (i.e. inclusion of additional letter sizes to allow testing to a limit equivalent to 6/2.4) and recruitment of experienced optometrists to perform functional evaluations at both study sites (WIT and DIT). BCVA among the study participants ranged from a minimum of 99 ($6/6^{-1}$) to a maximum of 118 ($6/2.4^{-2}$). MP, it appears, could account for the theoretical refinement of acuity by up to 0.1 log units in the study sample here. This represents a substantial contribution and might be equated to the elimination of up to 0.25 D of optical defocus, and appears to be consistent with previously reported limiting effects of chromatic aberration on the spatial MTF.[42]

This finding is, however, somewhat at odds with previously reported investigations of the ‘‘acuity hypothesis”. Engles *et al*. explored the relationship between MPOD and both gap and vernier acuity under ‘‘photopic” conditions.[43] They reported that neither gap acuity nor vernier acuity was significantly related to MPOD. Their findings however are not directly comparable to the results described here, and for a number of reasons. Specifically, their adopted background luminance levels were in the low photopic range (i.e. 17 cd m^{-2} for the achromatic condition, and 15.7 cd

m^{-2} for the chromatic condition). Also, gap, vernier and recognition acuity measures are not directly interchangeable, so it is entirely plausible that findings with relation to the "acuity hypothesis" might differ when different visual attributes are assessed. Despite the aforementioned methodological differences, the conflicting outcomes do serve to emphasise the challenges inherent in the evaluation of the role of MP on visual performance, particularly by associative means.

We also report that central MPOD (i.e. at 0.25° and at 0.5° of retinal eccentricity) is positively and significantly related to both mesopic and photopic contrast sensitivity at intermediate spatial frequencies (i.e. 7.5 and 11.8 cpd). Central MP appears to influence sensitivity at spatial frequencies to which the visual system is highly tuned.[44] However, and similar to the association between MP and BCVA, it is important to note that the 'r' values for MP's association with contrast sensitivity ranged from 0.167 to 0.220 and therefore the observed relationships can only explain 2.8 (r^2 = 0.28) – 4.8% (r^2 = 0.48) of the variability.

For photopic conditions, this finding might be attributable to the attenuation of the effects of chromatic aberration and light scatter, whereby image refinement potentially causes lateral inhibitory surround responses to be dampened, and the resultant ganglion cell response optimised.[45] Under mesopic conditions, it is more likely that enhanced visual performance is a consequence of the selective diminution of rod mediated signals. While rod and cone photoreceptors operate interactively in the high mesopic conditions employed here,[45] rods remain optimally sensitive to shorter wavelengths than cones (explaining the Purkinje shift in peak retinal spectral sensitivity towards blue under mesopic conditions). The pre-receptorial absorption of short wavelength light by MP might, therefore, serve to attenuate rod activity and allow cone mediated vision (which typically exhibits better contrast sensitivity,[46] to dominate further into the mesopic range. This theory is supported by the limited nature of the relationship observed between MP and contrast sensitivity, confined to the

most central anatomic locations where MP is highest and cone activity predominates.

Of note, this is the first study to report on the association between MP and contrast sensitivity in a young healthy population (not confounded by dietary supplementation or ocular pathology). Our findings are consistent with those of Kvansakul *et al*. who reported that MP augmentation, via supplementation, enhances contrast acuity thresholds (CATs) under mesopic conditions.[47]

Finally, we found that MPOD was not related to either glare disability or photostress recovery, as assessed here. At first glance, these findings might appear to conflict directly with a number of recent studies, which have reported positive and statistically significant associations between MP and several parameters of visual performance including: visual discomfort,[48] photophobia,[49] veiling glare[50] and photostress recovery.[50;51] The cited series of experimental analyses are consistent with the rationale whereby MP attenuates the effects of blue light, which is both valid and important. Fundamental methodological differences may, however, explain the differences between those reports and our observations.

Firstly, all the above studies employed a Maxwellian-view optical system to generate and present stimuli. While the rationale for doing so remains sound, in that it eliminates pupil diameter and pupil responses as a potential confounding factor, it is difficult to extrapolate their findings into a natural environment, outside of the laboratory, where changes in pupil diameter for example, are a natural consequence of the luminance changes typically observed on a daily basis, and may confer some level of protection against the deleterious effects of glare and excessive light stimulation. However, adoption of a natural pupil introduces other difficulties. Most importantly, the individual variation in pupil size, and the consequential variation in retinal illuminance, clouds the interpretation of MP's contribution to visual performance under glare conditions. It should therefore be conceded, that for a cross-sectional evaluation, the natural pupil

is less appropriate for a comprehensive evaluation of the role of MP, if any, in terms of its contribution to visual comfort and glare attenuation.

Secondly, the studies cited above invariably employed stimuli containing a strong short-wavelength blue light component. Again, there is an obvious rationale for doing so, as MP predominantly absorbs blue light. However, the concept of the environmental validity of such stimuli must again be questioned. Specifically, the most common light sources employed in industrial, commercial and home lighting systems typically contain significantly less blue light than those employed in cited studies. Tungsten and tungsten–halogen filament lighting systems, in fact, contain a minimal blue light component (see Figure 2.11). The absence of a strong blue light component in the photostress lamp, employed here, may partially explain the absence of any association between MP on PRT observed in our study. Our findings, therefore, in fact corroborate and extend the findings of Stringham *et al*.[52] and Stringham and Hammond[50] in that the associations between MP and glare are strongly wavelength dependent, and the influence of MP on glare disability is critically dependent on the spectral output of the source. It is worth noting, however, that the current trend for change to compact fluorescent and LED installations, which typically emit significantly more blue light (unpublished data from our laboratory suggests a twofold increase in blue light irradiance for compact fluorescent bulbs compared to tungsten), may render the role of MP for visual performance, if any, ever more important.

In conclusion, visual performance, as assessed by visual acuity and contrast sensitivity measures, appear to be weakly associated with MPOD. However, photostress recovery and visual performance under glare conditions were unrelated to this pigment. The lack of consistency between our findings and those of others possibly reflects the difficulties inherent in investigating the role of MP with respect to visual performance using a study of cross sectional design. Fundamental experimental design issues for visual performance evaluation must also be considered. There are no gold standard techniques, no means to accurately simulate the broad range of

environmental conditions experienced on a daily basis, so the selection of individual test parameters will influence both the results of the investigation, and any subsequent comparison with previous experimental results. The results of the current investigation should be interpreted with full appreciation of its design limitations, and conclusions should therefore be restricted to the specific testing conditions employed herein.

Visual acuity has been shown to relate to quality of life,[53] and is important in our highly visual society, where the demands for high quality visual resolution are constant. Contrast sensitivity correlates with various functional vision tasks such as mobility orientation, balance control, driving, face perception and reading performance,[54;55] and has been established as an important measure of visual function, which is related to quality of life.[54] These associations between MP and visual performance are likely to apply equally and possibly more substantially, in an older population, where, for example, the incidence of driving accidents and falls directly relate to visual performance.[55]

In summary, a P-controlled, randomised, L-based supplementation trial, designed to investigate if augmentation of MPOD enhances visual performance and/or comfort, is required to more adequately address this critical research question, and fully explore the proposed ‘‘optical” hypotheses of MP.

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-CHAPTER THREE-

STUDY TWO

The Impact of Macular Pigment Augmentation on Visual Performance in Normal Subjects: Longitudinal Investigation

3.1 INTRODUCTION

An average western diet contains about 1.3 to 3 mg/day of L and Z combined[1;2] with significantly more L than Z (represented by an estimated ratio of 7:1). Approximately 78% of dietary L and Z is sourced from vegetables.[3] L is found in highest concentrations in dark green leafy vegetables, such as spinach, kale, and collard greens.[3] Z is the major carotenoid found in orange peppers, and oranges, with a high mole percentage of both L and Z being found in egg yolk,[3] with comparable amounts of L and Z recently reported in corn and a variety of corn containing products (e.g. cornmeal and cereal).[4] Possible dietary sources of MZ include shrimp, certain marine fish, and turtles, none of which are found in a typical western diet,[5] however, it has recently been suggested that MZ may be present in some other, yet to be identified, foods.[6]

The macula is a specialised part of the retina, as it mediates central vision, provides sharpest visual acuity, and facilities best colour discrimination.[7] AMD is a disease of the macula and results in the loss of central and colour vision. AMD is the most common cause of blindness in the elderly population in the developed world.[8] It is now understood that oxidative stress,[9;10] exacerbated in part by cumulative short-wavelength visible light exposure,[11;12] is important in the aetiopathogenesis of AMD. MP is a short-wavelength (blue) light filter[13] and a powerful antioxidant,[14] and is therefore believed to protect against AMD.[15] This hypothesis, referred to as the "protective" hypothesis of MP, has been studied and reported on extensively.[15]

Beyond its "protective" hypothesis, MP's optical and anatomic properties have prompted the "optical" hypotheses of this pigment. The "optical" hypotheses of MP have been previously discussed by Reading *et al.*[16] and later by Nussbaum *et al.*[17] and include MP's putative ability to enhance visual performance and/or comfort by attenuation of the effects of chromatic aberration and light scatter, via its short wave light-filtering

properties.[18] This traditional description of the "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual performance, that are, perhaps, unrelated to the short wave filtration properties of MP. MP has been shown to exhibit dichroic properties[19] which may facilitate the reduction of glare disability through preferential absorption of polarised light. Higher MPOD has also been observed to relate to a trend towards lower root-mean-square wavefront aberrations (in particular, higher order aberrations), thereby enhancing visual performance.[20]

There is one additional, and important, mechanism, whereby MP may have a beneficial effect on visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons.

Many studies (to date mostly cross-sectional in design) have evaluated, and reported on the role of MP in visual performance, including: visual acuity; contrast sensitivity; glare disability; photostress recovery; CFF; temporal CSF (TCSF); colour vision; heterochromatic luminance contrast.[20-32] However, a P-controlled, randomised, L-based supplementation trial was needed to investigate if augmentation of MPOD actually enhances visual performance and/or comfort. This study was designed specifically to answer this important research question.

3.2 METHODS

3.2.1 SUBJECTS AND STUDY SITES

This study was conducted at WIT and DIT, vision science laboratories, located in the southeast and east of the Republic of Ireland, respectively. One hundred and twenty-one healthy subjects volunteered to participate in this two-centred study, which was approved by the research ethics committees at both study sites. Self-selected recruitment of subjects (WIT: n

= 61 and DIT: n = 60) was facilitated by poster and newsletter advertisement, and also by word of mouth, in the respective local communities. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

All subjects were aged between 18 to 41 years, in perfect general (self report) and ocular health (see below), and with visual acuity of at least 6/9 in the study eye, refractive error outside -6 D to +6 D. The study eye was selected on the basis of ocular dominance, determined using the Miles Test[33] with the dominant eye chosen as the study eye, except in cases of observed equidominance, in which case the right eye was selected.

A typical study visit lasted approximately four hours. Subjects were assessed at baseline, three, six, and 12 months (V1, V2, V3 and V4, respectively). All subjects recruited into the study were classed as naïve observers to the tests carried out (with the exception of the visual acuity test, with which all subjects were familiar). However, to optimise performance, and also to minimise any potential learning effects on performance, all subjects underwent a defined period of pre-test training. This training consisted of careful explanation of the nature of each test, pictorial and/or video demonstration of the test requirements and procedure, and was followed by a defined session of pre-test practice.

3.2.1.1 STUDY DESIGN AND FORMULATION

This is a registered trial on the ISRCTN database (number 35481392), and is a randomised, P-controlled clinical trial of oral supplementation with a formulation containing the macular carotenoids (L and Z) and co-antioxidants versus P. The tablets used in the current study were hard film coated tablets. The daily dose of two tablets for the A group consisted of 12 mg L, 1 mg Z (provided as ester), 120 mg vitamin C, 17.6 mg vitamin E, 10 mg zinc and 40 µg selenium. The P group consisted of cellulose, lactose and

magnesium stearate, and was manufactured to be identical to the A group preparation in terms of size and colour. The study tablets for the A and P groups were packaged into identical blister packs which contained the subjects' anonymised unique identification number and COMPASS study label information. Subjects were instructed to consume the daily dose of two tablets with a meal.

Compliance was assessed by tablet counting at each study visit, and encouraged by frequent reminder telephone calls and text messages by the study team. Compliance was also assessed at the end of the study by quantifying L and Z concentrations in serum, at each study visit, using HPLC.

3.2.2 DEMOGRAPHIC, MEDICAL, LIFESTYLE AND VISION CASE HISTORY QUESTIONNAIRES

For a detailed description of this method, please refer to *Section 2.2.2 - Demographic, Medical, Lifestyle and Vision Case History Questionnaires*

3.2.3 SPECTACLE REFRACTION, VISUAL ACUITY AND OCULAR DOMINANCE

For a detailed description of this method, please refer to *Section 2.2.3 - Spectacle Refraction, Visual Acuity, and Ocular Dominance*

3.2.4 GLARE DISABILITY

For a detailed description of this method, please refer to *Section 2.2.4 - Glare Disability*

3.2.5 CONTRAST SENSITIVITY FUNCTION

For a detailed description of this method, please refer to *Section 2.2.5 -*

Contrast Sensitivity Function

3.2.6 PHOTOSTRESS RECOVERY

For a detailed description of this method, please refer to *Section 2.2.6 – Photostress Recovery*

3.2.7 FUNDUS PHOTOGRAPHY

For a detailed description of this method, please refer to *Section 2.2.7 – Fundus Photography*

3.2.8 VISUAL FUNCTION IN NORMALS QUESTIONNAIRE

For a detailed description of this method, please refer to *Section 2.2.8 – Visual Function in Normals questionnaire*

3.2.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

For a detailed description of this method, please refer to *Section 2.2.9 – High Performance Liquid Chromatography*

3.2.10 FOOD FREQUENCY QUESTIONNAIRE

For a detailed description of this method, please refer to *Section 2.2.10 – Food Frequency Questionnaire*

3.2.11 MEASURMENT OF MACULAR PIGMENT OPTICAL DENSITY USING HETEROCHROMATIC FLICKER PHOTOMETRY

For a detailed description of this method, please refer to *Section 2.2.11 – Measurement of Macular Pigment Optical Density using Heterochromatic Flicker Photometry*

3.2.12 STATISTICAL ANALYSIS

The statistical software package PASW Statistics 17 (SPSS Inc, Chicago, Illinois) and the statistical programming language 'R' were used for analysis. It was determined at the outset of the study that a minimum sample size of 91 subjects was required in order to detect an effect size (correlation between two continuous variables) of 0.4 at the 5% level of significance with high power. However, 121 subjects were recruited into the study in order to allow for dropouts and for other possible analyses, in particular repeated measures analysis.

All continuous variables at baseline exhibited a typical normal distribution. Mean ± SDs are presented in the text and tables. Comparisons of A and P groups at baseline were conducted using independent samples *t*-tests and chi-square analysis, as appropriate.

We conducted repeated measures analysis of MPOD at each retinal eccentricity measured, for each of four study visits using a general linear model approach, with treatment (i.e. A and P) and smoking habits (non-smoker, past and current cigarette smoker) as between-subjects factors. Where appropriate we used the Greenhouse-Geisser correction for violation of sphericity. We used the 5% level of significance throughout our analysis, without adjustment for multiple testing.

Four visual performance variables (assessed subjectively by questionnaire) in this study were recorded as percentage change of V4 score compared to V1 score. Repeated measures analysis would not have been appropriate for these, and instead they were analysed using a general linear model with V4 percentage change as the dependent variable and fixed between-subjects factors treatment and smoking habits as explanatory variables.

3.3 RESULTS

3.3.1 BASELINE FINDINGS

The demographic, lifestyle, dietary and serum carotenoid concentrations, MPOD, and vision data of all 121 subjects recruited into the study, and divided by study arm (i.e. A or P group), are summarised in Table 3.1. As seen from this table, there was no significant difference between the A and P groups with respect to lifestyle, vision, and MP data, with the exception of a statistically significant difference between these groups for smoking habits (P = 0.046). Smoking status was therefore considered as a potential confounding variable and was controlled for throughout repeated measures analysis.

Table 3.1 Demographic, lifestyle, vision, and MP data at baseline visit.

Characteristic	**All** n***** = 121	**Active group** n = 61	**P group** n = 60	***P*-value**
Age	29 ± 7	29 ± 7	29 ± 6	0.864
Body mass index	26 ± 4	26 ± 4	25 ± 3	0.736
Best corrected visual acuity	113 ± 3	113 ± 3	112 ± 3	0.747
Macular pigment optical density				
0.25º	0.5 ± 0.19	0.49 ± 0.19	0.51 ± 0.20	0.458
0.5º	0.4 ± 0.17	0.39 ± 0.16	0.41 ± 0.18	0.425
1º	0.22 ± 0.13	0.20 ± 0.12	0.22 ± 0.15	0.433
1.75º	0.10 ± 0.11	0.09 ± 0.10	0.10 ± 0.11	0.376
3º	0.10 ± 0.10	0.08 ± 0.08	0.12 ± 0.12	0.058
Dietary carotenoids (mg/day)				
Lutein	1.26 ± 0.95	1.16 ± 0.96	1.36 ± 0.94	0.253
Zeaxanthin	0.21 ± 0.12	0.19 ± 0.10	0.23 ± 0.14	0.074
Serum carotenoids (µmol/L)				
Lutein	0.60 ± 0.32	0.57 ± 0.27	0.62 ± 0.36	0.399
Zeaxanthin	0.36 ± 0.17	0.36 ± 0.15	0.37 ± 0.18	0.623
Sex				
Male	69	34	35	
Female	52	27	25	0.773
Smoking habits†				
Never smoked	73	42	31	
Ex-smoker	21	11	10	
Current smoker	27	8	19	0.046

* n = sample size

† Smoking habits: ex-smoker = smoked ≥100 cigarettes in lifetime but none in last 12 months; current smoker = smoked ≥100 cigarettes in lifetime and at least 1 cigarette per week in last 12 months

3.3.2 LONGITUDINAL FINDINGS

3.3.2.1 SUPPLEMENT COMPLIANCE

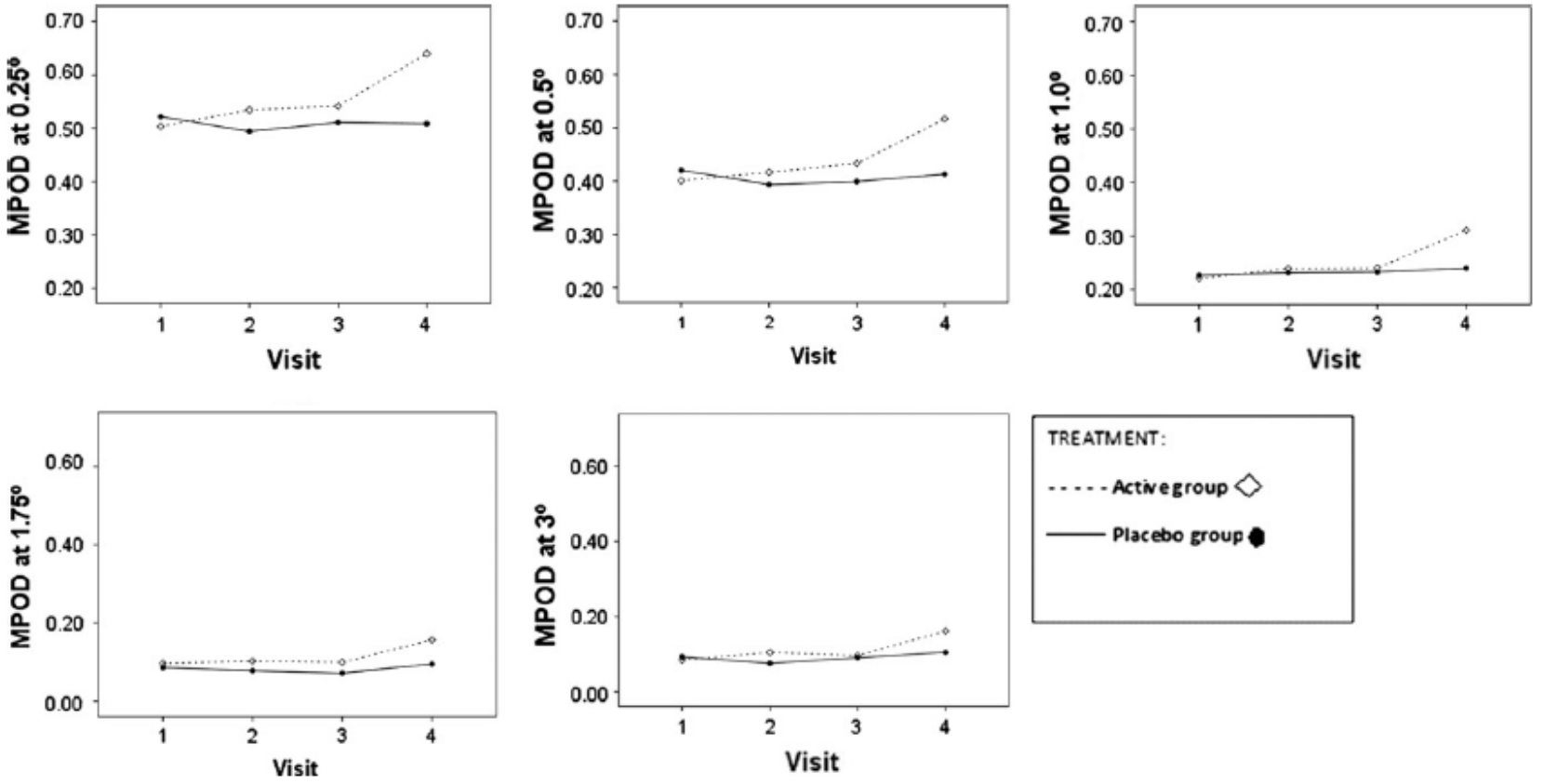
Seventy-six subjects returned tablets, and (based on the number of tablets returned) 94.7% of these subjects averaged at least one tablet per day. The average number of tablets per day was 1.57 in the A group and 1.65 in the P group, a difference that is not statistically significant (analysis of variance

(ANOVA), P = 0.32). In comparing change in MPOD and visual performance variables between A and P groups, therefore, it was not deemed necessary to control for differences in compliance in the two groups.

3.3.2.2 MACULAR PIGMENT OPTICAL DENSITY

We conducted repeated measures ANOVA of MPOD, for all retinal eccentricities measured (i.e. at 0.25º, 0.5º, 1.0º, 1.75º, and 3º), over time (i.e. over the study period [at V1, V2, V3, and V4, respectively]), using a general linear model approach, with two between-subjects factors: treatment (A, P) and smoking habits (never, past, current smoker). As seen in Figure 3.1, there was a trend (in the A group) towards an increase in MPOD at all eccentricities measured, but this increase was only statistically significant (at the 5% level) at the more central measured eccentricities (i.e. at 0.25º, 0.5º and 1.75º).

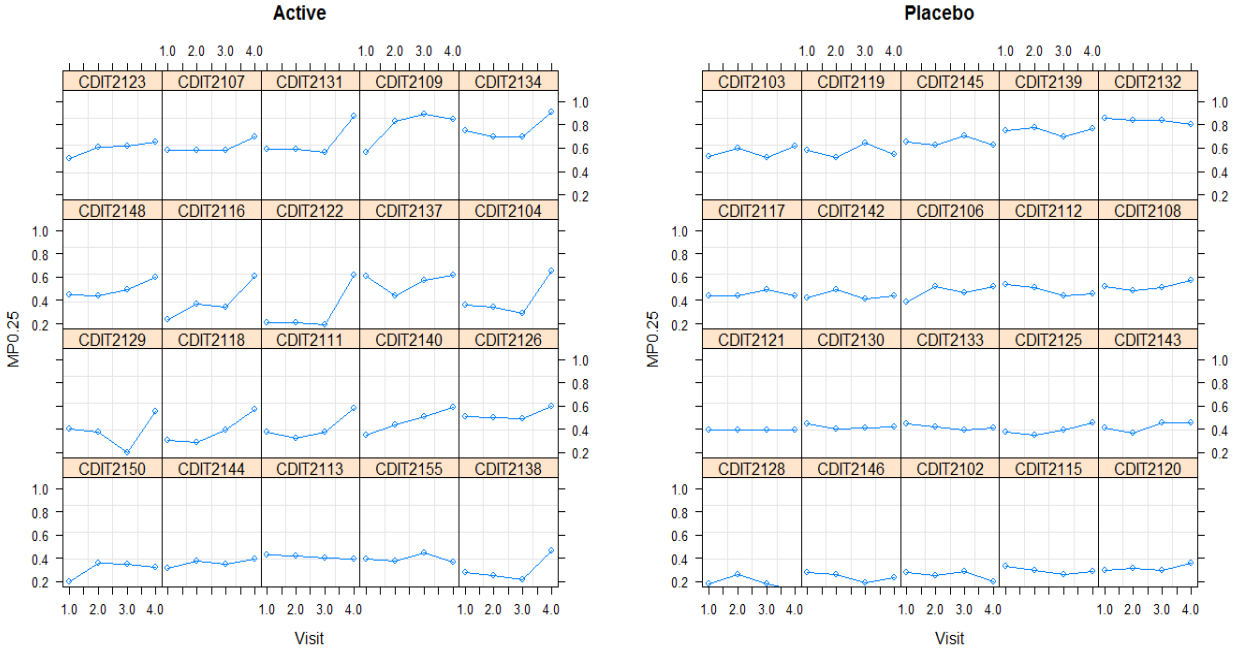
Figure 3.1 Change in MPOD at each retinal eccentricity measured, over the twelve month study period, following supplementation in both the A and P groups.



Repeated measure results for MPOD over the four study visits and analysing visit*treatment interaction at eccentricities 0.25º, 0.5º, 1.0º, 1.75º and 3º. The *P*-values reported are for the Greenhouse-Geisser correction for violation of sphericity and are as follows: MPOD 0.25º = $P < 0.001$; MPOD 0.5º = $P < 0.001$; MPOD 1.0º = 0.001; MPOD 1.75º = 0.585; MPOD 3.0º = 0.103. Subjects were assessed at baseline, three, six, and 12 months (V1, V2, V3 and V4, respectively).

Figure 3.2 (obtained from R statistical program) shows MPOD variation at 0.25º for 20 consecutive individual subjects from each of the A and P groups. The graphs are arranged so that those with lowest MP are in the bottom row, and only subjects who presented for all four visits are displayed.

Figure 3.2 Change in MPOD at 0.25º of retinal eccentricity for 20 subjects from each of A and P groups.



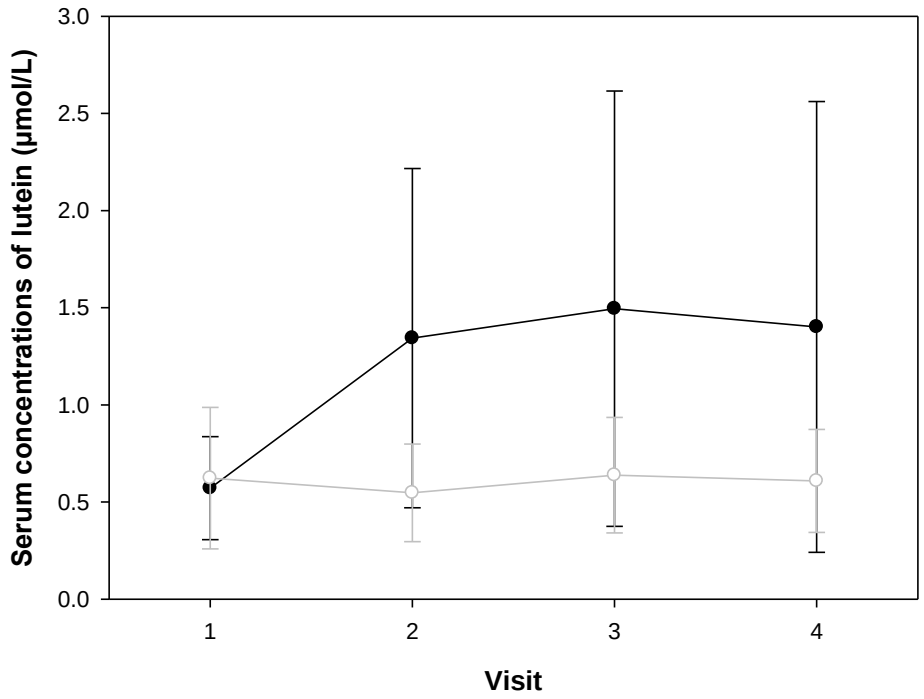
* MP 0.25° = macular pigment optical density at 0.25° of retinal eccentricity.

3.3.2.3 SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN

We conducted repeated measures analysis of serum concentrations of L and Z over time (i.e. over the study period) including all study visits (V1, V2, V3 and V4), using a general linear model approach, with treatment and cigarette smoking as between-subjects factors. As seen in Figure 3.3, there

was a statistically significant time/treatment interaction effect for serum concentrations of L, which remained significant ($P < 0.001$, for all) using any of the standard corrections for violation of sphericity. It is clear from the mean plots of Figure 3.3, how these significant time/treatment interaction effects came about: serum concentrations of L increased with time in the A group, but remained virtually static in the P group. This time/treatment effect was significant from V2 (as expected and confirmed using paired t-test analysis between V1 and V2, $P < 0.001$). There was no statistically significant time or time/treatment interaction effect for serum concentrations of Z over the study period ($P > 0.05$, for all tests); however, there was a trend towards an increase in the A group.

Figure 3.3 Change in serum concentrations of L over the twelve month study period, following supplementation in both the A and P groups



Mean (±SD) serum concentrations of L were quantified by HPLC at baseline, three, six, and 12 months (V1, V2, V3 and V4, respectively).

3.3.2.4 VISUAL PERFORMANCE

While the repeated measures ANOVA presented above is based on findings at all four study visits, it is apparent from the graphs (Figure 3.1 and Figure 3.2) that the largest differences in MPOD between A and P subjects are between V1 and V4. The analysis of visual performance variables which follows is, therefore, confined to V1 and V4 only (controlling for between-subjects factors: treatment and smoking habits).

Using repeated measures ANOVA or a general linear model, as appropriate, we report a statistically significant time/treatment effect in only one measure of visual performance, namely "daily tasks comparative analysis" assessed subjectively (P = 0.03); whereas all other measures of visual performance were statistically non-significant (P > 0.05, for all) [Table 3.2].

Table 3.2 Repeated measures assessment of all visual performance measures in study two.

Visual Performance Measure	Sub-Measure/Device	p-value
Glare disability	Medium glare (Functional Vision Analyzer™)	
	1.5 cpd	0.58
	3.0 cpd	0.94
	6.0 cpd	0.65
	12.0 cpd	0.96
	18.0 cpd	0.49
Glare disability	High glare (Functional Vision Analyzer™)	
	1.5 cpd	0.19
	3.0 cpd	0.99
	6.0 cpd	0.89
	12.0 cpd	0.41
	18.0 cpd	0.86

Glare questionnaire	Glare comparative analysis	0.32
	Glare change Analysis	0.88
	Glare situational analysis	0.74
	Glare subject satisfaction score	0.51
Visual acuity	BCVA (Test Chart 2000 Pro)	0.16
Visual acuity questionnaire	Acuity comparative analysis	0.08
	Acuity change analysis	0.15
	Acuity situational analysis	0.14
	Acuity subject satisfaction score	0.59
Daily tasks questionnaire	Daily tasks comparative analysis	0.03*
	Daily tasks change analysis	0.21
	Daily tasks situational analysis	0.27
	Daily tasks subject satisfaction score	0.41
Light/dark adaptation questionnaire	Light/dark comparative analysis	0.35
	Light/dark change analysis	0.15
	Light/dark situational analysis	0.75
	Light/dark subject satisfaction score	0.56
Mesopic contrast sensitivity	FACT™ (Functional Vision Analyzer™)	
	1.5 cpd	0.72
	3.0 cpd	0.77
	6.0 cpd	0.84
	12.0 cpd	0.66
	18.0 cpd	0.5
Mesopic contrast sensitivity	Metropsis	
	1.0 cpd	0.54
	4.1 cpd	0.79
	7.5 cpd	0.82
	11.8 cpd	0.18
	20.7 cpd	0.08
Photopic contrast sensitivity	Metropsis	
	1.0 cpd	0.95
	4.1 cpd	0.42
	7.5 cpd	0.31
	11.8 cpd	0.19
	20.7 cpd	0.87
Critical flicker fusion frequency	Macular Densitometer™	0.3

Foveal sensitivity	Humphrey® perimeter	0.93

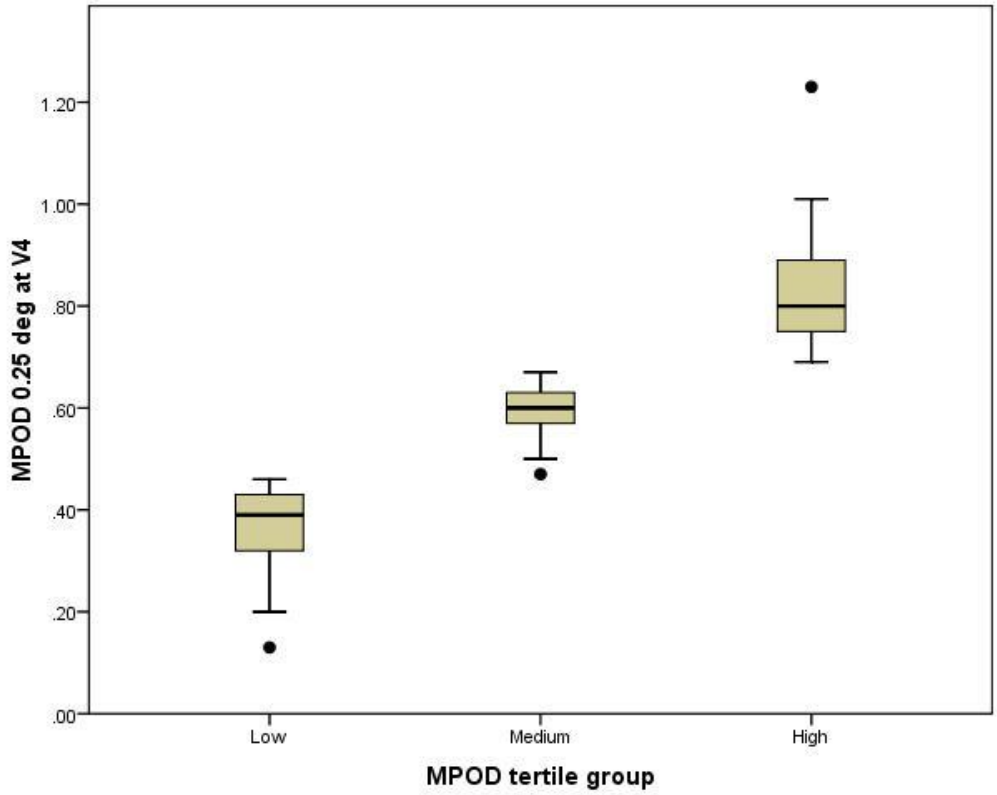
* Significant at the 0.05 level

Four visual performance variables in this study were recorded as percentage change of V4 score compared to V1 score. Repeated measures analysis would not have been appropriate for these, and instead they were analysed using a general linear model with V4 percentage change as the dependent variable and fixed between-subjects factors Treatment and Smoking as explanatory variables.

3.3.2.5 VISUAL PERFORMANCE DIFFERENCES: LOW MACULAR PIGMENT OPTICAL DENSITY SUBJECTS VERSUS HIGH MACULAR PIGMENT OPTICAL DENSITY SUBJECTS

We investigated whether subjects with high MPOD had significantly better visual performance scores than subjects with low MPOD following supplementation. We based this investigation, for the most part, on MPOD at 0.25º (peak MPOD) at V4. We used tertiles for V4 MPOD at 0.25º of retinal eccentricity to create low, medium and high MPOD groups, and then compared the low and high groups on a variety of visual performance measures assessed. The low group consisted of 31 subjects with V4 MPOD at or below 0.46 optical density and the high MPOD group had 29 subjects with V4 MPOD at or above 0.69 optical density [Figure 3.4]. Table 3.3 presents results for visual performance measures which differ significantly between these low and high MPOD groups. Table 3.3 also presents the corresponding results for V1. It should be noted that differences in these visual performance measures at V1 were not, in general, statistically significant.

Figure 3.4 Boxplots of V4 MPOD at 0.25º of retinal eccentricity showing range of values for each tertile group.



* MPOD 0.25° at visit 4 = macular pigment optical density at 0.25° of retinal eccentricity at visit four (12-months) presented for each tertile boxplot. Low, medium and high boxplots represent low tertile group, medium tertile group and high tertile groups with respect to MPOD measured at 0.25° of retinal eccentricity. Black dots represent extreme values (outliers).

Table 3.3 Comparing visual performance measures between low and high MPOD groups at visit 4 and visit 1

		Visit 4			Visit 1	
Visual Performance Variable	**MP Group***	**Mean (±SD)**	***P*-value**	**MP Group**	**Mean (±SD)**	***P*-value**
Best corrected visual acuity	High	113 (±3)		High	113 (±3)	
	Low	111 (±4)	0.038	Low	112 (±3)	0.045
Mesopic contrast sensitivity at 1.5 cpd under high glare†	High	28.6 (±15.8)		High	22.1 (±11.6)	
	Low	21.2 (±11.2)	0.042	Low	19.8 (±8.2)	0.337
Light/dark adaptation comparative analysis‡	High	70.3 (±17.4)		High	62.2 (±13.2)	
	Low	60.6 (±13.1)	0.018	Low	60.6 (±14.7)	0.624
Mesopic contrast at 20.7 cpd§	High	54.7 (±17.4)		High	57.1 (±15)	
	Low	62.9 (±10.9)	0.035	Low	59.2 (±12.1)	0.523

* Macular pigment optical density group tertile for 0.25º of retinal eccentricity: high = top tertile, low = bottom tertile

† Night-time contrast sensitivity at low spatial frequencies assessed under high glare conditions

‡ Self reported visual performance under changing light conditions compared to friends/family/peers

§ Night time contrast sensitivity measured at high spatial frequencies

3.4 DISCUSSION

This study was a randomised, P-controlled clinical trial of oral supplementation with a formulation containing the macular carotenoids (L and Z) and co-antioxidants versus P in young normal subjects. The pre-specified hypothesis was that supplementation, and consequential MPOD augmentation, would result in improved visual performance and/or comfort in those randomised to the A arm when compared with the P arm, by 12 months.

This study was designed to investigate whether augmentation of MP results in enhancement of visual performance and/or experience, regardless of the mechanism(s) whereby any such improvements may be realised. The optical and neuroprotective hypotheses around MP, which have been discussed previously by Reading & Weale[16], later by Nussbaum *et al*.[17] and are extended here, have generated interest among MP scientists, evident in a recent review.[34] In brief, some authors have suggested that MP may be important for visual performance and/or experience by at least one of a number of mechanisms, including the reduction of the effects of chromatic aberration, light scatter, higher order aberrations, and plane polarisation of light.[18;34] Importantly, however, and in theory at least, the macular carotenoids have the capacity to confer these optical advantages because of their light-filtering and dichroic properties and because of their central location within the retina and crystalline lens.

An additional consideration in relation to any trial investigating the impact of MP augmentation on visual performance and experience is the potential beneficial effect of MP on neurophysiological health. For example, the majority of studies investigating the effects of MP augmentation in ocular disease, including AMD (summarised by Loughman *et al*.),[34] have reported a beneficial effect on vision, and such findings are probably attributable to the neuroprotective, as opposed to the optical, properties of these intracellular

compounds. These studies have traditionally employed basic psychophysical outcome measures, including visual acuity and contrast sensitivity, and as such have not included stimuli likely to reveal improvements facilitated solely by image enhancement attributable to the optical properties of this pigment.

The study formulation used in the present study, in addition to L and Z, contained the co-antioxidants vitamin C, vitamin E, zinc and selenium. In contrast to the capacity to measure subjects' retinal response to supplementation with the macular carotenoids (i.e. by measuring MP) it was not possible to assess, or quantify, subjects' response to supplementation with the above named co-antioxidants. It is important to note that, as seen in the age-related eye disease study (AREDS),[35] that these antioxidants may have contributed to any benefits reported in visual performance in the present study.

Interestingly, several studies have reported, among normal subjects, findings which suggest that MP may play a key role in visual health through a complex interplay between the optical, neurological and physiological mechanisms underlying vision. These observations include (a) better CFF in the presence of higher MPOD[28], (b) associations between high MPOD and crystalline lens transparency and cataract formation[36-38], (c) the presence of L and Z in substantial concentrations in the primary visual cortex[39] and (d) higher PERG P50 amplitudes and better dark adapted cone sensitivities in association with higher MPOD[40].

The randomised design of the present study resulted in desirable baseline similarity between A and P groups on possible confounding variables, with the exception of smoking habits (which was controlled for throughout analysis, as appropriate). Significant efforts were made to encourage compliance during the study, and based on the number of tablets returned, we calculated that 95% of subjects averaged at least one tablet per day, with the

average number of tablets consumed per day statistically comparable between the A and P groups (at around 1.6 tablets per day).

Consistent with the positive tablet compliance, on average, serum L concentrations increased significantly over the course of the study in the A group with no significant change observed in the P group. Indeed, despite the slight drop in mean serum L concentrations between V3 and V4 in the A group, L concentrations more than doubled in the A group over the course of the study. This finding is consistent with other and recent L interventional studies.[41;42] However, while average serum L concentrations significantly increased in the A group and remained stable in the P group, it is important to point out that 9 (23%) of the A group shown negative or zero change in serum L concentrations. This "non-response" to L supplementation in serum is consistent with an observation by Hammond *et al*. in 1997 who reported that one subject (out of 11 measured) demonstrated no significant change in serum concentrations of L following consumption of ~12 mg of L per day over a 15 week study period (albeit L consumption in that study was achieved from diet [e.g. spinach and corn] and not from dietary supplements [as in the current study]).[47] To explain the high percentage of serum non-response in the current study, we propose the following possibilities: non-compliance with respect to consumption of the study tablet in these subjects: possible attenuation of the gastrointestinal absorption of supplemental L and Z if the subject fails to take the study tablet in the presence of synchronously ingested fat or oil (importantly, subjects were instructed to consume the daily dose of two tablets with a meal to facilitate the bioavailability of L from the tablet)**.** Indeed, it has been shown that the amount of fat in a person's diet significantly affects the absorption of L ester and its bioavailability, and given that the tablet used in the current study was a film coated tablet not containing oil, failure to consume the study formulation in the presence of fat and/or oil (i.e. with a meal) could significantly impact on the bioavailability of L[43] Mean serum concentrations of Z also increased in the A group, but the increase was not statistically

significant, probably due to the low concentration of this carotenoid in the study formulation (~1 mg/day).

Central MPOD increased significantly in the A group over the 12-month study period and remained stable in the P group. However, the observed increase in central MPOD in the A group only became apparent (significantly) at 12 months (whereas, as seen above, serum concentrations of L were significantly augmented in the A group at three months). This finding is consistent with previously published studies reporting slow uptake of L by the retina,[44;45] and inconsistent with others.[6] However, it should be noted that the retinal uptake in our study was much slower than any of these previously published studies. For example, Bone *et al*. (2003)[44] report that no significant change in MP was seen until after day 40 following supplementation with L and Z with up to 30 mg/day of each carotenoid and Johnson *et al*. (2000)[45] report a significant increase in MP after 4 weeks of consuming 60 g/day spinach and 150 g/day corn. However, the reason(s) for the difference seen between studies may be due to any (or a combination) of the following factors: dose of L and Z consumed per day; type of L and Z in the supplement (e.g. free versus ester) matrix in which carotenoids are consumed (e.g. oil versus micro-encapsulated); whether consumed alone or in the presence of other antioxidants; poor serum response to the supplement; non-compliance to the study supplement. Further, and detailed, study on this interesting topic is merited.

The average increase seen in the A group at 0.5º of retinal eccentricity (the standard and most commonly measured and reported MPOD eccentricity) over the 12-month study period was 0.11 ± 0.005 optical density, which is comparable to the findings of Trieschmann *et al*. (2007)[42] who reported an average increase in MP of 0.10 ± 0.009 optical density where they measured MPOD by 2-wavelength autofluorescence. Interestingly, Trieschmann *et al*. used the same study formulation (daily consumption of 12 mg of L provided as ester) over a 12-month study period as that used in the current study, but by

delivering four tablets per day (each containing 3 mg of L ester), whereas the current study achieved a daily consumption of 12 mg of L ester by delivering two tablets per day. Unlike the findings reported by Trieschmann *et al*. we report that the biggest gain in MPOD in the A group did not, in general, occur in subjects with lowest baseline MPOD values. However, consistent with the data reported by Trieschmann *et al*. who reported that 20 (21%) of 92 subjects assessed were retinal non-responders (at 0.5 º), we found that eight (17%) of the A group at 0.25º and nine (20%) of the A group at 0.5º shown negative or zero change in MP at 12 months.

In contrast with the MP measures discussed above, the visual performance measures assessed in the present study did not, in general, improve significantly over time in the A group. This would, superficially at least, seem to be at odds with the optical and visual health hypotheses of MP's function. Indeed, it is important to emphasise that, of all the visual performance measures assessed, and reported on, in the present study (48 variables in total; see Table 3.2) we report a statistically significant result for only one measure, namely "daily tasks comparative analysis", assessed subjectively. It is possible, therefore, as data from the current study suggest, that supplementation with the macular carotenoids, and consequential MP augmentation, has no major impact on visual performance and/or experience in young normal subjects (our primary research question and the main study hypothesis). This is, however, at odds with previous reports with respect to the impact of MPOD augmentation on glare disability.[21;23] This discrepancy with earlier findings may be explained, at least partly, by two fundamental differences between the relevant studies. Firstly, the present study was designed to evaluate glare disability under conditions approximating normal environmental experience. As such, testing was conducted using natural pupils, which typically constrict under glare conditions, and therefore confer protection against the effects of glare. The Maxwellian view system employed in other studies does not allow normal pupillary response, so, while MP was shown to impact glare disability under

these conditions, it is not clear whether the effect would have remained if a pupillary response had been allowed, which would have caused a variable reduction in retinal illuminance proportional to the magnitude of the pupillary response. Secondly, our findings can only be applied to the stimulus and glare intensity settings employed here, which, although informed by a detailed pilot study, are less comprehensive than the variable glare annulus intensity employed by Stringham & Hammond (2007 & 2008).[21;23]

Kvansakul *et al*. conducted a study to evaluate the effect of MP supplementation on mesopic CATs in normal subjects.[20] They reported a significant and beneficial effect of MP supplementation on mesopic CAT that was not evident in their P group, their findings therefore appearing to be at odds with those of the present study, probably reflecting a number of differences between the two studies in terms of methodology and design [e.g. stimuli, illumination levels (1 cd m^{-2} vs 3 cd m^{-2}), etc]. Also, the design by Kvansakul *et al*. did not incorporate longitudinal evaluation of MPOD, which was measured only at the final visit (interestingly the CATs reported by Kvansakul *et al*. shown no correlation with MPOD). Furthermore, CATs were not measured at baseline, but only after six months of supplementation and then again at the final 12 month visit. One cannot, therefore, draw meaningful conclusions with respect to the relationship, if any, between their mesopic CAT findings and MPOD, as there is no record of change in MPOD over their study period. A final point relates to the sample sizes of the two studies, the investigation by Kvansakul *et al*. being based on a P group of only five subjects and three groups of subjects receiving supplementation (containing three, five and five subjects respectively) and is thus not comparable with the present trial, involving 121 subjects.

There are however, a number of plausible explanations for the absence of any significant influence of MP augmentation on visual performance in our study. Firstly, it should be noted that the majority of study participants

exhibited average to high central MPOD pre-supplementation. Indeed, only a small number of subjects (~24%) were found to have central MPOD (at 0.5º of retinal eccentricity) less than 0.30 at baseline. Importantly, it has been suggested previously that MPOD levels greater than 0.30 might be superfluous to visual performance requirements,[16] due to the non-linear nature of the effect of MP on vision. Furthermore, the increase in MPOD observed in the A group did not become apparent until the final 12 month visit, and was relatively modest with an average increase of 0.11 ± 0.005 optical density (at 0.5º of retinal eccentricity), and unlike the findings reported by Trieschmann *et al.* (2007)[42] subjects (in the A group) in the current study with the lowest MP at baseline did not, in general, demonstrate the biggest increase in MPOD levels following supplementation with the study formulation. Indeed, even after 12-months of supplementation with 12 mg of L per day, over 15% of subjects in the A group retained central MPOD (at 0.5º of retinal eccentricity) values below 0.3 optical density. In other words, it is possible that the MP augmentation achieved in the present study was not sufficient (in an adequate number of subjects) to impact on visual performance, and that a greater increase in MPOD, particularly in the group with lowest baseline MPOD, might be required to elicit an improvement in visual performance. Also, as mentioned above, it is also likely that a significant number of subjects in the present study already had (at baseline) sufficient MP for optimal, measurable, and appreciable visual performance (i.e. 75% of subjects in the A group had baseline MP values ≥0.3 optical density) and therefore may explain, at least in part, the failure of the present study to demonstrate an improvement in visual performance following supplemental L.

In addition, the nature of the tests employed for visual performance testing in the present study also merits consideration and discussion. The investigators strategically chose to use tests that were either typically available in the average consulting room (to ensure applicability of findings to clinical practice), or designed to replicate typical environmental conditions. As such,

most of the tests did not contain substantial amounts of short wavelength light maximally absorbed by MP. The typical office or home environment (where the majority of us spend most of our time), does not have many short wave dominated light sources. Our results might, therefore, suggest that subjects' MP levels pre-supplementation were sufficient for optimal visual performance in this type of environment. Our results, therefore, cannot be extrapolated to short wave dominated visual scenes, such as against the background of a bright blue sky, which is difficult to replicate in an ecologically valid way. Importantly, the changing nature of internal and device lighting systems, such as the increased use of LED systems, and xenon car headlights, are extending our exposure to short wave light sources, and may enhance the applicable relevance of MP for visual performance.

However, given that our study subjects shown an extensive range of MP values, we considered it meaningful to compare visual performance and comfort measures for subjects with high MP (upper tertile) versus subjects with low MP (lower tertile). We made these comparisons at baseline and also at V4. At V1, the subjects in the low MP group (for central MP at 0.25º) were below 0.42 optical density, whereas subjects in the high MP group (for central MP at 0.25º) were above 0.59 optical density. At V4, the corresponding figures for low and high groups were 0.46 and 0.67 optical density. Supplementation with L, therefore, appears to have widened the gap in MP between the lower and upper tertiles. Of interest, at V4 we report statistically significant differences in some important visual performance measures, between lower and upper MP tertile groups, which were not present at V1.

The most significant finding is that of a ~30% greater contrast sensitivity under high glare conditions in those with highest MPOD following supplementation. Interestingly, of all the tests employed in the present study, the glare source contained the most substantial amount of short wave light (white LEDs used to generate glare contain a single "blue" peak around 460

nm). These results therefore would seem to corroborate previous findings which suggest a role for MP in the attenuation of glare disability,[21-23] and furthermore would seem to extend those findings to suggest that MP augmentation is beneficial for visual performance under glare conditions, even under the natural pupil conditions employed here. This finding and hypothesis is also supported by the results of the visual performance questionnaire. Subjects in the A group reported comparatively, and statistically significantly, better visual performance for daily visual tasks (including night driving against oncoming headlights). Furthermore, in the tertile analysis, those with the highest MP reported comparatively, and statistically significantly, better, capacity to deal with sudden changes in illumination (light/dark adaptation).

In conclusion, we report that a significant increase in central MP following L supplementation does not, in general, impact on visual performance in young normal subjects, and our pre-specified hypothesis that MP augmentation would result in improved visual performance and/or comfort by 12 months, in those randomised to the A arm, remains unproven. However, subjects with high MP following L supplementation demonstrate visual benefits with respect to glare disability and mesopic contrast sensitivity. Further study into MP and its relationship with visual performance is warranted to enhance our understanding of this pigment's role. However, in order to investigate the impact of MP augmentation on visual performance, the findings of our study suggest that we should direct our attention to a) subjects with low baseline central MP levels, b) subjects with suboptimal visual performance and c) subjects with symptoms of glare disability.

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-CHAPTER FOUR-

STUDY THREE

Macular Pigment and its Associations with Colour Discrimination and Matching:

Cross-Sectional Investigation

4.1 INTRODUCTION

As outlined previously, hue discrimination and colour vision in general are most acute at the fovea[1] corresponding to increased cone density, specialised anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good colour vision is possible beyond the fovea).[2] It is plausible that colour discrimination at a small angular subtense would be influenced by the optical density of MP at the fovea. Indeed it has long been speculated that inter-observer differences in colour matching by colour-normal observers are at least partially due to differences in macular pigmentation.[3;4] Also it is known that even subjects with ophthalmoscopically-normal fundi exhibit substantial variations in MPOD, contributing to a range of pre-receptorial light absorption at 460 nm from 3% to almost 100%.[5]

Since the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 458 nm,[6;24] it would seem likely that these pigments influence colour vision through selective absorption of short wavelengths, thereby influencing the short wave sensitive cones and the blue-yellow opponent-colour channel.

There is no consensus in the literature on the relationships, if any, between MPOD and colour vision parameters on the one hand, and mechanisms on the other hand. This may or may not simply reflect the innate differences between, for example, spectral sensitivity measurements of the isolated short wave sensitive cone mechanism and the overarching hue discrimination function at short wavelengths. It is also necessary to distinguish between the effects on colour vision (mechanisms, sensitivity or appearance) of (1) distribution of MP across the retina, and (2) variation of MPOD between subjects at a given retinal locus.

The objective of the present study was to evaluate, in a cross sectional manner, the associations between colour variables and MPOD, using a much larger sample of subjects than in most previous studies and a battery of colour assessments rather than relying on a single method of quantification.

4.2 METHODS

4.2.1 SUBJECTS AND STUDY SITES

One hundred and two subjects volunteered to participate in this study, which was approved by the research ethics committees at both WIT and DIT. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki. The study was conducted at WIT and DIT vision science laboratories, located in the southeast and east of the Republic of Ireland, respectively. Self-selected recruitment of subjects was facilitated by poster and newsletter advertisement, and also by word of mouth, in the respective local communities.

All subjects were aged between 18 and 41 years, in perfect general (self report) and ocular health. The exclusion criteria comprised: any ocular pathology (including abnormal macula appearance or cataract); BCVA <6/9 in the study eye; refractive error outside -6 D to +6 D; and defective colour vision. The eye with high BCVA was chosen as the study eye, except in cases of observed equal BCVA, in which case the right eye was selected. Full colour vision data were available for 84 subjects. All tests were conducted with the subject's optimal subjective refraction in place.

4.2.2 DEMOGRAPHIC, MEDICAL, LIFESTYLE AND VISION CASE HISTORY QUESTIONNAIRES

For a detailed description of this method, please refer to *Section 2.2.2 - Demographic, Medical, Lifestyle and Vision Case History Questionnaires*

4.2.3 SPECTACLE REFRACTION AND VISUAL ACUITY

For a detailed description of this method, please refer to *Section 2.2.3 - Spectacle Refraction, Visual Acuity.*

4.2.4 FARNSWORTH-MUNSELL 100 HUE TEST

The FM100 (X-Rite UK, Poynton) [Figure 4.1] was administered under colour-corrected fluorescent lighting supplied by a pair of 15W 46 cm lamps (The Daylight Co., London, UK) providing minimum luminance of 94 cd m^{-2} reflected from each colour sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was 12 cd m^{-2}. Colour temperature is rated at 6400° K. Subjects were allowed to review the arrangement in each tray if they so requested.

Individual error scores and total error scores (TES), summed across the visible spectrum and purple hues, were determined using the software supplied by the manufacturer. Partial error scores (PES) were used to assess hue discrimination specifically among blue and cyan hues using samples 50 to 68 and 36 to 54 respectively and were divided by TES to obtain percentage values (%PES). A typical FM100 test report is shown below [Figure 4.2]. Detailed procedure for the FM100 is given in the SOP for this test, provided in Appendix 7.13.

Figure 4.1 The Farnsworth-Munsell 100 hue test.

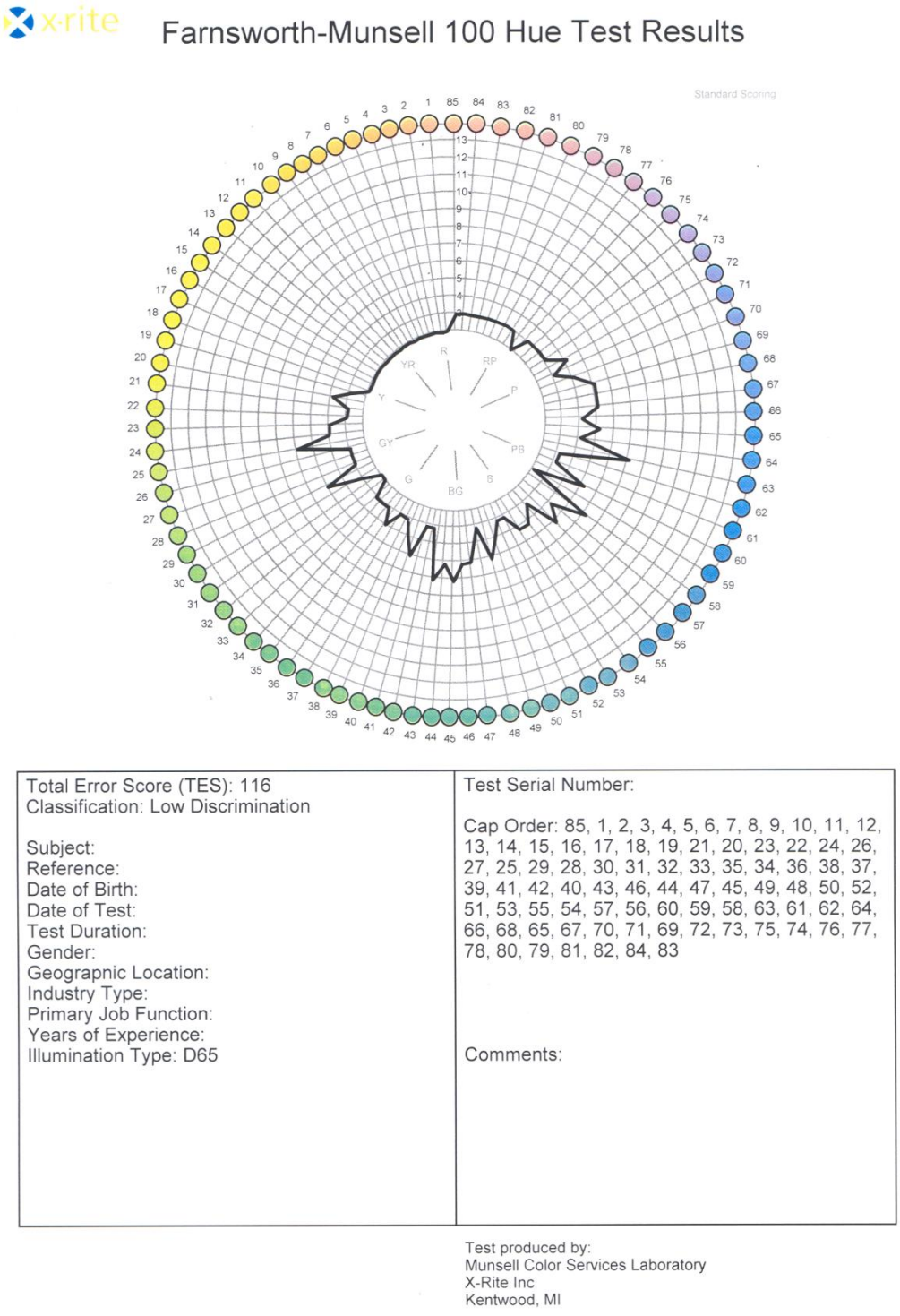

Figure 4.2 A typical test report of the Farnsworth-Munsell 100 hue test.

4.2.5 HEIDELBERG MULTI COLOUR ANOMALOSCOPE

This test was administered using the Moreland match on an HMC (Heidelberg Multi Colour) MR Anomaloscope (type 7700: Oculus, Wetzlar, Germany) [Figure 4.3]. This provides a 2° field within which 436 and 490 nm sources are matched to a mixture of 480 and 589 nm, the latter mixture providing a brightness match. Control of stimuli and calculation of blue/green mixture were achieved with the Anomaloscope under computer control using the manufacturer's software. Neutral pre-adaption was not used as this was found to produce transient adaptation effects on stimulus saturation. Stimuli were presented under continuous viewing mode. Following practice, subjects toggled the mixture to obtain 4 matches, 2 each with the mixture preset to blue bias and green bias. The mean of 4 blue/green matches was calculated for each subject to obtain the midpoint. A typical test report of Moreland match on the HMC Anomaloscope is shown below [Figure 4.4]. Detailed procedure for operation of the HMC Anomaloscope is given in the SOP for this instrument, provided in Appendix 7.14.

Figure 4.3 The HMC Anomaloscope.

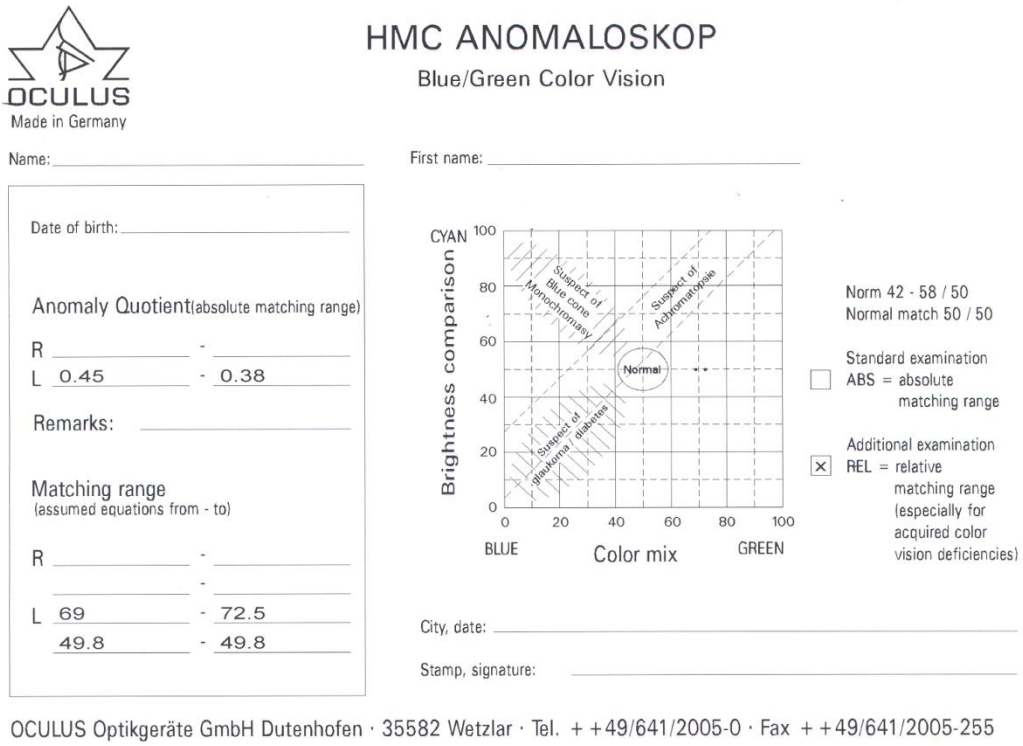

Figure 4.4 A typical test report of Moreland match on the HMC Anomaloscope.

4.2.6 CUSTOMISED SHORT WAVELENGTH AUTOMATED PERIMETRY

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard short wavelength automated perimetry (SWAP) routine on a Humphrey® field analyzer (Model 745*i* Carl Zeiss Meditec Inc. Dublin, CA, USA) [Figure 4.5]. Yellow (530nm) background luminance was 100 cd m^{-2}. Size V targets of 440 nm and 200msec duration subtending 1.7° at the eye were presented at 0°, 1°, 2°, 3°, 4° and 5° of retinal eccentricity from a fixation target. The number of targets at each retinal eccentricity beyond the foveal centre varied from 4 to 20. On each presentation, a single target was

presented. Increment thresholds were obtained using the SWAP adaptive staircase full thresholding technique. Subjects were given 3 minutes to adapt to the background before testing began. Sensitivity for each retinal eccentricity was the mean of values for all targets in the group at that retinal eccentricity. A typical test report of customised SWAP (cSWAP) is shown below [Figure 4.6]. Detailed procedure of cSWAP is given in the SOP for this test, provided in Appendix 7.16.

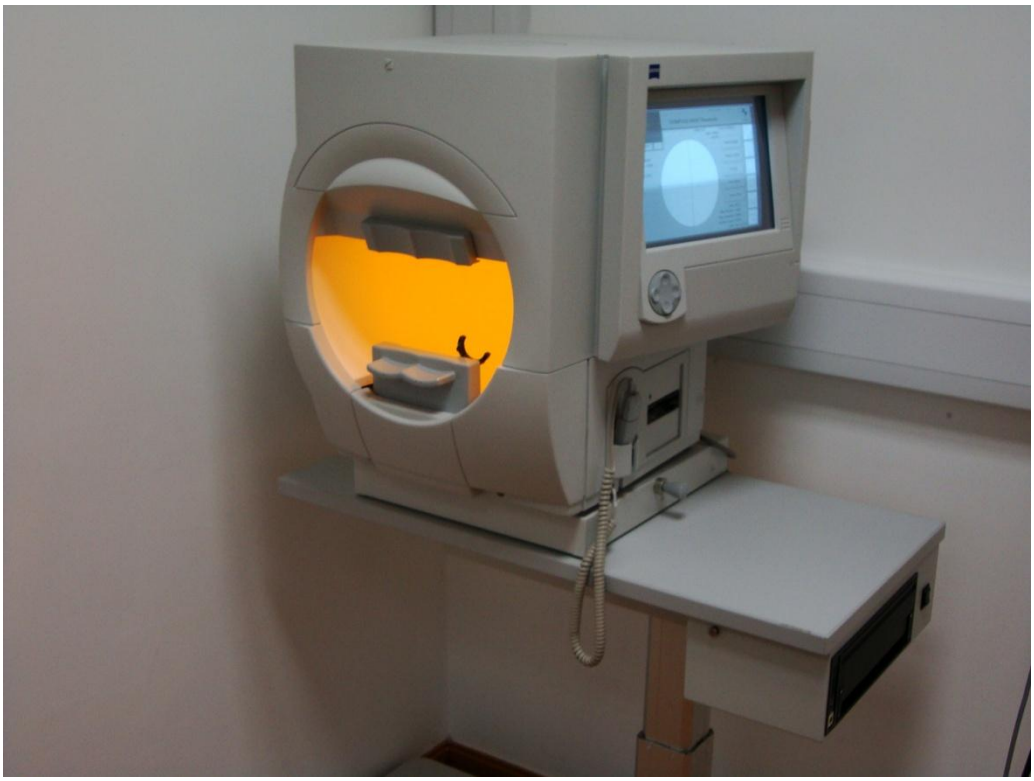

Figure 4.5 The Humphrey® field analyzer.

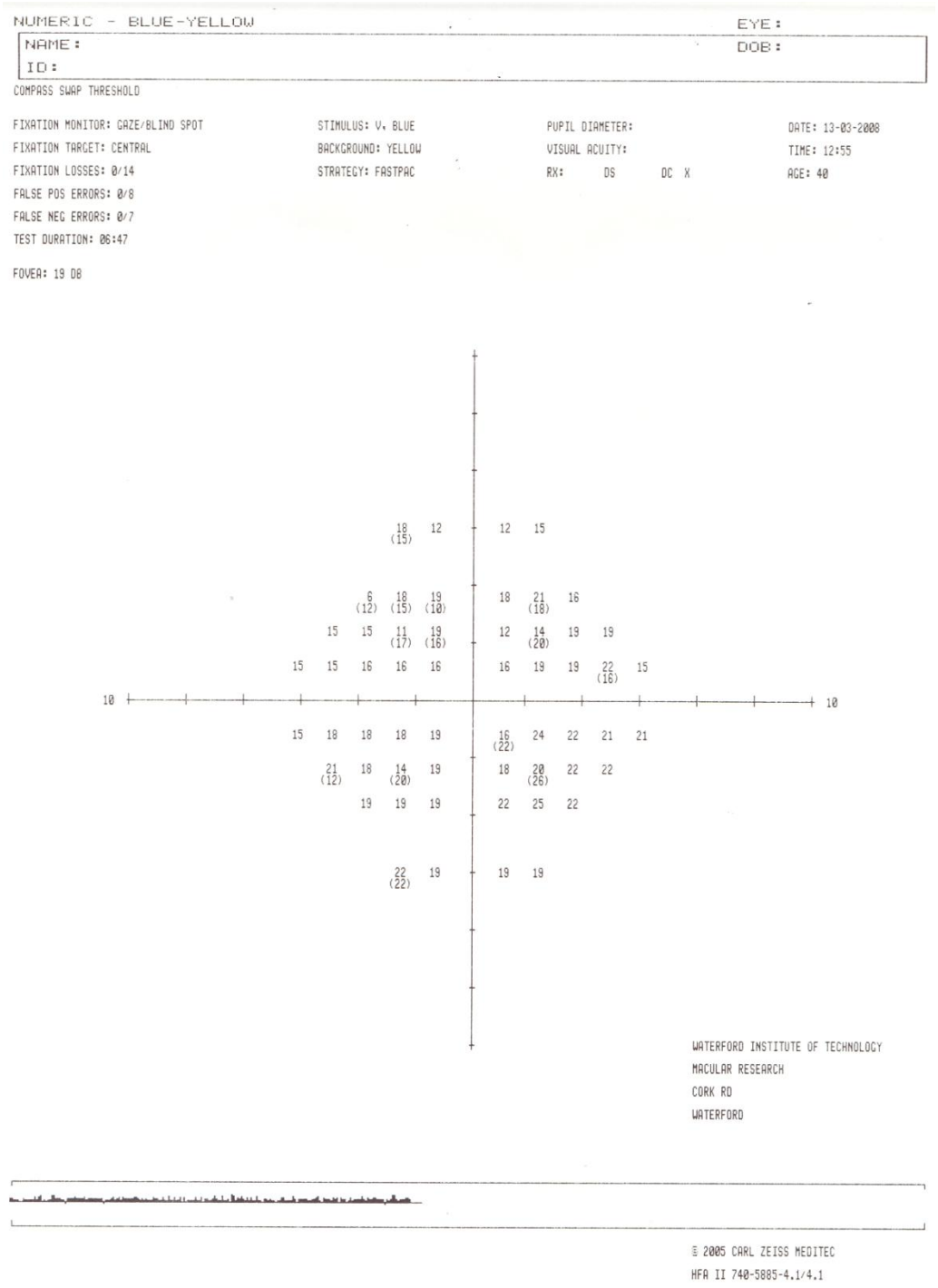

Figure 4.6 cSWAP test report showing a typical set of short wave sensitivity values recorded for a subject.

4.2.7 MEASURMENT OF MACULAR PIGMENT OPTICAL DENSITY USING HETEROCHROMATIC FLICKER PHOTOMETRY

For a detailed description of this method, please refer to *Section 2.2.11 – Measurement of Macular Pigment Optical Density using Heterochromatic Flicker Photometry*

4.2.8 STATISTICAL ANALYSIS

Data were analysed using PASW Statistics 17 (SPSS Inc, Chicago, Illinois). Correlation coefficients and first-order partial correlation coefficients were calculated using the Pearson product-moment method since scatter-plots shown no evidence of non-linearity. Statistical analysis was based on two-tailed tests and interpreted with reference to 0.05 significance levels and Bonferroni correction.

4.3 RESULTS

4.3.1 MEAN MACULAR PIGMENT OPTICAL DENSITY

Figure 4.7 shows the MPOD spatial profile. These data compare well with previously published data using the same cHFP method.[7] Mean (±SD) MPOD for the 0.25° stimulus was 0.45 (±0.18), range 0.16 to 0.93.

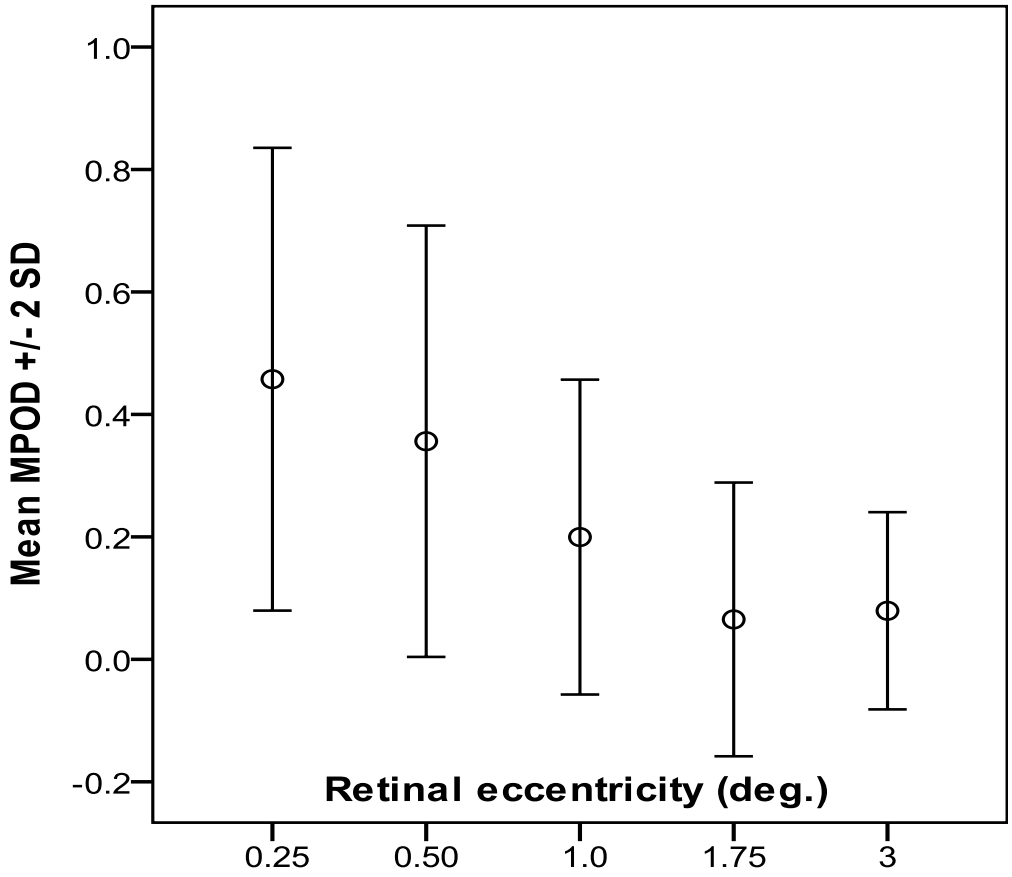

Figure 4.7 MPOD spatial profile. Abscissa: retinal eccentricity in degrees. Ordinate: mean MPOD across subjects ±2 SD.

4.3.2 MEAN TOTAL ERROR SCORE

Mean (±SD) hue discrimination TES for our subjects was 55 (±23), comparable to Kinnear and Sahraie's data for the 30-39 age group.[8]

4.3.3 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH ERROR SCORES

TES was found not to correlate significantly with MPOD ($P > 0.001$ after Bonferroni correction). Possible associations between MPOD and (1) short wavelength hue discrimination in the region of peak absorption by MP and (2) discrimination at the short wavelength end of the expected axis of a type III acquired colour vision defect were investigated by calculating %PES for colour samples 50-68 and 36-54 respectively, i.e.% (PES/TES). Both (1) and (2) were found to be non-significantly correlated ($P > 0.001$ with Bonferroni correction) to MPOD at all eccentricities (see Table 4.1 and Figure 4.8).

Table 4.1 Correlations between colour vision variables and MPOD.

MPOD		%PES		Moreland midpoint	cSWAP					
		B/G 36-54	B 50-68		Fovea	1	2	3	4	5
0.25 °	r_0	-.188	.114	.343	-.331	-.189	-.110	-.003	-.097	-.032
	r_1	-.183	.121	.343	-.328	-.186	-.106	.005	-.089	-.025
	p_0	.084	.301	.001**	.002*	.083	.314	.982	.378	.769
	df_0	83	83	91	83	83	83	83	83	83
0.5 °	r_0	-.142	.094	.298	-.267	-.191	-.116	-.047	-.134	-.063
	r_1	-.138	.099	.295	-.264	-.189	-.112	-.042	-.128	-.057
	p_0	.195	.393	.004*	.014*	.079	.292	.667	.223	.567
	df_0	83	83	91	83	83	83	83	83	83
1 °	r_0	-.219	.026	.329	-.285	-.180	-.200	-.132	-.165	-.125
	r_1	-.218	.028	.331	-.285	-.178	-.198	-.130	-.163	-.123
	p_0	.044*	.816	.001**	.008*	.100	.067	.229	.132	.256
	df_0	83	83	90	83	83	83	83	83	83
1.75 °	r_0	-.224	.113	.489	-.461	-.288	-.295	-.215	-.267	-.203
	r_1	-.217	.121	.484	-.458	-.284	-.291	-.209	-.261	-.196
	p_0	.040*	.304	.000**	.000**	.008*	.006*	.048*	.013*	.063
	df_0	83	83	90	83	83	83	83	83	83
3 °	r_0	-.177	.230	.387	-.393	-.288	-.317	-.249	-.307	-.283
	r_1	-.154	.258	.371	-.386	-.278	-.306	-.229	-.284	-.263
	p_0	.105	.034*	.000**	.000**	.008*	.003*	.021*	.004*	.009*
	df_0	83	83	90	83	83	83	83	83	83

r_0 = Pearson correlation coefficient; r_1 = 1st-order partial correlation coefficient controlling for age; p_0 = two-tailed significance for r_0; df_0 = degrees of freedom for r_0; *indicates $P < 0.05$ without Bonferroni correction; ** indicates significant with correction for a 5 by 9 correlation matrix; MPOD = macular pigment optical density at eccentricities 0.25º to 3º; %PES = FM100 percentage partial error scores; B/G 36-54 = blue/green caps (36-54); B 50-68 = blue caps (50-68); cSWAP = sensitivity values on customised short wavelength automated perimetry at fovea and eccentricities from 1º to 5º

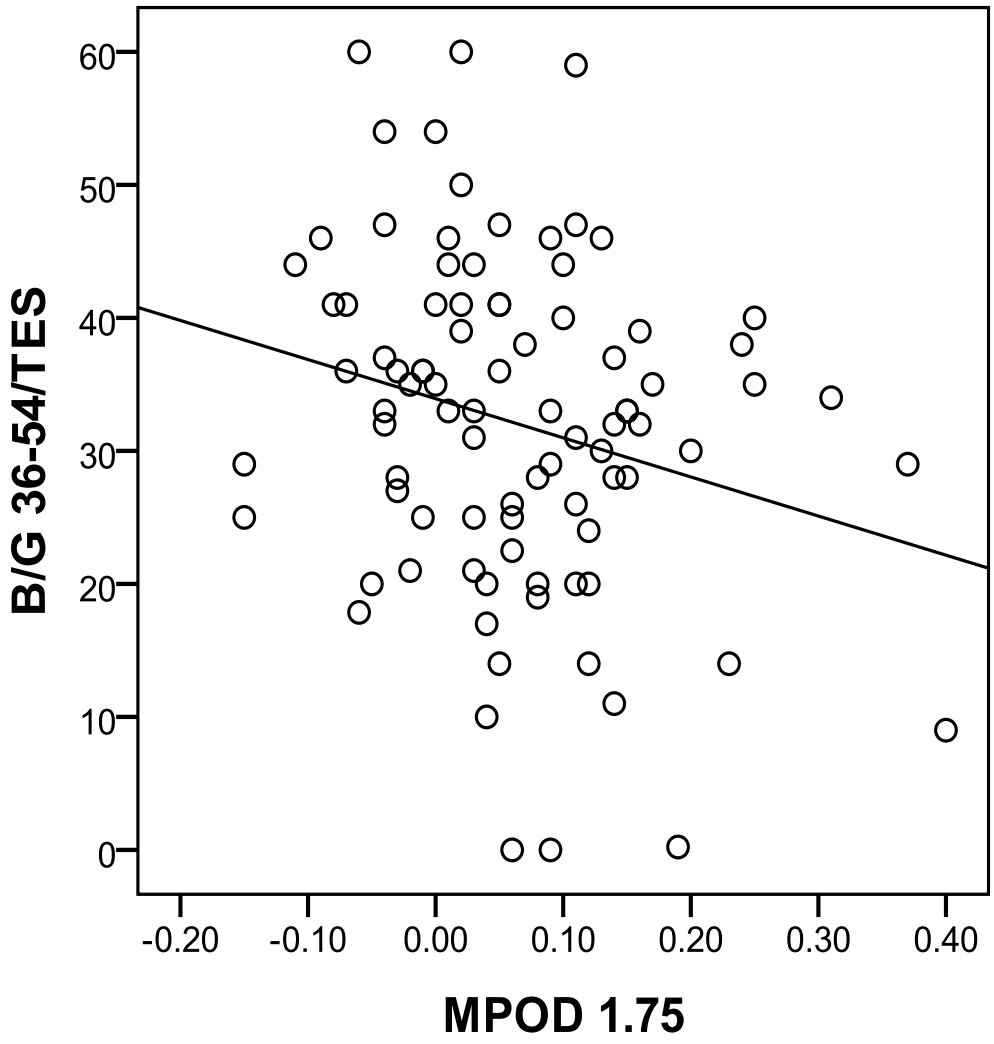

Figure 4.8 Scattergram of %PES for FM100 caps 36-54 against MPOD at 1.75º of retinal eccentricity. Solid line = linear model least-squares regression (%PES = -0.239 × MPOD + 33.92).

4.3.4 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH ANOMALOSCOPE MORELAND MATCH

The Anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (see Table 4.1 and Figure 4.9), indicating a shift towards green mixtures to match cyan. The coefficient was maximal for MPOD at 1.75º, corresponding to the Anomaloscope stimulus diameter of 2º. MPOD at 1.75º accounted for 23.9% of variability (r^2) in Moreland match data. Coefficients were still significant after Bonferroni correction at all eccentricities except at 0.5º.

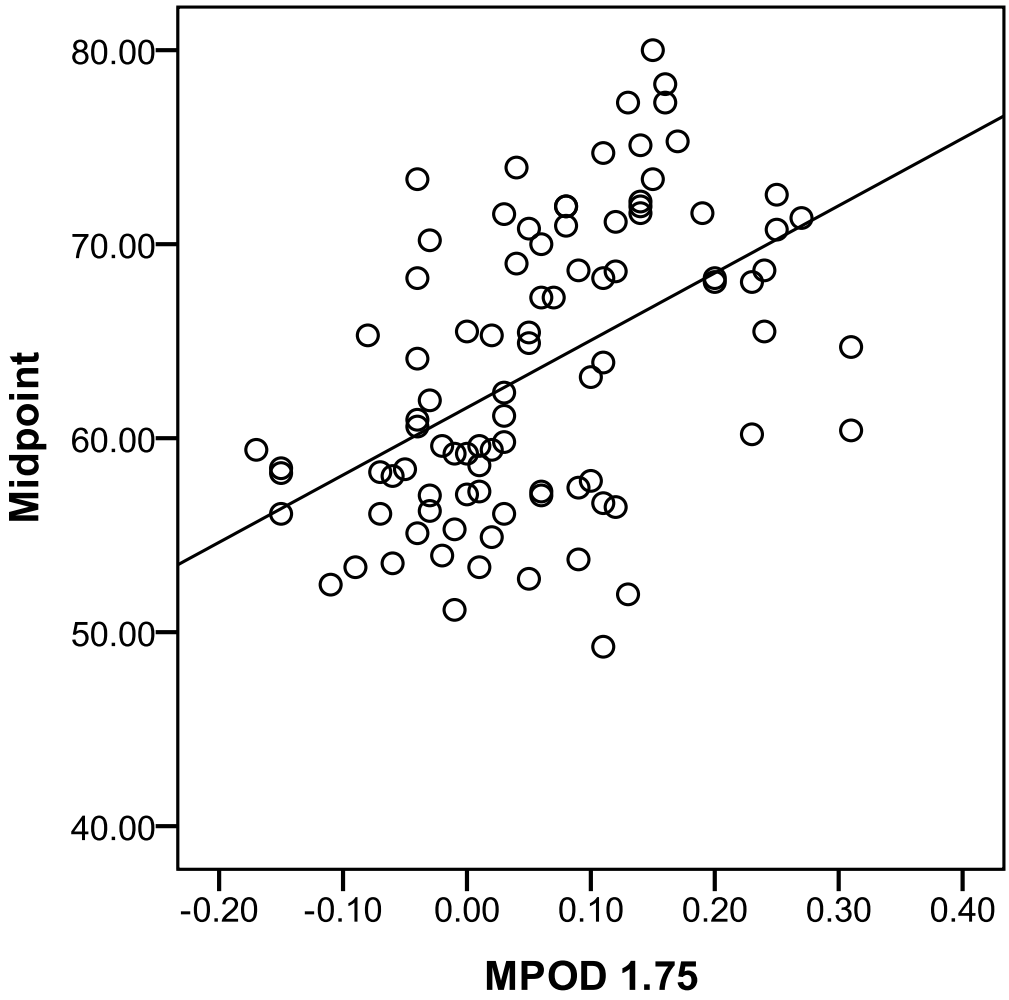

Figure 4.9 Scattergram of Anomaloscope Moreland match midpoints against MPOD at 1.75º of retinal eccentricity. Solid line = linear model least-squares regression. (Midpoint = 35.91 × MPOD + 61.46).

4.3.5 MACULAR PIGMENT OPTICAL DENSITY AND ITS RELATIONSHIP WITH CUSTOMISED SHORT WAVELENGTH AUTOMATED PERIMETRY

cSWAP data (sensitivity in dB) at all eccentricities measured were negatively correlated at high significance levels, with MPOD at both 1.75° and 3° of retinal eccentricity: see Table 4.1. Figure 4.10 is a scattergram of the data for cSWAP at 2° and MPOD at 1.75°. Furthermore, cSWAP at the fovea correlated negatively and significantly with MPOD at all eccentricities. Thus high cSWAP sensitivities were associated with low MPOD. However, after Bonferroni correction, only foveal cSWAP correlated significantly with MPOD at 1.75° and 3°. The maximal proportion of variability in cSWAP attributable to MPOD (r^2) is 21.2% (for foveolar cSWAP and MPOD at 1.75°).

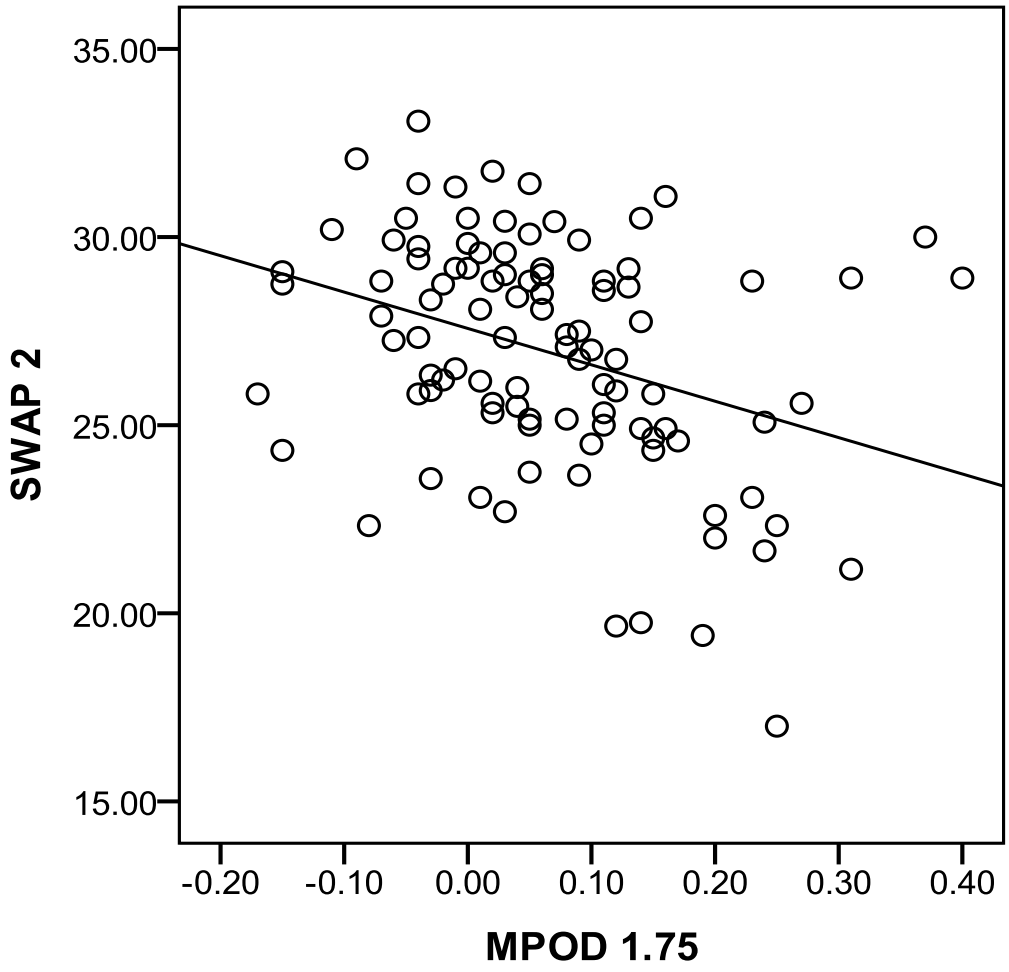

Figure 4.10 Scattergram of sensitivity data on cSWAP at 2º of retinal eccentricity against MPOD at 1.75º of retinal eccentricity. Solid line = linear model least-squares regression (cSWAP = -9.67 × MPOD + 27.57).

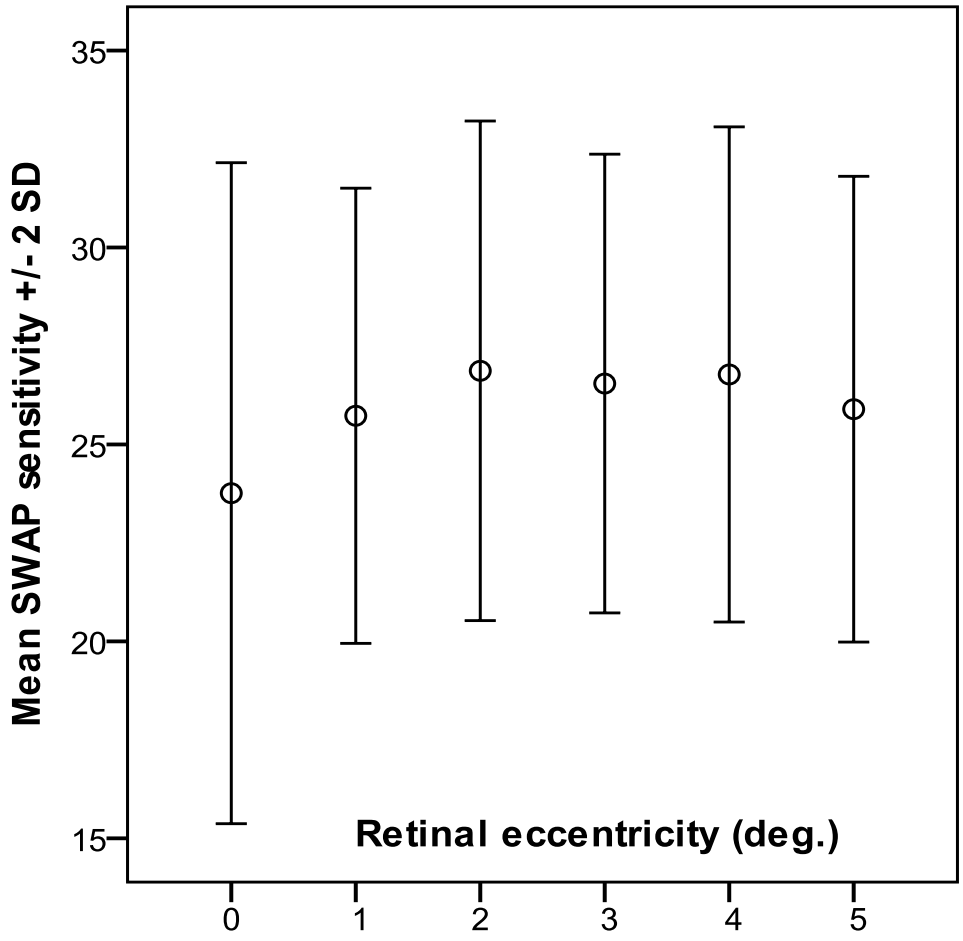

Figure 4.11 cSWAP spatial profile. Abscissa: retinal eccentricity in degrees. Ordinate: mean cSWAP sensitivity in dBs across subjects ± 2 SD.

4.4 DISCUSSION

Our hue discrimination data does not support the findings of Moreland and Dain (1995),[9] who found a significant increase in both TES and PES in the blue green region with their MP1 carotene filter of 1.0 maximum absorbance. We found no statistically significant association between MPOD at any retinal eccentricity and TES or PES after application of Bonferroni correction. This discrepancy may be a reflection of the nature of Moreland and Dain's filter, which was considerably denser than typical MPOD values; it exceeded the MPOD of all of our subjects at and between 1.75° and the foveola and did not provide an exact fit to the spectral absorbance of MP. It may also reflect a difference between a physiological filter, to which the visual system has adapted, and a filter placed before the eye.

It is possible that an artificial filter creates short-term changes in colour vision and that an autoregulatory process adjusts retinal and/or cortical colour mechanisms on a long-term basis in response to their naturally occurring MPOD. This hypothesis is supported by data showing a consistent shift in achromatic locus over a 3 month period for cataract patients post-surgery,[10] by colour constancy effects for blue and green targets despite crystalline lens brunescence (Hardy *et al*. 2005), and by evidence of plasticity of adult neural colour mechanisms.[11] Rodriguez Carmona *et al*. found no correlation between YB thresholds and MPOD using a technique in which threshold colour differences were measured for detection of movement of a stimulus within a checkered array.[12]

We did not assess the association, if any, of MPOD across subjects with colour appearance other than by using the HMC Anomaloscope Moreland match. Using this technique, we found that midpoint data were surprising in that subjects with high MPOD required less blue to match cyan; this finding

was consistent for MPOD at all eccentricities. No directly comparable data exists in the literature, though Stringham and Hammond[5] found that YB cancellation thresholds were constant across the retina despite significant MPOD variability across the retinal region tested. It is of interest that in one study of Moreland match midpoint data, no difference was reported between post-cataract patients with short wavelength-absorbing IOLs and those with clear IOLs.[13]

The cSWAP data show relatively constant sensitivity across the retina beyond the foveola (Figure 4.11) despite substantial differences in MPOD across the retina (Figure 4.7). This finding is consistent with that of Stringham *et al*.[14] who used Maxwellian-view multi-channel optics except that they found slightly lower sensitivity at the foveola compared to parafovea using 16 subjects of similar age to those in the present study. This suggests that parafoveal (but not foveolar) cSWAP may provide a valid clinical test of short wave sensitive cone function. The fact that we found statistically significant inverse correlations between short-wave sensitivity for the foveal stimulus and MPOD at two eccentricities does not in fact contradict Stringham *et al.*'s conclusions; our correlations relate to differences between subjects rather than to averaged measures across the retina which would not take into account the effects of inter-subject variance in both short wave sensitive cone sensitivity and MPOD at any single retinal locus.

We hypothesise that the fact that short wave sensitive cone sensitivity exhibited significant inverse associations with MPOD, while hue discrimination thresholds shown no significant associations with MPOD, may be related to temporal differences between the 2 measures. It is possible that, by using short stimulus presentations, the cSWAP technique (200 msec) produces transient effects quite different to those found with much longer presentations such as those of the FM100 test.

Confounding variables which might influence the relationship between MPOD and colour vision include: iris and choroidal pigmentation, age, stimulus size, and pupil diameter. The effect of iris pigment density has been studied by Woo and Lee (2002),[15] who found that Asians have poorer PES in the blue quadrant, and by Hammond and Caruso-Avery (2000),[16] who reported that subjects with darker irides had higher MPOD. Since all subjects in the present study were Caucasian, the density range of both iris pigment and choroidal pigment was limited, and yet MPOD was found to correlate significantly with colour sensitivity across a variety of measures. We suggest that our findings are independent of iris pigmentation, though such pigmentation is a factor in a less racially homogenous group of subjects.[17]

The effect of age on hue discrimination, in the blue-green spectral region in particular, is well known[18] and is partly due to wavelength-selective loss of light transmission by the aging crystalline lens.[19] An age effect on MPOD has also been reported, some studies having shown a statistically significant age related decline in MPOD.[16;20] It is therefore possible that age is a confounding factor influencing our findings on MPOD and hue discrimination in the blue-green spectral region. A similar age effect is possible in relation to short wave sensitive cone function as measured by cSWAP.[21;22] Although our subjects were restricted to the age range 18 to 40 years, and our exclusion criteria included any evidence of cataract, potentially confounding contributions attributable to age cannot be dismissed. However, inspection of Table 4.1 shows that first-order partial correlation coefficients with age as the control variable are very similar to zero order coefficients. In no case did a significance level change from significant to non-significant by controlling for age. We therefore suggest that our observed associations between MPOD and both Moreland midpoint and cSWAP are independent of age within the age range of

the present study (18 to 40 years, mean age ± SD = 29 ± 6 years). However, the age factor may be important in older subjects.

Stimulus size and location are known to affect both colour vision[23] and measures of MPOD.[7] In the present study MPOD was measured using targets subtending between 30 minutes and 3.5° at eccentricities between 0° and 3°. Colour thresholds were measured using centrally fixated targets subtending approximately 1.5° (FM100), 2° (Anomaloscope), and 1.7° at between 0° and 5° of retinal eccentricity (cSWAP). A clear pattern is evident from our data: MPOD correlated consistently across size and retinal eccentricity parameters with cSWAP and Moreland midpoint. MPOD values were reported in this study at a range of eccentricities in order to assess the consistency of correlations, and because retinal images extend beyond their geometric optical limits as a result of aberrations, diffraction and scatter. Furthermore eye movements produce translational shift of retinal images in a natural viewing environment.

The practical implications of the present study are two-fold. Firstly, dietary supplementation to increase MPOD is not likely to adversely affect hue discrimination. However, a longitudinal study of the effects of supplementation on colour vision is needed to support this. Secondly, we have shown that appropriate customisation of a standard clinical automated perimetry test (cSWAP) provides a potential clinical test for foveal short wave sensitive cone sensitivity, though this awaits confirmation by a concordance study using Maxwellian view instrumentation.

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-CHAPTER FIVE-

DISCUSSION

5. DISCUSSION

5.1 SUMMARY

First, we report that measures of central visual function, including BCVA and contrast sensitivity are positively associated with MPOD ($P < 0.05$, for all). Photostress recovery and glare sensitivity were unrelated to MPOD ($P > 0.05$).

Second, we report that a significant increase in central MP following L supplementation does not, in general, impact on visual performance in young normal subjects, and our pre-specified hypothesis that MP augmentation would result in improved visual performance and/or comfort by 12 months, in those randomised to the A arm, remains unproven. However, subjects with high MP following L supplementation demonstrate visual benefits with respect to glare disability and mesopic CS. Further study into MP and its relationship with visual performance is warranted to enhance our understanding of this pigment's role.

Third, our findings suggest that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. The association of MPOD with cSWAP may be a temporally limited effect to which the visual system normally adapts. We suggest that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity.

5.2 IMPLICATIONS OF RESULTS FOR CLINICAL PRACTICE

The results of this thesis combined with other studies suggests to clinicians that, augmentation of MPOD, through the use of daily supplementation of 12 mg of

L and 1 mg of Z can (a) increase MPOD by a value of more than 0.1 optical density in healthy eyes, and (b) can enhance visual performance in those with the highest MPOD values. Even in such cases, no adverse effect on colour vision might be expected. Clinicians, therefore, should seek to understand the benefits of MP, not only for AMD, but also from a visual performance perspective.

However, further study into MP and its relationship with visual performance is warranted to enhance our understanding of this pigment's role.

5.3 FUTURE CONSIDERATIONS

5.3.1 MESO-ZEAXANTHIN (+ LUTEIN & ZEAXANTHIN) AND HIGHER DOSE SUPPLEMENT

No study investigating the effect of MP on visual performance has emphasised or evaluated the potential role of MZ. Aside from its obvious protective function, MZ is somewhat intriguing from a visual performance perspective for a number of reasons.

Firstly MZ is the dominant carotenoid at the central macula (where visual performance is maximal); secondly it expands the range of pre-receptorial blue light filtration capacity at the retina; thirdly it has been shown that in older subjects and cigarette smokers (two of the most significant known risk factors for AMD development) that the central MP is sometimes deficient and can be rebuilt through MZ supplementation;[1;2] and finally, it has been suggested that some individuals may lack the capacity to convert retinal L to MZ, possibly predisposing these individuals not only to an increased risk of AMD development, but also the possibility of visual performance deterioration even in the absence of "disease" per se.

The combination of all the above factors, in particular the capacity of MZ to filter more blue light from the region of the macula responsible for most acute and detailed form vision, would suggest that MZ is particularly important from a visual performance perspective.

An appropriately powered, randomised, controlled trial, with MZ (+L & Z) in young and old healthy eyes, people with low MP, people with glare disability, other ocular diseases will improve our understanding of the benefits of MZ supplementation from a visual performance perspective.

5.3.2 ENVIRONMENTAL CONSIDERATIONS

Blue light transmission into the eye was previously limited to the daylight hours only. Even with the invention of the light bulb, the filaments used did not radically alter our blue light exposure. In the modern world however, technological advances (and environmental concerns) have resulted in an unprecedented explosion in the nature and amount of blue light irradiating the average retina. Important sources of "modern" blue light stimulation include:

- full spectrum lighting systems with correlated colour temperatures > 5000K, these often have enhanced levels of ultra violet (UV) and blue light.
- fluorescent lighting systems incorporate an enhanced 400-480nm band to help imitate natural daylight.
- LEDs are seen as a modern solution to environmental issues associated with lighting – blue LEDs, invented only about eight years ago are brighter than red or green alternatives (~ x20) – are now widely used e.g. electrical devices, PC monitors (large displays employ a three colour LED mix), backlighting liquid crystal display (LCD) television displays, Christmas lights, internal car instrument lighting, mobile

phones etc. The problem with blue LEDs is that they emit a single intense wavelength blue instead of a broad spectrum; this can be focussed by the eye to a high intensity image. Even white LEDs are often now actually blue LEDs coated in a yellow phosphor to make them appear white (giving the characteristic lunar white appearance).

- modern compact fluorescent lamp (CFL) light bulbs emit more than double the amount of blue light emitted from their tungsten predecessors (data on file from DIT laboratory).
- bright light therapy systems – used to treat psychological conditions such as seasonal affective disorder (SAD), thought only to be effective at night- so increased blue exposure at night time - it is also often deemed appropriate on awakening (NB- the waking eye is dark adapted and more sensitive).
- xenon car headlights – an increasing source of "blue" glare light.
- other sources include – street lighting, medical lighting (phototherapy, dentistry applications, surgical lighting etc), industrial lighting- (UV curing/sterilisation, photolithography, forensics, quality control etc.).

We are therefore continuously and cumulatively exposed to blue light sources, whether in the home, the office, the car, while on the computer, watching TV and even in the bedroom (alarm clocks, air condition system LEDs, battery chargers and other gadgets). Continuous low and high level exposure must have increased impact on longitudinal retinal integrity, both in terms of disease development and for visual performance quality and preservation. People are more affected by glare than ever before, photophobia is an increasing clinical problem, and the long term effects of this blue light exposure will likely result in significantly more difficulties in later life.

The acute and chronic effects of such increased short wavelength light exposure warrant further exploration. This is particularly important in relation to the potential capacity of MP to optimise and preserve vision quality across

the lifespan, especially given the ever-increasing life expectancy in the developed world.

5.3.3 ADDITIONAL MACULAR PIGMENT STUDIES

In order to investigate the impact of MP augmentation on visual performance, the findings of this thesis suggest that we should direct our attention to a) subjects with low baseline central MP levels (<0.2 optical density), b) people with glare symptoms (e.g. post laser-assisted in situ keratomileusis (LASIK), early cataract or retinal disease etc.), c) investigate the effect of MP augmentation on presbyopia, d) investigate the effect of MP augmentation on cataract, e) investigate MP and its functions in non-caucasian population, where dietary habits are different from the western world.

5.3.4 OPTIMAL MACULAR PIGMENT OPTICAL DENSITY LEVELS

The optical, physiological and neurological interactions that contribute to vision suggest that the optimal level of MPOD, from a visual performance perspective, may be personal to an individual eye. In other words, and for example, even if MP is found to be important for visual performance and experience, exceeding a particular optical density of the pigment may yield no further measurable or appreciable advantage, and this level may vary substantially from one individual to the next. Determining the range of optimal MPOD levels will be helpful for clinicians, in prescribing macular carotenoids.

5.3.5 VISUAL HEALTH VERSUS OPTICAL FILTRATION

The traditional description of the MP's "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual performance, that are, perhaps, unrelated to the short wave filtration properties of MP. As mentioned above, MP may play a key role in visual health through a complex interplay between the optical, neurological and physiological mechanisms underlying vision. Several studies, which have been discussed in Table 1.1 & 1.2 support this hypothesis. In order to investigate this visual health hypothesis, we suggest that future studies employ additional techniques including pattern and multifocal electroretinography, and visual evoked potential (VEP) over a period of time following supplementation with macular carotenoids.

There is one additional, and important, mechanism, whereby MP may have a beneficial effect on visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons, there by improving retinal health. Measuring retinal oxidative stress over a period of time following supplementation with macular carotenoids will be helpful in understanding the antioxidant properties of macular carotenoids.

5.4 CONCLUSIONS

The findings of this thesis suggest that MP is important from a visual performance perspective. Longer life expectancy, lack of macular carotenoids in modern western diet, increased exposure to short wavelength light (ancestors had little or no short wavelength light exposure after dark), increased effects of scatter from expanding smog and haze, modern visual requirements and the ever-increasing incidence of AMD heightens the importance of both optimising

(and possibly enhancing) visual performance in the working population, and preserving such performance into old age. Clinicians needs to understand that the primary role of MP rests on its contribution to visual performance and experience, although the pigment may also longitudinally contribute to the preservation of macular function by preventing or delaying the onset of retinal disease such as AMD through its protection against chronic (photo)-oxidative damage.

Bibliography – Discussion

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PUBLICATIONS AND PRESENTATIONS

6. PUBLICATIONS AND PRESENTATIONS

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POSTER PRESENTATIONS

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2. **Akkali MC**, Nolan JM, Beatty S, Davison PA, O'Dwyer V, Scanlon G, Major P, Loughman J. The relationship between macular pigment and visual performance. *Invest Ophthalmol Vis Sci* 2010;51: E-Abstract 1286. *ARVO Annual Meeting, Fort Lauderdale, Florida, USA, May 2010*
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OPTOMETRY AND VISION SCIENCE

ORIGINAL ARTICLE

Macular Pigment: Its Associations with Color Discrimination and Matching

Peter Davison*, Mukunda Akkali†, James Loughman‡, Grainne Scanlon§, John Nolan‖, and Stephen Beatty¶

ABSTRACT

Purpose. Macular pigment (MP) acts as a prereceptoral filter which selectively absorbs short wavelengths. It has the potential to alter color vision but the literature is conflicting on whether it does and, if so, to what extent, possibly reflecting differences between color mechanisms and color tests. This study was designed to identify and investigate relationships, if any, between MP optical density (MPOD) and color sensitivity using a battery of techniques to quantify the color vision of color-normal observers.

Methods. Color vision was assessed with the Farnsworth-Munsell 100-Hue test (FM100), Moreland match on the HMC anomaloscope, and a customized short wavelength automated perimetry (SWAP) technique at the foveola and at 1, 2, 3, 4, and 5° eccentricity. MPOD spatial profile was measured using customized heterochromatic flicker photometry.

Results. Total error scores and % partial error scores on the FM100 were uncorrelated to MPOD. Moreland matches showed a significant long wavelength shift with MPOD at between 1 and 3° (at 1.75°, $r = 0.489$, $p < 0.001$). Sensitivities on customized SWAP (cSWAP) using foveal targets were significantly inversely correlated with MPOD at both 1.75° ($r = -0.461$, $p < 0.001$) and 3° ($r = -0.393$, $p < 0.001$). Partial correlation analysis suggests that none of these findings can be attributed to age effects within the range 18 to 40 years.

Conclusions. Our findings suggest that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. The association of MPOD with cSWAP may be a temporally limited effect to which the visual system normally adapts. We suggest that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity. (Optom Vis Sci 2011;88:1–●●●)

Key Words: hue discrimination, anomaloscope, SWAP, macular pigment

Macular pigment (MP), consisting of the carotenoids lutein, zeaxanthin, and meso-zeaxanthin, is concentrated at the macula and is not detectable optically beyond about 7° from the foveal center.[1] Of these carotenoids, the zeaxanthins predominate at the fovea whereas lutein dominates beyond the fovea.[2] The extent of macular pigmentation has recently been found to be related to the width of the foveal cup, as assessed by optical coherence tomography.[3] Because these pigments are located in the fibers of Henle at the foveola and in the inner nuclear layer beyond the foveola,[4] they act as a prereceptoral filter and are believed to contribute a variety of potentially beneficial properties for vision, including reduction of the effects of chromatic aberration[5] (though not supported by Engles et al.,[6]), improvement of spatial vision and contrast enhancement,[7] increased photopic increment sensitivity,[8] reduced glare sensitivity in some studies[9,10] but not others,[11] and increased critical flicker frequency.[12]

Hue discrimination and color vision in general are most acute at the fovea[13] corresponding to increased cone density, specialized anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good color vision is possible beyond the fovea[14]). It is plausible that color discrimination at a small angular subtense would be influenced by the optical density (OD) of MP at the fovea. Indeed, it has long been speculated that interobserver differences in color matching by color-normal observers are at least partly because of differences in macular pigmentation.[15,16] Also, it is known that even subjects with ophthalmoscopically normal fundi exhibit substantial variations in MPOD, contributing to a range of prerecep-

*MSc, PhD
†BS(Optom)
‡PhD, FAOI
§DipOptom, FAOI
‖BSc, PhD
¶MD, FRCOphth
Macular Pigment Research Group (MPRG), Department of Optometry, Dublin Institute of Technology, Ireland (PD, JL, GS), African Vision Research Institute, University of Kwazulu Natal, Durban, Republic of South Africa (JL) and MPRG, Department of Chemical and Life Sciences, Waterford Institute of Technology, Ireland (MK, JN, SB).

toral light absorption at 460 nm from 3% to almost 100%.[17] Dietary supplementation with the macular carotenoids has been shown to increase MPOD[18] and may retard development of age-related macular degeneration because of its antioxidant and short wavelength light filtering properties. Such hypotheses are currently the subject of a major randomized controlled clinical study (AREDS 22)[19] and follows potentially significant results from the LAST II study.[20]

Because the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 460 nm,[21] it would appear likely that these pigments influence color vision through selective absorption of short wavelengths, thereby influencing the short-wave sensitive (SWS) cones and the blue-yellow opponent-color channel. Moreland and Dain[22] reported that hue discrimination, measured using the Farnsworth-Munsell 100-Hue test (FM100), is indeed adversely affected primarily for short wavelengths by simulation of high MPOD using liquid filters containing carotene in a benzene solution. Comparing the results with those obtained with a neutral filter, they concluded that this effect was not simply the result of reduced retinal illuminance. However, to our knowledge, there are no published studies on the effects of actual (rather than simulated) MPOD on conventional measurements of hue discrimination thresholds. Further evidence supporting an effect of MPOD on short wavelength vision has been obtained from studies of SWS cone sensitivity.[8,23] Finally, it has been shown that color discrimination measured by a color matching technique is influenced by MPOD.[24,25]

However, two recent studies using alternative methods, produced conclusions differing from those of the above mentioned studies. First, a study of the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP [measured by heterochromatic flicker photometry (HFP)] and red-green (RG) or yellow-blue (YB) color discrimination thresholds, although it was reported that RG vision tends to improve with augmentation of MP.[26] Second, RG cancellation profiles have been reported to be highly correlated with MPOD, whereas profiles for YB were independent of both eccentricity and MPOD.[17] However, changes in spectral sensitivity across the fovea, macula, and paramacula are accompanied by relatively little change in color appearance, depending on whether corrections are made for MP absorption.[27,28]

Thus, there is no consensus in the literature on the relationships, if any, between MPOD and color vision parameters on the one hand, and mechanisms on the other hand. This may or may not simply reflect the innate differences between, for example, spectral sensitivity measurements of the isolated SWS cone mechanism and the overarching hue discrimination function at short wavelengths. It is also necessary to distinguish between the effects on color vision (mechanisms, sensitivity, or appearance) of (1) distribution of MP across the retina and (2) variation of MPOD between subjects at a given retinal locus.

The objective of this study was to evaluate, in a cross-sectional manner, the associations between color variables and MPOD, using a much larger sample of subjects than in most previous studies and a battery of color assessments rather than relying on a single method of quantification. This study was part of a larger study of the association between MPOD and a wide range of vision parameters.[11]

The color vision tests used in this study were (a) hue discrimination using the FM100 test, (b) hue matching using the Moreland match on an anomaloscope, and (c) short wavelength automated perimetry (SWAP) increment thresholds using a customized procedure (cSWAP) to provide optimal foveal and parafoveal stimuli. This study has clinical implications for the visual effects of dietary supplementation of patients with age-related macular degeneration and at-risk patients.

METHODS

Identical instrumentation and test protocols were used in the Macular Pigment Research Group laboratories in Dublin and Waterford, Ireland.

Subjects

One hundred two healthy subjects aged 18 to 40 years and resident in either Dublin or Waterford, Ireland, were recruited to participate in this dual-center study, which was approved by Research Ethics Committees of Waterford Institute of Technology and of Dublin Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Potential subjects underwent a full eye examination. The exclusion criteria comprised: any ocular pathology (including abnormal macula appearance or cataract); corrected visual acuity $<6/9$ in the better eye; refractive error outside -6 to $+6$ diopters; and defective color vision. One eye only of each subject was tested, that with better corrected acuity. Full color vision data were available for 84 subjects.

Color Threshold/Sensitivity Techniques

The FM100 test (X-Rite UK, Poynton)

This test was administered under color-corrected fluorescent lighting supplied by a pair of 15W 46 cm lamps (The Daylight Co., London, UK) providing minimum luminance of 94 cd.m^{-2} reflected from each color sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was 12 cd.m^{-2}. Color temperature is rated at 6400° K. Subjects were allowed to review the arrangement in each tray if they so requested.

Individual error scores and total error scores (TES), summed across the visible spectrum and purple hues, were determined using the software supplied by the manufacturer. Partial error scores (PES) were used to assess hue discrimination specifically among blue and cyan hues using samples 50 to 68 and 36 to 54, respectively, and were divided by TES to obtain percentage values (%PES).

Anomaloscope

This test was administered using the Moreland match on an HMC MR anomaloscope (type 7700: Oculus, Wetzlar, Germany). This provides a 2° field within which 436 and 490 nm sources are matched to a mixture of 480 and 589 nm, the latter mixture providing a brightness match. Control of stimuli and calculation of blue/green mixture were achieved with the anomaloscope under computer control using the manufacturer's software. Neutral preadaption was not used as this

was found to produce transient adaptation effects on stimulus saturation. Stimuli were presented under continuous viewing mode. After practice, subjects toggled the mixture to obtain four matches, two each with the mixture preset to blue bias and green bias. The mean of six blue/green matches was calculated for each subject to obtain the midpoint.

Customized Short-Wavelength Automated Perimetry

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard SWAP routine on a Humphrey Field Analyzer 2i (Carl Zeiss Medetec, Jena, Germany). Yellow (530 nm) background luminance was 100 cd.m^2. Size V targets of 440 nm and 200 ms duration subtending 1.7° at the eye were presented at 0, 1, 2, 3, 4, and 5° eccentricity from a fixation target. The number of targets at each eccentricity beyond the foveal center varied from 4 to 20. On each presentation, a single target was presented. Increment thresholds were obtained using the SWAP adaptive staircase full thresholding technique. Subjects were given 3 min to adapt to the background before testing began. Sensitivity for each eccentricity was the mean of values for all targets in the group at that eccentricity.

Macular Pigment Optical Density

MPOD was measured by customized HFP (cHFP) using a densitometer (Macular Metrics Corp., Providence, RI), which alternates 460 and 550 nm stimuli, the former being maximally absorbed by MP whereas the latter is not absorbed by MP. A spatial profile of MPOD was obtained by performing five measurements at each eccentricity (0.25, 0.5, 1, 1.75, and 3°), and at 7°, to provide a reference point at which MP is optically undetectable. Further details have been published elsewhere.[29] This instrument and technique have been shown to be valid and have high reproducibility.[30]

Statistical Methods

Data were analyzed using PASW Statistics 17 (SPSS, Chicago, IL). Correlation coefficients and first-order partial correlation coefficients were calculated using the Pearson product-moment method because scatter-plots showed no evidence of non-linearity. Statistical analysis was based on two-tailed tests and interpreted with reference to 0.05 significance levels and Bonferroni correction.

RESULTS

Fig. 1 shows the MPOD spatial profile. These data compare well with previously published data using the same cHFP method.[3] Mean (±SD) MPOD for the 0.25° stimulus was 0.45 (±0.18), range 0.16 to 0.93.

Mean (±SD) hue discrimination TES for our subjects was 55 (±23), comparable with Kinnear and Sahraie's data for the 30 to 39 age group.[31] TES was found not to correlate significantly ($p > 0.001$ after Bonferroni correction). Possible associations between MPOD and (1) short wavelength hue discrimination in the region of peak absorption by MP and (2) discrimination at the short wavelength end of the expected axis of a type III acquired color vision defect were investigated by calculating %PES for color samples 50 to 68 and 36 to 54, respectively, i.e., %(PES/TES). An example of this analysis is provided in Fig. 2, which is a scattergram of % PES for FM100 samples 36 to 54 against MPOD at 1.75° eccentricity. Despite an apparent trend of increased %PES with higher MPOD, both (1) and (2) were found to be non-significantly

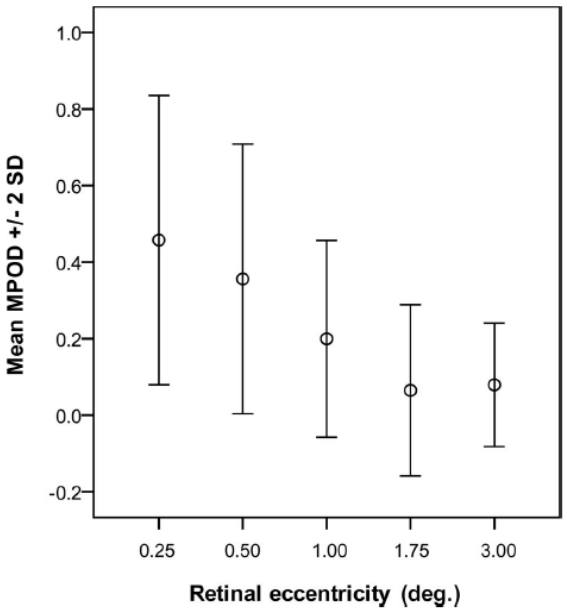

FIGURE 1.
Spatial profile of MPOD. Abscissa: eccentricity in degrees. Ordinate: mean MPOD across subjects ± 2 SDs.

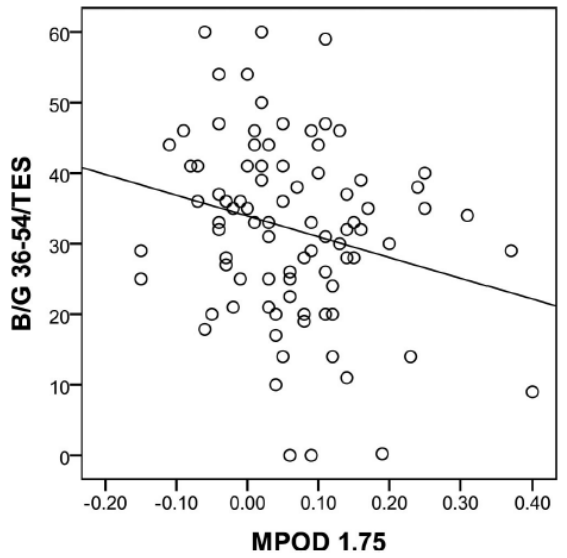

FIGURE 2.
Scattergram of %PES for FM100 caps 36 to 54 against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (%PES = −0.239 × MPOD + 33.92).

TABLE 1.
Correlations between color vision variables and MPOD

	%PES		Moreland	cSWAP					
MPOD	B/G 36–54	B 50–68	midpoint	Fovea	1	2	3	4	5
0.25°									
r_o	−0.188	0.114	0.343	−0.331	−0.189	−0.110	−0.003	−0.097	−0.032
r_1	−0.183	0.121	0.343	−0.328	−0.186	−0.106	0.005	−0.089	−0.025
p_o	0.084	0.301	0.001[a]	0.002[b]	0.083	0.314	0.982	0.378	0.769
df_o	83	83	91	83	83	83	83	83	83
0.5°									
r_o	−0.142	0.094	0.298	−0.267	−0.191	−0.116	−0.047	−0.134	−0.063
r_1	−0.138	0.099	0.295	−0.264	−0.189	−0.112	−0.042	−0.128	−0.057
p_o	0.195	0.393	0.004[b]	0.014[b]	0.079	0.292	0.667	0.223	0.567
df_o	83	83	91	83	83	83	83	83	83
1°									
r_o	−0.219	0.026	0.329	−0.285	−0.180	−0.200	−0.132	−0.165	−0.125
r_1	−0.218	0.028	0.331	−0.285	−0.178	−0.198	−0.130	−0.163	−0.123
p_o	0.044[b]	0.816	0.001[a]	0.008[b]	0.100	0.067	0.229	0.132	0.256
df_o	83	83	90	83	83	83	83	83	83
1.75°									
r_o	−0.224	0.113	0.489	−0.461	−0.288	−0.295	−0.215	−0.267	−0.203
r_1	−0.217	0.121	0.484	−0.458	−0.284	−0.291	−0.209	−0.261	−0.196
p_o	0.040[b]	0.304	0.000[a]	0.000[a]	0.008[b]	0.006[b]	0.048[b]	0.013[b]	0.063
df_o	83	83	90	83	83	83	83	83	83
3°									
r_o	−0.177	0.230	0.387	−0.393	−0.288	−0.317	−0.249	−0.307	−0.283
r_1	−0.154	0.258	0.371	−0.386	−0.278	−0.306	−0.229	−0.284	−0.263
p_o	0.105	0.034[b]	0.000[a]	0.000[a]	0.008[b]	0.003[b]	0.021[b]	0.004[b]	0.009[b]
df_o	83	83	90	83	83	83	83	83	83

[a]Significant with correction for a 5 by 9 correlation matrix.
[b]$p \leq 0.05$ without Bonferroni correction.
r_o, Pearson correlation coefficient; r_1, first-order partial correlation coefficient controlling for age; p_o, 2-tailed significance for r_o; df_o, degrees of freedom for r_o; B/G 36–54, blue/green caps (36–54); B 50–68, blue caps (50–68).

correlated ($p > 0.001$ with Bonferroni correction) to MPOD at all eccentricities.

The anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (Table 1 and Fig. 3), indicating a shift toward green mixtures to match cyan. The coefficient was maximal for MPOD at 1.75°, corresponding to the anomaloscope stimulus diameter of 2°. MPOD at 1.75° accounted for 23.9% of variability (r^2) in Moreland match data. Coefficients were still significant after Bonferroni correction at all eccentricities except at 0.5°.

cSWAP data (sensitivity in dB) at all eccentricities measured were negatively correlated at high significance levels, with MPOD at both 1.75 and 3° of retinal eccentricity (Table 1). Fig. 4 is a scattergram of the data for cSWAP at 2° and MPOD at 1.75°. Furthermore, cSWAP at the fovea correlated negatively and significantly with MPOD at all eccentricities. Thus, high cSWAP sensitivities were associated with low MPOD. However, after Bonferroni correction, only foveal cSWAP correlated significantly with MPOD at 1.75 and 3°. The maximal proportion of variability in cSWAP attributable to MPOD (r^2) is 21.2% (for foveolar cSWAP and MPOD at 1.75°).

DISCUSSION

Our hue discrimination data do not support the findings of Moreland and Dain,[22] who found a significant increase in both TES and PES in the blue-green region with their MP1 carotene filter of 1.0 maximum absorbance. We found no statistically significant association between MPOD at any retinal eccentricity and TES or PES after application of Bonferroni correction. This discrepancy may be a reflection of the nature of Moreland and Dain's filter, which was considerably denser than typical MPOD values; it exceeded the MPOD of all our subjects at and between 1.75° and the foveola) and did not provide an exact fit to the spectral absorbance of MP. It may also reflect a difference between a physiological filter, to which the visual system has adapted, and a filter placed before the eye.

It is possible that an artificial filter creates short-term changes in color vision and that an autoregulatory process adjusts retinal and/or cortical color mechanisms on a long-term basis in response to their naturally occurring MPOD. This hypothesis is supported by data showing a consistent shift in achromatic locus over a 3 months period for cataract patients postsurgery,[32] by color con-

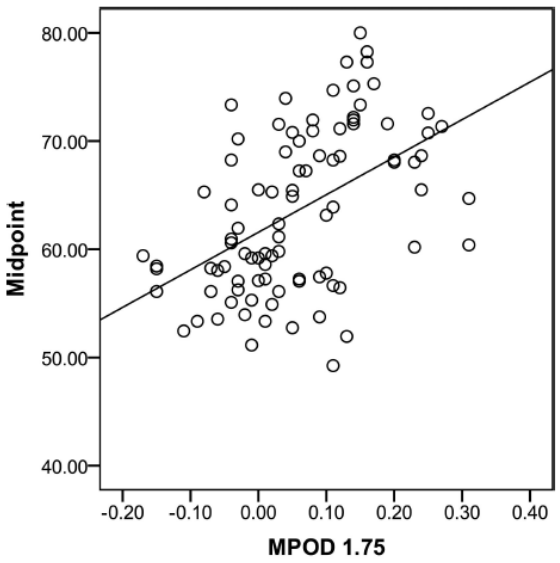

FIGURE 3.

Scattergram of anomaloscope Moreland match midpoints against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (midpoint = 35.91 × MPOD + 61.46).

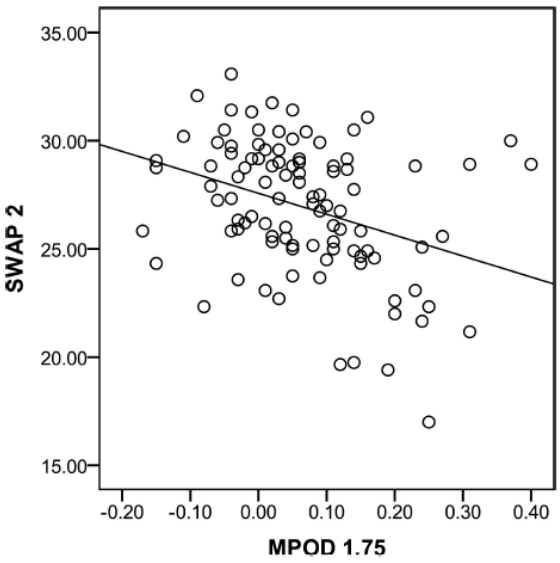

FIGURE 4.

Scattergram of sensitivity data on customized shortwave automated perimetry (cSWAP) at 2° eccentricity against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (cSWAP = −9.67 × MPOD + 27.57).

stancy effects for blue and green targets despite crystalline lens brunescence,[33] and by evidence of plasticity of adult neural color mechanisms.[34] Rodriguez-Carmona et al.[26] found no correlation between YB thresholds and MPOD using a technique in which threshold color differences were measured for detection of movement of a stimulus within a checkered array.

We did not assess the association, if any, of MPOD across subjects with color appearance other than by using the HMC anomaloscope Moreland match. Using this technique, we found that midpoint data were surprising in that subjects with high MPOD required less blue to match cyan; this finding was consistent for MPOD at all eccentrities. No directly comparable data exist in the literature, although Stringham and Hammond[17] found that YB cancellation thresholds were constant across the retina despite significant MPOD variability across the retinal region tested. It is of interest that in one study of Moreland match midpoint data, no difference was reported between postcataract patients with short wavelength-absorbing intraocular lenses and those with clear intraocular lenses.[35]

The cSWAP data show relatively constant sensitivity across the retina beyond the foveola (Fig. 5) despite substantial differences in MPOD across the retina (Fig. 1). This finding is consistent with that of Stringham et al.[36] who used Maxwellian-view multichannel optics except that they found slightly lower sensitivity at the foveola compared with parafovea using 16 subjects of similar age to those in this study. This suggests that parafoveal (but not foveolar) cSWAP may provide a valid clinical test of SWS cone function. The fact that we found statistically significant inverse correlations between short-wave sensitivity for the foveal stimulus and MPOD at two eccentricities does not in fact contradict Stringham et al.'s conclusions; our correlations relate to differences between subjects rather than to averaged measures across the retina which would not take into account the effects of intersubject variance in both SWS cone sensitivity and MPOD at any single retinal locus.

We hypothesize that the fact that SWS cone sensitivity exhibited significant inverse associations with MPOD, whereas hue discrimination thresholds showed no significant associations with

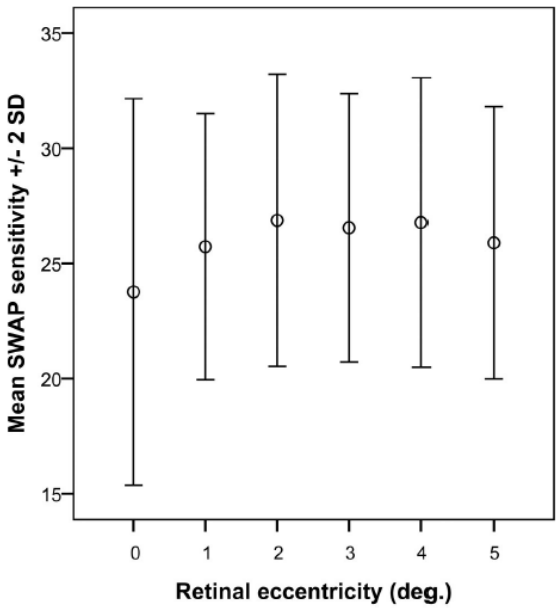

FIGURE 5.

cSWAP spatial profile. Abscissa: eccentricity in degrees. Ordinate: mean cSWAP sensitivity in decibels across subjects ± 2 SDs.

MPOD, may be related to temporal differences between the two measures. It is possible that, by using short stimulus presentations, the cSWAP technique (200 ms) produces transient effects quite different to those found with much longer presentations such as those of the FM100 test.

Confounding variables which might influence the relationship between MPOD and color vision include iris and choroidal pigmentation, age, stimulus size, and pupil diameter. The effect of iris pigment density has been studied by Woo and Lee,[37] who found that Asians have poorer PES in the blue quadrant, and by Hammond and Caruso-Avery,[38] who reported that subjects with darker irides had higher MPOD. Because all subjects in this study were white, the density range of both iris pigment and choroidal pigment was limited, and yet MPOD was found to correlate significantly with color sensitivity across a variety of measures. We suggest that our findings are independent of iris pigmentation, although such pigmentation is a factor in a less racially homogenous group of subjects.[39]

The effect of age on hue discrimination, in the blue-green spectral region in particular, is well known[40] and is partly because of wavelength-selective loss of light transmission by the aging crystalline lens.[41] An age effect on MPOD has also been reported, some studies having shown a statistically significant age-related decline in MPOD.[38,42] It is therefore possible that age is a confounding factor influencing our findings on MPOD and hue discrimination in the blue-green spectral region. A similar age effect is possible in relation to SWS cone function as measured by cSWAP.[43,44] Although our subjects were restricted to the age range 18 to 40 years, and our exclusion criteria included any evidence of cataract, potentially confounding contributions attributable to age cannot be dismissed. However, inspection of Table 1 shows that first-order partial correlation coefficients with age as the control variable are very similar to 0-order coefficients. In no case did a significance level change from significant to non-significant by controlling for age. We therefore suggest that our observed associations between MPOD and both Moreland midpoint and cSWAP are independent of age within the age range of this study (18 to 40 years, mean age $\pm$SD = 29 $\pm$ 6 years). However, the age factor may be important in older subjects.

Stimulus size and location are known to affect both color vision[45] and measures of MPOD.[3] In this study, MPOD was measured using targets subtending between 30 min and 3.5° at eccentricities between 0 and 3°. Color thresholds were measured using centrally fixated targets subtending ~1.5° (FM100), 2° (anomaloscope), and 1.7° at between 0 and 5° eccentricity (cSWAP). A clear pattern is evident from our data: MPOD correlated consistently across size and eccentricity parameters with cSWAP and Moreland midpoint. MPOD values were reported in this study at a range of eccentricities to assess the consistency of correlations, and because retinal images extend beyond their geometric optical limits as a result of aberrations, diffraction, and scatter. Furthermore eye movements produce translational shift of retinal images in a natural viewing environment.

The practical implications of this study are two-fold. First, dietary supplementation to increase MPOD is not likely to adversely affect hue discrimination. However, a longitudinal study of the effects of supplementation on color vision is needed to support this. Second, we have shown that appropriate customization of a standard clinical automated perimetry test (cSWAP) provides a potential clinical test for foveal SWS-cone sensitivity, although this awaits confirmation by a concordance study using Maxwellian view instrumentation.

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Peter A. Davison
National Optometry Centre
Dublin Institute of Technology
Kevin Street, Dublin 8
Ireland
e-mail: peter.davison@dit.ie

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The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS

John M. Nolan [a,*,2], James Loughman [b,2], Mukunda C. Akkali [a,1], Jim Stack [a,2], Grainne Scanlon [b,1], Peter Davison [b], Stephen Beatty [a,2]

[a] Macular Pigment Research Group, Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Ireland
[b] Macular Pigment Research Group, Optometry Department, Dublin Institute of Technology, Dublin, Ireland

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ABSTRACT

This study was conducted to investigate whether augmentation of macular pigment (MP) enhances visual performance (VP). 121 normal subjects were recruited. The active (A) group consumed 12 mg of lutein (L) and 1 mg of zeaxanthin (Z) daily. MP optical density (MPOD) was assessed by customized heterochromatic flicker photometry. VP was assessed as best corrected visual acuity (BCVA), mesopic and photopic contrast sensitivity (CS), glare disability, photostress, and subjective visual function. Subjects were assessed at baseline; 3; 6; 12 months (V1, V2, V3 and V4, respectively). Central MPOD increased significantly in the A group ($p < 0.05$) but not in the placebo group ($p > 0.05$). This statistically significant increase in MPOD in the A group was not, in general, associated with a corresponding improvement in VP ($p > 0.05$, for all variables), with the exception of a statistically significant time/treatment effect in "daily tasks comparative analysis" ($p = 0.03$). At V4, we report statistically significant differences in mesopic CS at 20.7 cpd, mesopic CS at 1.5 cpd under high glare conditions, and light/dark adaptation comparative analysis between the lower and the upper MP tertile groups ($p < 0.05$) Further study into the relationship between MP and VP is warranted, with particular attention directed towards individuals with low MP and suboptimal VP.

1. Introduction

The dietary carotenoids zeaxanthin (Z) and lutein (L) and L's retinal isomer *meso*-zeaxanthin (*meso*-Z) are lipid-like molecules that accumulate at the macula, where they are collectively referred to as macular pigment (MP) (Bone, Landrum, Hime, Cains, & Zamor, 1993). An average western diet contains about 1.3–3 mg/day of L and Z combined (Nebeling, Forman, Graubard, & Snyder, 1997a, 1997b), with significantly more L than Z (represented by an estimated ratio of 7:1). Approximately 78% of dietary L and Z is sourced from vegetables (Sommerburg, Keunen, Bird, & van Kuijk, 1998). L is found in highest concentrations in dark green leafy vegetables, such as spinach, kale, and collard greens (Sommerburg et al., 1998). Z is the major carotenoid found in orange peppers, and oranges, with a high mole percentage of both L and Z being found in egg yolk (Sommerburg et al., 1998), with comparable amounts of L and Z recently reported in corn and a variety of corn containing products (e.g. cornmeal and cereal) (Perry, Rasmussen, & Johnson, 2009). Possible dietary sources of *meso*-Z include shrimp, certain marine fish, and turtles, none of which are found in a typical western diet (Maoka, Arai, Shimizu, & Matsuno, 1986), however, it has recently been suggested that MZ may be present in some other, yet to be identified, foods (Connolly et al., 2010).

The macula is a specialized part of the retina, as it mediates central vision, provides sharpest visual acuity, and facilities best color discrimination (Hirsch & Curcio, 1989). Age-related macular degeneration (AMD) is a disease of the macula and results in the loss of central and color vision. AMD is the most common cause of blindness in the elderly population in the developed world (Congdon et al., 2004). It is now understood that oxidative stress (Beatty, Koh, Henson, & Boulton, 2000; Winkler, Boulton, Gottsch, & Sternberg, 1999), exacerbated in part by cumulative short-wavelength visible light exposure (Algvere, Marshall, & Seregard, 2006; Fletcher et al., 2008), is important in the aetiopathogenesis of AMD. MP is a short-wavelength (blue) light filter (Bone, Landrum, & Cains, 1992) and a powerful antioxidant (Khachik, Bernstein, & Garland, 1997), and is therefore believed to protect against AMD (Loane, Kelliher, Beatty, & Nolan, 2008). This hypothesis, referred to as the "protective" hypothesis of MP, has been studied and reported on extensively (Loane et al., 2008).

* Corresponding author.
E-mail address: jmnolan@wit.ie (J.M. Nolan).
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Beyond its "protective" hypothesis, MP's optical and anatomic properties have prompted the "optical" hypotheses of this pigment. The "optical" hypotheses of MP have been previously discussed by Reading and Weale (1974) and later by Nussbaum, Pruett, and Delori (1981), and include MP's putative ability to enhance visual performance and/or comfort by attenuation of the effects of chromatic aberration and light scatter, via its short wave light-filtering properties (Walls & Judd, 1933). This traditional description of the "optical hypothesis" does not account for additional mechanisms whereby MP may enhance visual performance, that are, perhaps, unrelated to the short wave filtration properties of MP. MP has been shown to exhibit dichroic properties (Hemenger, 1982) which may facilitate the reduction of glare disability through preferential absorption of polarized light. Higher MPOD has also been observed to relate to a trend towards lower root-mean-square wavefront aberrations (in particular, higher order aberrations), thereby enhancing visual performance (Kvansakul et al., 2006).

There is one additional, and important, mechanism, whereby MP may have a beneficial effect on visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate or prevent the deleterious effects of free radical damage on the physiological functions of the photoreceptors and their axons.

Many studies (to date mostly cross-sectional in design) have evaluated, and reported on the role of MP in visual performance, including: visual acuity; contrast sensitivity; glare disability; photostress recovery; critical flicker fusion frequency (CFF); color vision (amongst others) (Bartlett & Eperjesi, 2008; Engles, Wooten, & Hammond, 2007; Hammond & Wooten, 2005; Kvansakul et al., 2006; Loughman et al., 2010; Rodriguez-Carmona et al., 2006; Stringham, Fuld, & Wenzel, 2004; Stringham & Hammond, 2007, 2008; Wooten & Hammond, 2002). However, a placebo-controlled, randomized, L-based supplementation trial was needed to investigate if augmentation of MPOD actually enhances visual performance and/or comfort. The Collaborative Optical Macular Pigment ASsessment Study (COMPASS), presented here, was designed specifically to answer this important research question.

2. Methods

2.1. Subjects and study sites

COMPASS was conducted at Waterford Institute of Technology (WIT) and Dublin Institute of Technology (DIT), vision science laboratories, located in the southeast and east of the Republic of Ireland, respectively. One hundred and twenty-one healthy subjects volunteered to participate in this two-centered study, which was approved by the research ethics committees at both study sites. Self-selected recruitment of subjects (WIT: $n = 61$ and DIT: $n = 60$) was facilitated by poster and newsletter advertisement, and also by word of mouth, in the respective local communities. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

All subjects were aged between 18 to 41 years, in perfect general (self report) and ocular health (see below), and with visual acuity of at least 20/30 in the study eye. A typical study visit lasted approximately 4 h. Subjects were assessed at baseline, three, six, and 12 months (V1, V2, V3 and V4, respectively). All subjects recruited into the study were classed as naïve observers to the tests carried out (with the exception of the visual acuity test, with which all subjects were familiar). However, to optimize performance, and also to minimize any potential learning effects on performance, all subjects underwent a defined period of pre-test training. This training consisted of careful explanation of the nature of each test, pictorial and/or video demonstration of the test requirements and procedure, and was followed by a defined session of pre-test practice.

2.2. Study design and formulation

COMPASS is a registered trial on the ISRCTN database (number 35481392), and is a randomized placebo-controlled clinical trial of oral supplementation with a formulation containing the macular carotenoids (L and Z) and co-antioxidants versus placebo. The tablets used in the current study were hard film coated tablets. The daily dose of two tablets for the active (A) group consisted of 12 mg L, 1 mg Z (provided as ester), 120 mg vitamin C, 17.6 mg vitamin E, 10 mg zinc and 40 μg selenium. The placebo (P) consisted of cellulose, lactose and magnesium stearate, and was manufactured to be identical to the A preparation in terms of size and color. The study tablets for the A and P groups were packaged into identical blister packs which contained the subjects' anonymized unique identification number and COMPASS study label information. Subjects were instructed to consume the daily dose of two tablets with a meal.

Compliance was assessed by tablet counting at each study visit, and encouraged by frequent reminder telephone calls and text messages by the study COMPASS research team. Compliance was also assessed at the end of the study by quantifying L and Z concentrations in serum, at each study visit, using high performance liquid chromatography (HPLC).

2.3. Demographic, medical history, lifestyle and vision case history questionnaires

The following details were recorded, for each volunteer, on a purpose designed case report form: demographics; general health status; smoking habits (never, current or past); alcohol consumption (average unit weekly intake); exercise (minutes per week); body mass index (BMI, kg/m^2); blood pressure; ethnicity; marital status; education; occupation.

A vision case history was also performed, and details reported included: time since last eye examination; spectacles or contact lens use; history of ocular treatment or surgery; history of occlusion therapy or visual training in childhood; family history of eye disease; current problems with vision; asthenopia associated with computer use; history of headaches.

2.4. Diet and serum concentrations of lutein and zeaxanthin

Dietary intakes of L and Z were quantified using a self-administrated, semiquantitative food frequency questionnaire developed by the Scottish Collaborative Group at the University of Aberdeen (Scotland UK), recently described by O'Connell et al. (O'Connell et al. 2008) Serum concentrations of L and Z were quantified by HPLC using an assay previously reported by Loane et al.(Loane, Nolan, & Beatty 2010).

2.5. Spectacle refraction, visual acuity, and ocular dominance

Each subject underwent precise spectacle refraction by an experienced optometrist to determine refractive error and best corrected visual acuity (BCVA) for each eye. A computer generated LogMAR test chart (Test Chart 2000 Pro; Thomson Software Solutions) was used to determine BCVA at a viewing distance of 4 m, using a Sloan ETDRS letterset. BCVA was determined as the average of three measurements, with letter and line changes facilitated by the software pseudo-randomization feature. Best corrected visual acuity was recorded using a letter-scoring visual acuity rating, with 20/20 visual acuity assigned a value of 100. Best corrected visual acuity was scored relative to this value, with each letter correctly identified assigned a nominal value of one, so that, for example, a BCVA of 20/20^{+1} equated to a score of 101, and 20/20^{-1} to 99. The study eye was selected on the basis of ocular dominance, determined using the Miles Test (Roth, Lora, & Heilman, 2002) with

the dominant eye chosen as the study eye, except in cases of observed equidominance, in which case the right eye was selected. All subsequent tests were conducted with the subject's optimal subjective refraction in place.

2.6. Glare disability

Glare disability is a term used to describe the degradation of visual performance typically caused by loss of retinal image contrast. Glare disability is often caused, for example, by surface light reflections, or bright light sources such as car headlights, and typically is a consequence of increased forward light scatter within the eye. Glare disability was assessed using a Functional Acuity Contrast Test (FACT) (Hitchcock, Dick, & Krieg, 2004; Terzi, Buhren, Wesemann, & Kohnen, 2005), displayed using the Functional Vision Analyzer (Hohberger, Laemmer, Adler, Juenemann, & Horn, 2007) (Stereo Optical Co., Inc., Chicago, IL), which is a desktop device that allows the measurement of contrast sensitivity, and includes a customized internal glare source for assessing the impact of glare on this measure of visual performance. The test comprised linear, vertically oriented, sine wave gratings presented at five different spatial frequencies including 1.5, 3, 6, 12 and 18 cycles per degree (cpd). Nine circular patches were presented at each spatial frequency, the contrast of each patch decreasing by 0.15-log units from the previous. Gratings were tilted −15°, 0° or +15° with respect to the vertical, to keep them within the orientation bandwidth of the visual channel. The background was tapered into a grey field in order to keep retinal illumination constant and avoid ghost imaging. Baseline contrast sensitivity was determined on the basis of the lowest contrast compatible with accurate determination of patch orientation across all five spatial frequencies for mesopic [three candelas per meter squared (cdm^{-2})] instrument background conditions, initially in the absence of a glare source. Subjects were asked to identify grating orientation, starting with the patch at highest contrast, and continuing until identification was no longer possible due to reducing contrast. Subjects were instructed not to guess, but to respond "don't know" if patch orientation could not be correctly identified. As this procedure represented a non-standard psychophysical method of threshold detection, each subject was required to re-identify the orientation of certain gratings in a pseudo-random fashion in order to confirm the validity of the subject responses at each spatial frequency. Glare disability was assessed using a radial glare source consisting of 12 white LEDs arranged circumferentially in an oval pattern surrounding the grating charts (ranging from 4.5° to 6° from central fixation). These LEDs have a color temperature of 6500 K, and the spectral emission profile demonstrated a single large peak at 453 nm (close to MP peak absorption), where the spectral irradiance was approximately double that of the peak emissions in the flatter emission spectrum across mid to long wavelengths. Two customized intensity settings were used to determine the effect of different levels of glare on contrast sensitivity. Glare source settings were set at a medium intensity of 42 Lux and a higher intensity of 84 Lux. All correct responses were entered into the Eyeview software provided, and contrast sensitivity scores for no glare, medium and high glare conditions were determined for the respective spatial frequencies.

2.7. Visual Function in Normals questionnaire

A 30-part, non-validated, Visual Function in Normals questionnaire (VFNq30) was designed specifically for the study (JL). The design was based loosely on a previously validated visual activities questionnaire (Sloane, Ball, Owsley, Bruni, & Roenker, 1992), but adapted to suit a normal, young and healthy population sample. This questionnaire allowed the subject to quantify their visual performance using three separate metrics: situational analysis (SA) which required the subject to rate their visual performance in specified daily life situations; comparative analysis (CA) which required the subject to compare their perceived visual performance to that of their peers/family/friends; subject satisfaction score (SSS) which required the subject to provide an overall estimate of their perceived quality of vision. Each of the three metrics above was computed to give a performance score for five different functional aspects of their vision: acuity/spatial vision; glare disability; light/dark adaptation; daily visual tasks; color discrimination.

2.8. Contrast sensitivity function

A Dell Dimension 9200 computer and a Metropsis Visual Stimulus Generation device (VSG (ViSaGe S/N: 81020197), Cambridge Research Systems Ltd., Cambridge, UK) were used to generate and control the stimuli. The VSG provided 14-bit output resolution per phosphor. The stimuli were displayed on a 19″ ViewSonic professional series p227f color CRT flat screen monitor with a frame rate of 119.98 Hz. The resolution of the monitor was set to 1024×769 pixels. Non-linearities in the screen luminance output were eliminated by gamma correction prior to testing using a photometer system (Opti-Cal; Minolta, Japan). The Metropsis software calculated the inverse curves required to correct for the monitor's non-linearities.

The Metropsis contrast sensitivity system generated luminance modulated sine gratings (Gabor patches). The orientation of the stimuli was vertical. The Gabor patches were presented on the CRT monitor and subtended a visual angle of 4.2°. The mean luminance was used as the background luminance. The Gabor had a two-dimensional spatial Gaussian envelope and was radially symmetrical with equal standard deviations, δx and δy.

Contrast sensitivity functions were determined under both mesopic and photopic conditions. Each subject was seated at a fixed viewing distance of 1.5 m from the CRT monitor. Natural pupils were used throughout the experiment. The non-dominant eye was occluded. Testing was carried out in a light free (other than CRT background mesopic and photopic light) environment. The subject was dark adapted for 5 min and a 5-min training session was given prior to testing under mesopic conditions. Subject responses were recorded using a handheld responder (CR6, Cambridge Research Systems Ltd., Cambridge, UK), which communicated with the VSG device via an infra red link. A four alternate forced choice testing system was used, with four possible target locations. The stimuli were randomly presented at 2° spatial offset from the central cross target. The subject indicated the location of the target in relation to the fixation cross using the appropriate button on the responder box. The subject's contrast sensitivity was determined for five different spatial frequencies (1.0, 4.1, 7.5, 11.8 and 20.7 cpd) under both mesopic and photopic conditions, all at a mean luminance of 3 cdm^{-2} (mesopic) and 100 cdm^{-2} (photopic).

A linear staircase method was used to determine the contrast threshold. The first Gabor at a particular location was presented at an initial contrast level where it was anticipated that the observer would be able to detect the Gabor patch for that particular spatial frequency (initial contrast settings were informed by a brief pilot study involving five young healthy subjects). Subsequently, the contrast of the Gabor patch was varied using an adaptive staircase procedure, which was computer controlled and depended upon the subject's responses. The stimulus contrast was reduced in steps of 0.3 log units until the subject did not detect the Gabor patch (first reversal). The contrast was subsequently increased by 0.15-log unit steps until the subject saw the Gabor patch and responded correctly (second reversal). The Metropsis software calculated the contrast threshold for each location and spatial frequency by taking the mid-point between the mean for peaks and

troughs for 12 reversal points. The standard deviation was calculated by taking the deviations of the peak reversals from their peak means and using the average square of these deviations to calculate a peak variance. This method was repeated for the troughs. The square root of both variances were then calculated and averaged to provide the threshold standard deviation.

For each subject, the Metropsis software plotted the inverse of the contrast threshold against the range of spatial frequencies tested to provide a contrast sensitivity function under both mesopic and photopic conditions.

2.9. Photostress recovery

Photostress recovery time (PRT) was calculated using a macular automated photostress (MAP) test (Dhalla & Fantin, 2005; Dhalla, Fantin, Blinder, & Bakal, 2007). MAP is a novel photostress method for the evaluation of macular function using the Humphrey® field analyzer (Model 745*i* Carl Zeiss Meditec Inc. Dublin, CA, USA). The foveal threshold feature of the field analyzer was used to establish baseline foveal sensitivity as the average of three consecutive foveal sensitivity measurements recorded in decibels (dB), with each dB representing a 0.1 log unit sensitivity variation.

Following baseline foveal sensitivity calculation, the subject was exposed to a photostress stimulus, which consisted of a 5-s exposure to a 300-W, 230-V tungsten lamp head from a viewing distance of 1 m. The spectral irradiance in the wavelength range, 300–800 nm, was measured using a Bentham DMc 150 double monochromator scanning spectroradiometer. The input optic consisted of a very high precision cosine response diffuser (f2 error < 1%) and the measurements were performed in 1 nm intervals. Calibration was carried out with reference to a quartz-halogen lamp traceable to the UK National Physical Laboratory. The illuminance at 1 m was obtained by using the photopic weighting function.

Immediately post-photostress, a continuous and timed cycle of foveal sensitivity measurements were conducted and recorded for each subject. The reduction in foveal sensitivity from baseline, along with the time taken to recover to baseline foveal sensitivity, was recorded.

2.10. Macular pigment optical density

We used the Macular Densitometer™, a device developed and originally described by Wooten, Hammond, Land, and Snodderly (1999), to measure MPOD, including its spatial profile across the retina (i.e. 0.25°, 0.5°, 1.0°, 1.75° and 3° of retinal eccentricity). The Macular Densitometer™ uses heterochromatic flicker photometry (HFP) to obtain a valid measure of MPOD at a given retinal location.(Hammond, Wooten, & Smollon, 2005) This method has recently been refined and is now referred to as customized HFP or cHFP. For a detailed description of this protocol please see recent publications by our research group and others (Loane, Stack, Beatty, & Nolan, 2007; Nolan et al., 2009; Stringham et al., 2008). One subject (cwit2553) was excluded from analysis due to inability to use the Densitometer to obtain reliable MPOD data.

2.11. Fundus photography

Fundus photographs were obtained in both eyes using a NIDEK non-mydriatic fundus camera (AFC-230). Fundus photographs were assessed by an expert eyecare professional to exclude fundoscopically evident retinal pathology.

2.12. Statistical analysis

The statistical software package SPSS (version 17) and the statistical programming language R were used for analysis. It was determined at the outset of the study that a minimum sample size of 91 subjects was required in order to detect an effect size (correlation between two continuous variables) of 0.4 at the 5% level of significance with high power. However, 121 subjects were recruited into the study in order to allow for dropouts and for other possible analyses, in particular repeated measures analysis.

All continuous variables at baseline exhibited a typical normal distribution. Mean ± SDs are presented in the text and tables. Comparisons of A and P groups at baseline were conducted using independent samples *t*-tests and chi-square analysis, as appropriate.

We conducted repeated measures analysis of MPOD at each retinal eccentricity measured, for each of four study visits using a general linear model approach, with treatment (i.e. A and P) and smoking habits (non-smoker, past and current cigarette smoker) as between-subjects factors. Where appropriate we used the Greenhouse–Geisser correction for violation of sphericity. We used the 5% level of significance throughout our analysis, without adjustment for multiple testing.

Four visual performance (VP) variables (assessed subjectively by questionnaire) in this study were recorded as percentage change of V4 score compared to V1 score. Repeated measures analysis would not have been appropriate for these, and instead they were analysed using a general linear model with V4 percentage change as the dependent variable and fixed between-subjects factors treatment and smoking habits as explanatory variables.

3. Results

3.1. Baseline findings

The demographic, lifestyle, dietary and serum carotenoid concentrations, MPOD, and vision data of all 121 subjects recruited into the study, and divided by study arm (i.e. A or P group), are summarized in Table 1. As seen from this table, there was no

Table 1
Demographic, lifestyle, vision, and macular pigment data at baseline.

Characteristic	All [a] $n = 121$	A $n = 61$	P $n = 60$	**Sig.
Age	29 ± 7	29 ± 7	29 ± 6	0.864
Body mass index	26 ± 4	26 ± 4	25 ± 3	0.736
Best corrected visual acuity	113 ± 3	113 ± 3	112 ± 3	0.747
Macular pigment optical density				
0.25°	0.5 ± 0.19	0.49 ± 0.19	0.51 ± 0.20	0.458
0.5°	0.4 ± 0.17	0.39 ± 0.16	0.41 ± 0.18	0.425
1°	0.22 ± 0.13	0.20 ± 0.12	0.22 ± 0.15	0.433
1.75°	0.10 ± 0.11	0.09 ± 0.10	0.10 ± 0.11	0.376
3°	0.10 ± 0.10	0.08 ± 0.08	0.12 ± 0.12	0.058
Dietary carotenoids (mg/day)				
Lutein	1.26 ± 0.95	1.16 ± 0.96	1.36 ± 0.94	0.253
Zeaxanthin	0.21 ± 0.12	0.19 ± 0.10	0.23 ± 0.14	0.074
Serum carotenoids (μmol/L)				
Lutein	0.60 ± 0.32	0.57 ± 0.27	0.62 ± 0.36	0.399
Zeaxanthin	0.36 ± 0.17	0.36 ± 0.15	0.37 ± 0.18	0.623
Sex				
Male	69	34	35	
Female	52	27	25	0.773
Smoking habits[b]				
Never smoked	73	42	31	
Ex-smoker	21	11	10	
Current smoker	27	8	19	0.046

[a] n = sample size.
[b] Smoking habits: ex-smoker = smoked ⩾ 100 cigarettes in lifetime but none in last 12 months; current smoker = smoked ⩾ 100 cigarettes in lifetime and at least 1 cigarette per week in last 12 months; A = active group and P = Placebo group.
** Sig. = probability significance value.

significant difference between the A and P groups with respect to lifestyle, vision, and MP data, with the exception of a statistically significant difference between these groups for smoking habits ($p = 0.046$). Smoking status was therefore considered as a potential confounding variable and was controlled for throughout repeated measures analysis. The COMPASS baseline findings have already been published in a separate manuscript in this journal and, therefore, are not discussed in the current manuscript (Loughman, Akkali et al., 2010).

3.2. Longitudinal findings

3.2.1. Supplement compliance

Seventy-six subjects returned tablets, and (based on the number of tablets returned) 94.7% of these subjects averaged at least one tablet per day. The average number of tablets per day was 1.57 in the A group and 1.65 in the P group, a difference that is not statistically significant (ANOVA, $p = 0.32$). In comparing change in MPOD and VP variables between A and P groups, therefore, it was not deemed necessary to control for differences in compliance in the two groups.

3.2.2. Macular pigment optical density

We conducted repeated measures ANOVA of MPOD, for all retinal eccentricities measured (i.e. at 0.25°, 0.5°, 1.0 °, 1.75 °, and 3°), over time (i.e. over the study period [at V1, V2, V3, and V4, respectively]), using a general linear model approach, with two between-subjects factors: treatment (A, P) and smoking habits (never, past, current smoker). As seen in Fig. 1, there was a trend (in the A group) towards an increase in MPOD at all eccentricities measured, but this increase was only statistically significant (at the 5% level) at the more central measured eccentricities (i.e. at 0.25°, 0.5° and 1.75°).

Fig. 2 (obtained from R statistical program) shows MPOD variation at 0.25° for 20 consecutive individual subjects from each of the A and P groups. The graphs are arranged so that those with lowest MP are in the bottom row, and only subjects who presented for all four visits are displayed.

3.2.3. Serum concentrations of lutein and zeaxanthin

We conducted repeated measures analysis of serum concentrations of L and Z over time (i.e. over the study period) including all study visits (V1, V2, V3 and V4), using a general linear model approach, with treatment and cigarette smoking as between-subjects factors. As seen in Fig. 3, there was a statistically significant time/treatment interaction effect for serum concentrations of L, which remained significant ($p < 0.001$, for all) using any of the standard corrections for violation of sphericity. It is clear from the mean plots of Fig. 3, how these significant time/treatment interaction effects came about: serum concentrations of L increased with time in the A group, but remained virtually static in the P group. This time/treatment effect was significant from V2 (as expected and confirmed using paired t-test analysis between V1 and V2, $p < 0.001$). There was no statistically significant time or time/treatment interaction effect for serum concentrations of Z over the study period ($p > 0.05$, for all tests); however, there was a trend towards an increase in the A group.

3.2.4. Visual performance

While the repeated measures ANOVA presented above is based on findings at all four study visits, it is apparent from the graphs (Figs. 1 and 2) that the largest differences in MPOD between A and P subjects are between V1 and V4. The analysis of VP variables which follows is, therefore, confined to V1 and V4 only (controlling for between-subjects factors: treatment and smoking habits).

Using repeated measures ANOVA or a general linear model, as appropriate, we report a statistically significant time/treatment effect in only one measure of VP, namely "daily tasks comparative analysis" assessed subjectively ($p = 0.03$); whereas all other measures of VP were statistically non-significant ($p > 0.05$, for all) [see Table 2].

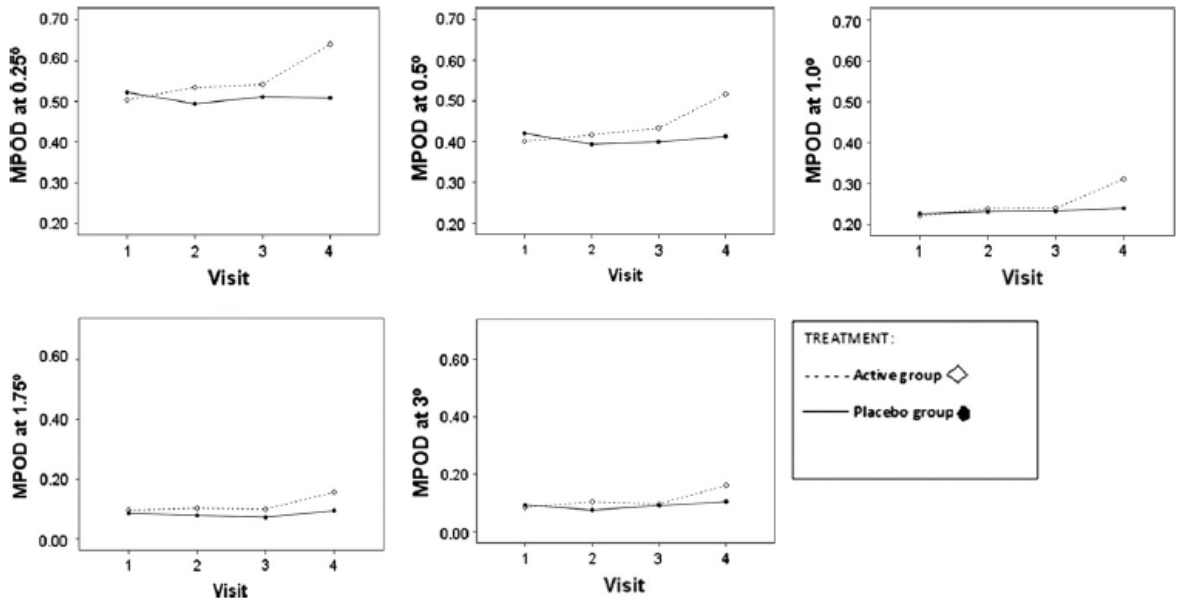

Fig. 1. Change in MPOD at each eccentricity measured, over the 12-month study period, following supplementation in both the active and placebo groups. Repeated measure results for MPOD over the four study visits and analyzing visit * treatment interaction at eccentricities 0.25°, 0.5°, 1.0°, 1.75° and 3°. The *p*-values reported are for the Greenhouse–Geisser correction for violation of sphericity and are as follows: MPOD 0.25 = $p < 0.001$; MPOD 0.5 = $p < 0.001$; MPOD 1.0 = 0.001; MPOD 1.75 = 0.585; MPOD 3.0 = 0.103. Subjects were assessed at baseline, 3, 6, and 12 months (V1, V2, V3 and V4, respectively).

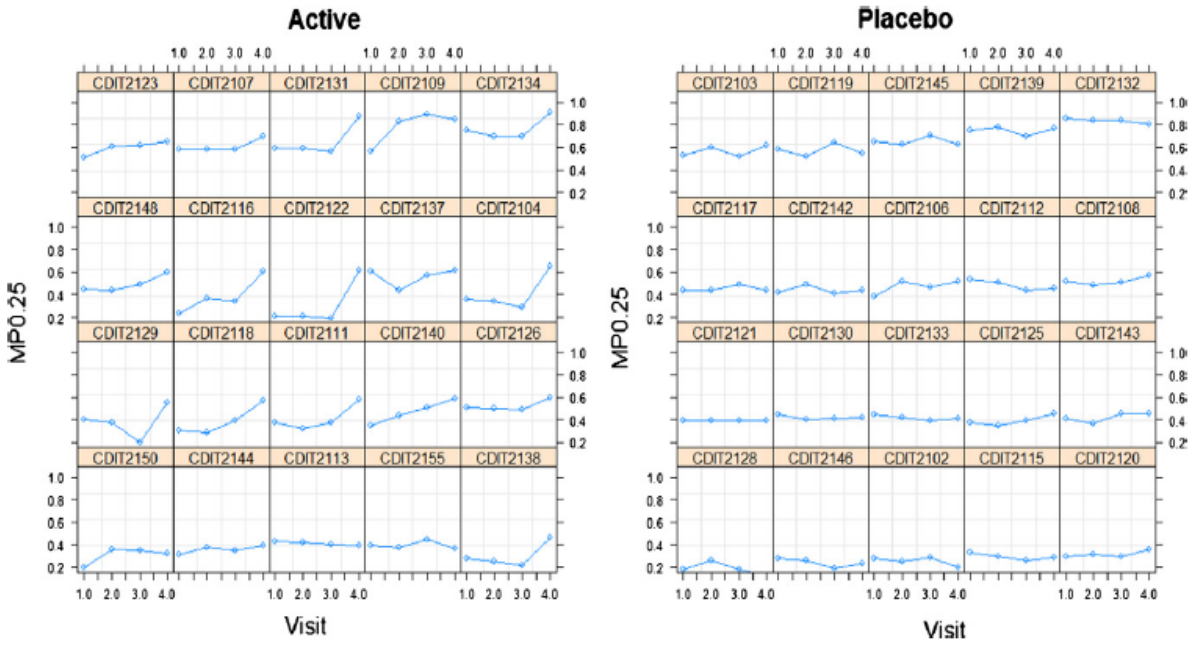

Fig. 2. Change in MPOD at 0.25° eccentricity for 20 subjects from each of active and placebo groups. *MP 0.25° = macular pigment optical density at 0.25° degrees of eccentricity

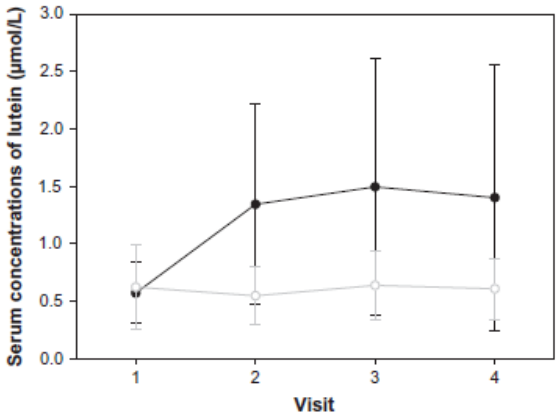

Fig. 3. Change in serum concentrations of lutein over the 12-month study period, following supplementation in both the active and placebo groups. Mean (±SD) serum concentrations of lutein were quantified by high-performance liquid chromatography at baseline, 3, 6, and 12 months (V1, V2, V3 and V4, respectively)

3.2.5. Visual performance differences: low MPOD versus high MPOD subjects

We investigated whether subjects with high MPOD had significantly better VP scores than subjects with low MPOD following supplementation. We based this investigation, for the most part, on MPOD at 0.25° at V4. We used tertiles for V4 MPOD at 0.25° eccentricity to create low, medium and high MPOD groups, and then compared the low and high groups on a variety of VP measures assessed. The low group consisted of 31 subjects with V4 MPOD at or below 0.46 optical density and the high MPOD group had 29 subjects with V4 MPOD at or above 0.69 optical density (Fig. 4). Table 3 presents results for VP measures which differ significantly between these low and high MPOD groups. Table 3 also presents the corresponding results for V1. It should be noted that differences in these VP measures at V1 were not, in general, statistically significant.

4. Discussion

COMPASS is a randomized placebo-controlled clinical trial of oral supplementation with a formulation containing the macular carotenoids (L and Z) and co-antioxidants versus placebo in young normal subjects The pre-specified hypothesis was that supplementation, and consequential MPOD augmentation, would result in improved visual performance and/or comfort in those randomized to the A arm when compared with the P arm, by 12 months.

COMPASS was designed to investigate whether augmentation of MP results in enhancement of visual performance and/or experience, regardless of the mechanism(s) whereby any such improvements may be realized. The optical and neuroprotective hypotheses around MP, which have been discussed previously by Reading and Weale (1974), later by Nussbaum et al. (1981) and are extended here, have generated interest amongst macular pigment scientists, evident in a recent review (Loughman, Davison, Nolan, Akkali, & Beatty, 2010). In brief, some authors have suggested that MP may be important for visual performance and/or experience by at least one of a number of mechanisms, including the reduction of the effects of chromatic aberration, light scatter, higher order aberrations, and plane polarization of light (Loughman, Davison et al., 2010; Walls & Judd, 1933). Importantly, however, and in theory at least, the macular carotenoids have the capacity to confer these optical advantages because of their light-filtering *and* dichroic properties and because of their central location within the retina and crystalline lens.

An additional consideration in relation to any trial investigating the impact of MP augmentation on visual performance and experience is the potential beneficial effect of MP on neurophysiological health. For example, the majority of studies investigating the effects of MP augmentation in ocular disease, including AMD

Table 2
Repeated measures assessment of all VP measures in COMPASS.

Visual performance measure	Sub-measure/device	p-value
Glare disability	Medium glare (Optec)	
	1.5 cpd	0.58
	3.0 cpd	0.94
	6.0 cpd	0.65
	12.0 cpd	0.96
	18.0 cpd	0.49
Glare disability	High glare (Optec)	
	1.5 cpd	0.19
	3.0 cpd	0.99
	6.0 cpd	0.89
	12.0 cpd	0.41
	18.0 cpd	0.86
Glare questionnaire	Glare comparative analysis	0.32
	Glare change analysis	0.88
	Glare situational analysis	0.74
	Glare subject satisfaction score	0.51
Visual acuity	BCVA (Thomson Chart)	0.16
Visual acuity questionnaire	Acuity comparative analysis	0.08
	Acuity change analysis	0.15
	Acuity situational analysis	0.14
	Acuity subject satisfaction score	0.59
Daily tasks questionnaire	Daily tasks comparative analysis	0.03*
	Daily tasks change analysis	0.21
	Daily tasks situational analysis	0.27
	Daily tasks subject satisfaction score	0.41
Light–dark adaptation questionnaire	Light–dark comparative analysis	0.35
	Light–dark change analysis	0.15
	Light–dark situational analysis	0.75
	Light–dark subject satisfaction score	0.56
Mesopic contrast sensitivity	F.A.C.T. (Optec)	
	1.5 cpd	0.72
	3.0 cpd	0.77
	6.0 cpd	0.84
	12.0 cpd	0.66
	18.0 cpd	0.5
Mesopic contrast sensitivity	Metropsis	
	1.0 cpd	0.54
	4.1 cpd	0.79
	7.5 cpd	0.82
	11.8 cpd	0.18
	20.7 cpd	0.08
Photopic contrast sensitivity	Metropsis	
	1.0 cpd	0.95
	4.1 cpd	0.42
	7.5 cpd	0.31
	11.8 cpd	0.19
	20.7 cpd	0.87
Critical flicker fusion frequency	Densitometer	0.3
Foveal sensitivity	Humphrey perimeter	0.93

VP = visual performance; **sig. = probability significance value.
Four VP variables in this study were recorded as percentage change of V4 score compared to V1 score. Repeated measures analysis would not have been appropriate for these, and instead they were analysed using a general linear model with V4 percentage change as the dependent variable and fixed between-subjects factors treatment and smoking as explanatory variables.

(summarized by Loughman, Davison et al. (2010)), have reported a beneficial effect on vision, and such findings are probably attributable to the neuroprotective, as opposed to the optical, properties of these intracellular compounds. These studies have traditionally employed basic psychophysical outcome measures, including visual acuity and contrast sensitivity, and as such have not included stimuli likely to reveal improvements facilitated solely by image enhancement attributable to the optical properties of this pigment.

The study formulation used in COMPASS, in addition to L and Z, contained the co-antioxidants vitamin C, vitamin E, zinc and selenium. In contrast to the capacity to measure subjects' retinal response to supplementation with the macular carotenoids (i.e. by measuring MP) it was not possible to assess, or quantify, subjects' response to supplementation with the above named co-antioxidants. It is important to note that, as seen in the age-related eye disease study (AREDS) (Kassoff & The AREDS research group, 2001), that these antioxidants may have contributed to any benefits reported in visual performance in the current study.

Interestingly, several studies have reported, amongst normal subjects, findings which suggest that MP may play a key role in visual health through a complex interplay between the optical, neurological and physiological mechanisms underlying vision. These observations include (a) better critical flicker fusion frequency (CFF) in the presence of higher MPOD (Hammond & Wooten, 2005), (b) associations between high MPOD and crystalline lens transparency and cataract formation (Brown et al., 1989; Chasan-Taber et al., 1999; Hammond, Wooten, & Snodderly, 1997), (c) the presence of L and Z in substantial concentrations in the primary visual cortex (Craft, Haitema, Garnett, Fitch, & Dorey, 2004) and (d) higher pattern electroretinogram (PERG) P50 amplitudes and better dark adapted cone sensitivities in association with higher MPOD (Carboni, Forma, Mutolo, Jennings, & Iannaccone, 2010) (Carboni et al., 2010 ARVO Abstract 1293-A105).

The randomized design of COMPASS resulted in desirable baseline similarity between A and P groups on possible confounding variables, with the exception of smoking habits (which was controlled for throughout analysis, as appropriate). Significant efforts were made to encourage compliance during the study, and based on the number of tablets returned, we calculated that 95% of subjects averaged at least one tablet per day, with the average number of tablets consumed per day statistically comparable between the A and P groups (at around 1.6 tablets per day).

Consistent with the positive tablet compliance, on average, serum L concentrations increased significantly over the course of the study in the A group with no significant change observed in the P group. Indeed, despite the slight drop in mean serum L concentrations between V3 and V4 in the A group, L concentrations more than doubled in the A group over the course of the study. This finding is consistent with other and recent L interventional studies (Bone & Landrum, 2010; Trieschmann et al., 2007). However, while average serum L concentrations significantly increased in the A group and remained stable in the P group, it is important to point out that 9 (23%) of the A group showed negative or zero change in serum L concentrations. This "non-response" to L supplementation in serum is consistent with an observation by Hammond et al. in 1997 who reported that one subject (out of 11 measured) demonstrated no significant change in serum concentrations of L following consumption of ~12 mg of L per day over a 15 week study period (albeit L consumption in that study was achieved from diet [e.g. spinach and corn] and not from dietary supplements [as in the current study]). To explain the high percentage of serum non-response in the current study, we propose the following possibilities: non-compliance with respect to consumption of the study tablet in these subjects: possible attenuation of the gastrointestinal absorption of supplemental L and Z if the subject fails to take the study tablet in the presence of synchronously ingested fat or oil (importantly, subjects were instructed to consume the daily dose of two tablets with a meal to facilitate the bioavailability of L from the tablet). Indeed, it has been shown that the amount of fat in a person's diet significantly affects the absorption of L ester and its bioavailability, and given that the tablet used in the current study was a film coated tablet not containing oil, failure to consume the study formulation in the presence of fat and/or oil (i.e. with a meal) could significantly impact on the bioavailability of L (Roodenburg,

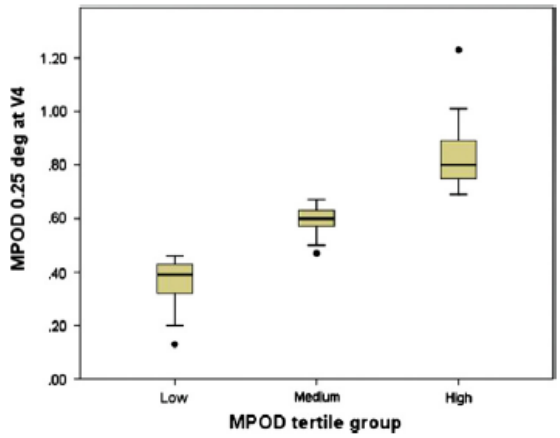

Fig. 4. Boxplots of V4 MPOD at 0.25° showing range of values for each tertile group. *MPOD 0.25° at visit 4 = macular pigment optical density at 0.25° degrees of eccentricity at visit four (12-months) presented for each tertile boxplot. Low, medium and high boxplots represent low tertile group, medium tertile group and high tertile groups with respect to MPOD measured at 0.25° degrees of eccentricity. Black dots represent extreme values (outliers).

Leenen, van het Hof, Weststrate, & Tijburg, 2000). Mean serum concentrations of Z also increased in the A group, but the increase was not statistically significant, probably due to the low concentration of this carotenoid in the study formulation (~1 mg/day).

Central MPOD increased significantly in the A group over the 12-month study period and remained stable in the P group. However, the observed increase in central MPOD in the A group only became apparent (significantly) at 12 months (whereas, as seen above, serum concentrations of L were significantly augmented in the A group at three months). This finding is consistent with previously published studies reporting slow uptake of L by the retina (Bone, Landrum, Guerra, & Ruiz, 2003; Johnson et al., 2000), and inconsistent with others (Connolly et al. (2010)). However, it should be noted that the retinal uptake in our study was much slower than any of these previously published studies. For example, Bone et al. report that no significant change in MP was seen until after day 40 following supplementation with L and Z with up to 30 mg/day of each carotenoid and Johnson et al. report a significant increase in MP after 4 weeks of consuming 60 g/day spinach and 150 g/day corn. However, the reason(s) for the difference seen between studies may be due to any (or a combination of) the following factors: dose of L and Z consumed per day; type of L and Z in the supplement (e.g. free versus ester) matrix in which carotenoids are consumed (e.g. oil versus micro-encapsulated); whether consumed alone or in the presence of other antioxidants; poor serum response to the supplement; non-compliance to the study supplement. Further, and detailed, study on this interesting topic is merited.

The average increase seen in the A group at 0.5° of retinal eccentricity (the standard and most commonly measured and reported MPOD eccentricity) over the 12-month study period was 0.11 ± 0.005 optical density, which is comparable to the findings of Trieschmann et al. who reported an average increase in MP of 0.10 ± 0.009 optical density where they measured MPOD by 2-wavelength autofluorescence. Interestingly, Trieschmann et al. used the same study formulation (daily consumption of 12 mg of L provided as ester) over a 12-month study period as that used in the current study, but by delivering four tablets per day (each containing 3 mg of L ester), whereas the current study achieved a daily consumption of 12 mg of L ester by delivering two tablets per day (Trieschmann et al., 2007). Unlike the findings reported by Trieschmann et al., we report that the biggest gain in MPOD in the A group did not, in general, occur in subjects with lowest baseline MPOD values. However, consistent with the data reported by Trieschmann et al., who reported that 20 (21%) of 92 subjects assessed were retinal non-responders (at 0.5 °), we found that eight (17%) of the A group at 0.25° and nine (20%) of the A group at 0.5° showed negative or zero change in MP at 12 months.

In contrast with the MP measures discussed above, the VP measures assessed in the current study did not, in general, improve significantly over time in the A group. This would, superficially at least, seem to be at odds with the optical and visual health hypotheses of MP's function. Indeed, it is important to emphasise that, of all the VP measures assessed, and reported on, in COMPASS (48 variables in total; see Table 2) we report a statistically significant result for only one measure, namely "daily tasks comparative analysis", assessed subjectively. It is possible, therefore, as data from the current study suggest, that supplementation with the macular carotenoids, and consequential MP augmentation, has no major impact on visual performance and/or experience in young normal subjects (our primary research question and the main study hypothesis). This is, however, at odds with previous reports with respect to the impact of MPOD augmentation on glare disability (Stringham & Hammond, 2005, 2008). This discrepancy with earlier findings may be explained, at least partly, by two fundamental differences between the relevant studies. Firstly, COMPASS was designed to evaluate glare disability under conditions approximating normal environmental experience. As such, testing was conducted using natural pupils, which typically constrict under glare conditions, and therefore confer protection against the effects of glare. The Maxwellian view system employed in other studies does not

Table 3
Comparing visual performance measures between low and high macular pigment optical density groups at visit 4 and visit 1.

Visual performance variable	Visit 4			Visit 1		
	MP group[a]	Mean (±SD)	Sig.	MP group	Mean (±SD)	Sig.
Best corrected visual acuity	High	113 (3)		High	113 (3)	
	Low	111 (4)	0.038	Low	112 (3)	0.045
Mesopic CS at 1.5 cpd under high glare[b]	High	28.6 (15.8)		High	22.1 (11.6)	
	Low	21.2 (11.2)	0.042	Low	19.8 (8.2)	0.337
Light/dark adaptation comparative analysis[c]	High	70.3 (17.4)		High	62.2 (13.2)	
	Low	60.6 (13.1)	0.018	Low	60.6 (14.7)	0.624
Mesopic contrast sensitivity at 20.7 cpd Ω	High	54.7 (17.4)		High	57.1 (15.0)	
	Low	62.9 (10.9)	0.035	Low	59.2 (12.1)	0.523

[a] MP group = macular pigment optical density group tertile for 0.25° eccentricity: high = top tertile, low = bottom tertile.
[b] Mesopic CS at 1.5 cpd under high glare = night-time contrast sensitivity at low spatial frequencies assessed under high glare conditions.
[c] Light/dark adaptation comparative analysis = self reported visual performance under changing light conditions compared to friends/family/peers; Ω = Mesopic contrast sensitivity at 20.7 cpd = night time contrast sensitivity measured at high spatial frequencies.

allow normal pupillary response, so, while MP was shown to impact glare disability under these conditions, it is not clear whether the effect would have remained if a pupillary response had been allowed, which would have caused a variable reduction in retinal illuminance proportional to the magnitude of the pupillary response. Secondly, our findings can only be applied to the stimulus and glare intensity settings employed here, which, although informed by a detailed pilot study, are less comprehensive than the variable glare annulus intensity employed by Stringham & Hammond.

Kvansakul et al. (Kvansakul et al., 2006) conducted a study to evaluate the effect of MP supplementation on mesopic contrast acuity thresholds (CAT) in normal subjects.(Kvansakul et al., 2006) They reported a significant and beneficial effect of MP supplementation on mesopic CAT that was not evident in their placebo group, their findings therefore appearing to be at odds with those of the current study, probably reflecting a number of differences between the two studies in terms of methodology and design [e.g. stimuli, illumination levels ($1 cdm^{-2}$ vs $3 cdm^{-2}$), etc.]. Also, the design by Kvansakul et al. did not incorporate longitudinal evaluation of MPOD, which was measured only at the final visit (interestingly the CATs reported by Kvansakul showed no correlation with MPOD). Furthermore, contrast acuity thresholds were not measured at baseline, but only after six months of supplementation and then again at the final 12 month visit. One cannot, therefore, draw meaningful conclusions with respect to the relationship, if any, between their mesopic CAT findings and MPOD, as there is no record of change in MPOD over their study period. A final point relates to the sample sizes of the two studies, the investigation by Kvansakul et al. being based on a placebo group of only five subjects and three groups of subjects receiving supplementation (containing three, five and five subjects respectively) and is thus not comparable with the COMPASS trial, involving 121 subjects.

There are however, a number of plausible explanations for the absence of any significant influence of MP augmentation on visual performance in our study. Firstly, it should be noted that the majority of study participants exhibited average to high central MPOD pre-supplementation. Indeed, only a small number of subjects (~24%) were found to have central MPOD (at 0.5° eccentricity) less than 0.30 at baseline. Importantly, it has been suggested previously that MPOD levels greater than 0.30 might be superfluous to visual performance requirements (Reading & Weale, 1974), due to the non-linear nature of the effect of MP on vision. Furthermore, the increase in MPOD observed in the A group did not become apparent until the final 12 month visit, and was relatively modest with an average increase of 0.11 ± 0.005 optical density (at 0.5° eccentricity), and unlike the findings reported by Trieschmann et al., subjects (in the A group) in the current study with the lowest MP at baseline did not, in general, demonstrate the biggest increase in MPOD levels following supplementation with the study formulation. Indeed, even after 12-months of supplementation with 12 mg of L per day, over 15% of subjects in the A group retained central MPOD (at 0.5° eccentricity) values below 0.3 optical density. In other words, it is possible that the MP augmentation achieved in the current study was not sufficient (in an adequate number of subjects) to impact on visual performance, and that a greater increase in MPOD, particularly in the group with lowest baseline MPOD, might be required to elicit an improvement in visual performance. Also, as mentioned above, it is also likely that a significant number of subjects in the current study already had (at baseline) sufficient MP for optimal, measurable, and appreciable visual performance (i.e. 75% of subjects in the A group had baseline MP values $\geqslant 0.3$ optical density) and therefore may explain, at least in part, the failure of the current study to demonstrate an improvement in VP following supplemental L.

In addition, the nature of the tests employed for visual performance testing in COMPASS also merits consideration and discussion. The investigators strategically chose to use tests that were either typically available in the average consulting room (to ensure applicability of findings to clinical practice), or designed to replicate typical environmental conditions. As such, most of the tests did not contain substantial amounts of short wavelength light maximally absorbed by MP. The typical office or home environment (where the majority of us spend most of our time), does not have many short wave dominated light sources. Our results might, therefore, suggest that subjects' MP levels pre-supplementation were sufficient for optimal visual performance in this type of environment. Our results, therefore, cannot be extrapolated to short wave dominated visual scenes, such as against the background of a bright blue sky, which is difficult to replicate in an ecologically valid way. Importantly, the changing nature of internal and device lighting systems, such as the increased use of LED systems, and xenon car headlights, are extending our exposure to short wave light sources, and may enhance the applicable relevance of MP for visual performance.

However, given that our study subjects showed an extensive range of MP values, we considered it meaningful to compare VP and comfort measures for subjects with high MP (upper tertile) versus subjects with low MP (lower tertile). We made these comparisons at baseline and also at V4. At V1, the subjects in the low MP group (for central MP at 0.25°) were below 0.42 optical density, whereas subjects in the high MP group (for central MP at 0.25°) were above 0.59 optical density. At V4, the corresponding figures for low and high groups were 0.46 and 0.67 optical density. Supplementation with L, therefore, appears to have widened the gap in MP between the lower and upper tertiles. Of interest, at V4 we report statistically significant differences in some important VP measures, between lower and upper MP tertile groups, which were not present at V1.

The most significant finding is that of a ~30% greater CS under high glare conditions in those with highest MPOD following supplementation. Interestingly, of all the tests employed in COMPASS, the glare source contained the most substantial amount of short wave light (white LEDs used to generate glare contain a single "blue" peak around 460 nm). These results therefore would seem to corroborate previous findings which suggest a role for MP in the attenuation of glare disability (Stringham and Hammond, 2007, 2008; Stringham et al., 2004), and furthermore would seem to extend those findings to suggest that MP augmentation is beneficial for visual performance under glare conditions, even under the natural pupil conditions employed here. This finding and hypothesis is also supported by the results of the visual performance questionnaire. Subjects in the A group reported comparatively, and statistically significantly, better visual performance for daily visual tasks (including night driving against oncoming headlights). Furthermore, in the tertile analysis, those with the highest MP reported comparatively, and statistically significantly, better, capacity to deal with sudden changes in illumination (light/dark adaptation).

In conclusion, we report that a significant increase in central MP following L supplementation does not, in general, impact on VP in young normal subjects, and our pre-specified hypothesis that MP augmentation would result in improved VP and/or comfort by 12 months, in those randomized to the A arm, remains unproven. However, subjects with high MP following L supplementation demonstrate visual benefits with respect to glare disability and mesopic CS. Further study into MP and its relationship with VP is warranted to enhance our understanding of this pigment's role. However, in order to investigate the impact of MP augmentation on visual performance, the findings of our study suggest that we should direct our attention to a) subjects with low baseline central

MP levels, b) subjects with suboptimal visual performance and c) subjects with symptoms of glare disability.

Grant information

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.visres.2010.12.016.

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Retina

A Central Dip in the Macular Pigment Spatial Profile Is Associated with Age and Smoking

Mark L. Kirby, Stephen Beatty, Edward Loane, Mukunda C. Akkali, Eithne E. Connolly, Jim Stack, and John M. Nolan

Purpose. To investigate the relationship between specific macular pigment (MP) spatial profiles and risk factors for age-related macular degeneration (AMD).

Methods. The MP spatial profile of 484 healthy subjects was measured with customized heterochromatic flicker photometry (cHFP) and categorized into one of two profile types: typical exponential or atypical "central dip." Data on risk factors for AMD were obtained with a general health and lifestyle questionnaire. Dietary and serum concentrations of lutein (L) and zeaxanthin (Z) were also assessed.

Results. The presence of the central dip MP spatial profile was significantly more common in older subjects (the mean ± SD age of subjects with a central dip MP spatial profile was 46.9 ± 12 years, whereas the mean age of subjects with a typical MP spatial profile was 41.8 ± 12 years; $P = 0.004$) and in current cigarette smokers ($P = 0.031$). Also, there was a significant age-related decline in central MP optical density (MPOD; 0.25° retinal eccentricity), but in the men only ($r = -0.146$, $P = 0.049$).

Conclusions. A central dip in the MP spatial profile, seen in older subjects and in cigarette smokers, may represent an undesirable feature of macular pigmentation. Further research is needed in this area. (*Invest Ophthalmol Vis Sci.* 2010;51: 6722–6728) DOI:10.1167/iovs.10-5344

The macula contains the highest density of cone photoreceptors in the retina and is responsible for detailed central color vision.[1] Age-related macular degeneration (AMD) is the leading cause of age-related blindness in the developed world.[2,3] Increasing age, family history of AMD, and cigarette smoking[4–6] are the three major risk factors for AMD; other putative risk factors include being of the female sex, obesity, light iris color, low dietary intake and low serum concentrations of lutein (L) and zeaxanthin (Z), and low macular pigment optical density (MPOD).[7–9]

At the macula, the carotenoids L, Z, and *meso*-Z (generated from retinal L) accumulate at high concentrations (to the exclusion of all other carotenoids) and are collectively referred to as macular pigment (MP).[10–12] MP is a short-wavelength (blue) light filter[1] and a powerful antioxidant[11] and is therefore believed to protect against AMD.[13,14] Consistent with the suggested protection that MP may afford against AMD, a recent study has shown that risk factors for AMD (including the three established risk factors: increasing age, family history of AMD, and cigarette smoking) are associated with a relative lack of MP[7]; however, the relationship between the spatial profile of MP and risk factors for this disease, if any, is not yet known.

To date, studies investigating the spatial profile of MP have reported its distribution as a first-order exponential decline with increasing retinal eccentricity.[15–18] However, variations in its distribution have been reported.[15,16,19] Recently, it has been shown that atypical MP spatial profiles are reproducible, when measured with customized heterochromatic flicker photometry (cHFP).[19]

The importance of such variations, if any, in the spatial profile of MP (e.g., the presence of a central dip) is not yet known, but may be related to the putative protective role of this pigment. For example, reduced MPOD at the center of the macula (i.e., the presence of a central dip) may be associated with increased risk of AMD (given the lower antioxidant activity and short-wavelength light-filtering capacity of the affected individual, when compared with an individual without such a central dip). Indeed, and consistent with this hypothesis, Trieschmann et al.,[20] in a study of 400 subjects (253 with signs of early AMD, 147 without AMD), reported that eyes with AMD are more likely to display low central MPOD when compared with non-AMD eyes.

It appears that L, Z, and *meso*-Z play central roles in the macula; however, determinants of their concentration and factors that influence their spatial distribution remain unclear. The purpose of this study was to investigate the association, if any, between the MP spatial profile and established (and putative) risk factors for AMD.

Methods

Subjects

Four hundred eighty-four subjects were recruited for this single-visit study. The subjects were recruited by a local poster campaign, by word of mouth in the college community, and by invitation. The study was approved by the Research Ethics Committee of Waterford Institute of Technology. The subjects were required to sign an informed-consent document before participating, and all experimental procedures adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria for participation were as follows: Caucasian race, age between 18 and 70 years, no evidence of ocular disease, visual acuity 6/12 or better in the study eye, and no current consumption of L and/or Z dietary supplements.

From the Macular Pigment Research Group, Department of Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Ireland.

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Corresponding author: John M. Nolan, Macular Pigment Research Group, Department of Chemical and Life Sciences, Waterford Institute of Technology, Cork Road, Waterford, Ireland; jmnolan@wit.ie.

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Personal Details, Lifestyle, and Risk Factor Assessment

The following details were recorded for each subject by questionnaire: demographic data, best corrected visual acuity, refractive status, family history of eye disease, height (meters), weight (kilograms), body mass index (BMI, kilograms divided by meters squared), personal ophthalmic and medical history, medication use, smoking status (never, past, or current smoker), alcohol consumption, iris color, ethnicity, and ocular and dermatologic sun sensitivity.

Fundus and Iris Color Assessment

Fundus photography was performed with a nonmydriatic auto fundus camera (AFC-210; Nidek, Gamagori, Japan). Both eyes of each subject were photographed to screen for signs of any retinal disease. Iris photography was also performed to document and categorize subjects with respect to iris color. Subjects with brown or hazel iris color were classified as having dark irides, whereas subjects with blue, green, or gray iris color were classified as having light irides.

Measurement of MPOD

The spatial profile of MP was measured by using a customized version of heterochromatic flicker photometry (cHFP). We used a Macular Densitometer (Macular Metrics, Corp., Providence, RI), an HFP instrument that was slightly modified from a device described by Wooten and Hammond.[21] A detailed description of the principle of HFP and its customization to accurately measure MP has been published by Kirby et al.[19]

To measure the spatial profile of MP, we performed measurements at the following degrees of eccentricity: 0.25°, 0.5°, 1°, 1.75°, and 7° (the reference point), obtained with the following sized target diameters: 30-minute, 1°, 2°, 3.5°, and 2°, respectively. Stimulus 5, our reference point, is a 2° diameter disc with its center located 7° from a red fixation point (i.e., the average of the inner arc, which defines the disc at 6.5° and the outer arc which defines the disc at 7.5°), as MPOD at this location is assumed to be optically undetectable and its distribution at this location is essentially flat.

The spatial profile for each subject was classified into one of two profile types based on individual MPOD results: The "exponential MP spatial profile" describes a steady decline in MPOD from the center (0.25°) to the periphery (7°), with each successive MPOD lower than the previous one (group 1; Fig. 1). The "central dip" MP spatial profile describes a dip in the central MP (0.25° retinal eccentricity), followed by an increase in MPOD at 0.5° retinal eccentricity, and finally a steady decline to the periphery. Subjects were classified as having a central dip MP spatial profile if the central MPOD (i.e., 0.25°) was lower than that at 0.5° (group 2; Fig. 2).

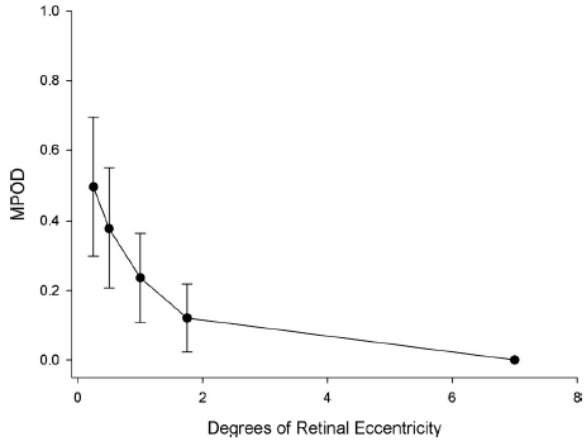

FIGURE 1. Average MP spatial profile for group 1, the typical exponential MP spatial profile. Data are expressed as the mean ± SD.

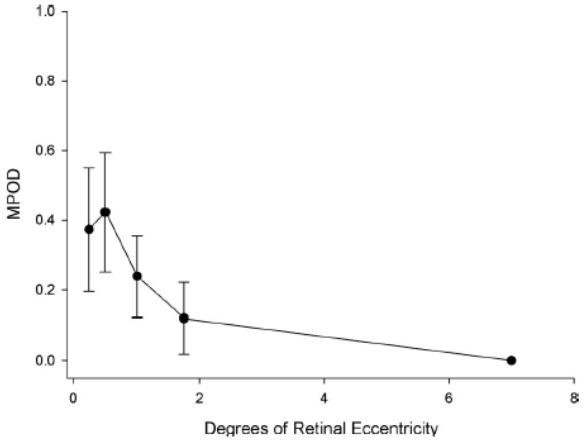

FIGURE 2. Average MP spatial profile for group 2, the central dip MP spatial profile. Data are expressed as the mean ± SD.

For each subject, the area of MPOD under the spatial profile curve was calculated by using the trapezoid rule, as follows: MPOD area = ({[(MPOD at 0.25° + MPOD at 0.5°)/2]*0.25} + {[(MPOD at 0.5° + MPOD at 1°)/2]*0.5} + {[(MPOD at 1° + MPOD at 1.75°)/2]*0.75} + {[(MPOD at 1.75° + MPOD at 7°)/2]*5.25}), assuming an MPOD of 0 at 7° retinal eccentricity and also assuming a linear point-to-point fit between each successive point of measurement of MPOD, but a nonlinear MP spatial profile overall. This MPOD area may give a better representation of actual MP quantity across the macula than does an individual optical density measurement at a single point of retinal eccentricity.

Food Frequency Questionnaire

Dietary intake was assessed with a self-administered, semiquantitative food frequency questionnaire developed by the Scottish Collaborative Group at the University of Aberdeen (Scotland, UK) and has previously been described by O'Connell et al.[22]

High-Performance Liquid Chromatography

Serum concentrations of L and Z were analyzed by using high-performance liquid chromatography (HPLC). A description of our extraction procedure and detailed specifications of the HPLC device can be found in a paper recently published by Loane et al.[23]

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards used to generate the response factors that were employed in calculating serum concentrations of L and Z. An internal standard, α-tocopherol acetate made up in ethanol (0.25 mg/L), was used to correct for the recovery of extractions for HPLC analysis and to assist in the quantification (α-tocopherol acetate recovery, 94% ± 4%). All chromatograms were integrated manually by drawing a baseline and dropping perpendicular lines to quantify the peaks of interest (ChemStation software; Agilent Technologies, Palo Alto, CA).

Statistical Analysis

Pearson correlation coefficients were calculated to investigate the relationship between continuous variables (e.g., MPOD, at each degree of retinal eccentricity, and age). One-way analysis of variance and/or independent-samples *t*-tests were used to analyze the relationship between a continuous variable (e.g., MPOD) and a categorical variable (e.g., MP profile group, sex). Box plots and histograms were used to graphically illustrate these relationships. Our main method of analysis was multiple linear regression with MPOD as the dependent variable (individual models were generated for each degree of retinal eccen-

TABLE 1. Demographic and Anthropometric Data for All Subjects, Group 1 and Group 2

	All Subjects (n = 484)	Group 1 (n = 426)*	Group 2 (n = 58)†
Age, y‡	42 ± 13	41.8 ± 12.8	46.9 ± 12.1
Sex, n (%)			
Male	183 (37.8)	161 (37.8)	22 (37.9)
Female	301 (62.2)	265 (62.2)	36 (62.1)
Family history of AMD, n (%)			
Positive	157 (32.4)	140 (89.2)	17 (10.8)
Negative	322 (66.5)	281 (87.3)	41 (12.7)
Body mass index	26.4 ± 4.4	26.4 ± 4.5	25.9 ± 4.2
Smoking status, n (%)			
Never‡	272 (56.2)	248 (91.2)	24 (8.8)
Past	127 (26.2)	109 (85.8)	18 (14.2)
Current‡	85 (17.6)	69 (81.2)	16 (18.8)
Iris color, n (%) (n = 442)			
Light	320 (66)	288 (90)	32 (10)
Dark	164 (33.9)	138 (84.1)	26 (15.9)
Weekly alcohol intake, n (%)			
0 Units	96 (19.8)	83 (86.5)	13 (13.5)
1 Unit	54 (11.2)	49 (90.7)	5 (9.3)
2–5 Units	103 (21.3)	91 (83.3)	12 (11.7)
6–10 Units	133 (27.5)	116 (87.2)	17 (12.8)
>10 Units	96 (19.8)	86 (89.6)	10 (10.4)
MPOD			
0.25°§‖	0.48 ± 0.19	0.49 ± 0.19	0.37 ± 0.17
0.5°	0.38 ± 0.17	0.37 ± 0.17	0.42 ± 0.17
1.0°	0.23 ± 0.13	0.23 ± 0.12	0.23 ± 0.11
1.75°	0.12 ± 0.10	0.12 ± 0.09	0.11 ± 0.10
MPOD area	0.71 ± 0.42	0.71 ± 0.42	0.71 ± 0.42
Diet, mg/day			
Diet L	1.38 ± 1.21	1.36 ± 1.2	1.59 ± 1.3
Diet Z	0.20 ± 0.12	0.2 ± 0.12	0.2 ± 0.11
Serum, μmol/L			
Serum L	0.45 ± 0.23	0.44 ± 0.23	0.46 ± 0.23
Serum Z	0.21 ± 0.11	0.21 ± 0.12	0.2 ± 0.09

* Exponential MP spatial profile type.
† Central dip MP spatial profile type.
‡ Significant difference between groups at the 0.05 level.
§ Degree of retinal eccentricity.
‖ Significant difference between groups at the 0.01 level.

tricity measured) and risk factors for AMD as potential explanatory variables. Binary logistic regression was used to analyze the relationship between MPOD spatial profile group and risk factors for AMD (all analyses: SPSS, ver. 15; SPSS, Chicago, IL).

RESULTS

Demographics

The anthropometric and lifestyle data for all subjects are presented in Table 1.

Macular Pigment Optical Density

Mean ± SD MPOD (at each measured degree of retinal eccentricity) and MPOD area for all subjects are presented in Table 1. Four hundred twenty-six (88%) of the 484 subjects had an exponential MP spatial profile (group 1; Fig. 1), and 58 (12%) had a central dip MP spatial profile (group 2; Fig. 2).

MPOD with Respect to Established Risk Factors for AMD

Age. There was no relationship between age and MPOD at any of the eccentricities measured (0.25°, 0.5°, 1.0°, or 1.75°) or between age and MPOD area ($P > 0.05$, for all). However, when the data were analyzed separately in the male and female subjects, there was a statistically significant inverse relationship between increasing age and MPOD at 0.25° retinal eccentricity, for men only ($r = -0.146$; $P = 0.049$; Fig. 3).

The mean ± SD age of the subjects in group 1 (exponential MP spatial profile) was 41.8 ±13 years, whereas the mean ± SD age of the subjects in group 2 (central dip MP spatial

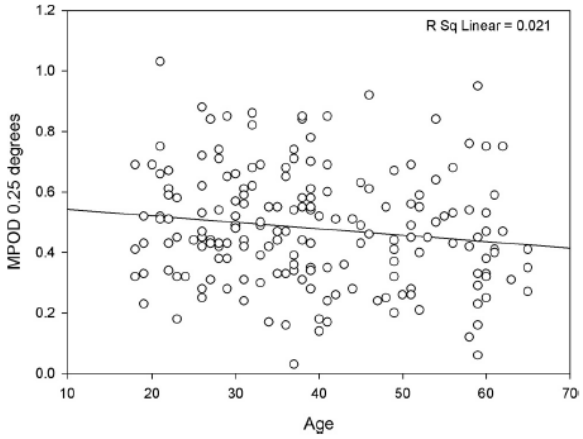

FIGURE 3. MPOD at 0.25° retinal eccentricity versus age.

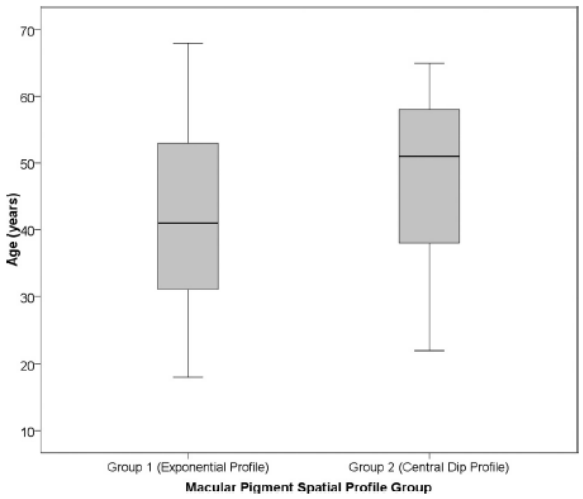

FIGURE 4. Age with respect to MP spatial profile group.

profile) was 46.9 ± 12 years (Table 1). There was a statistically significant difference between these two groups with respect to age ($P = 0.004$; Fig. 4).

Family History of AMD. The subjects with a confirmed family history of AMD ($n = 157$) had a mean ± SD MPOD at all eccentricities measured (0.25°, 0.5°, 1.0°, and 1.75°) that was statistically comparable to that in the subjects with no known family history of disease ($n = 322$; $P > 0.05$, for all eccentricities). The presence or absence of a confirmed family history of AMD was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.549$; Table 1).

Cigarette Smoking. There was no statistically significant difference in MPOD, at any of the eccentricities measured, nor in MPOD area, attributable to smoking status (i.e., never, past, current; $P > 0.05$ for all). However, there was a statistically significant relationship between MP spatial profile group type and cigarette smoking (Fig. 5, Table 2 [$P = 0.031$]; Table 3 [$P = 0.021$]). The percentage of subjects with a central dip MP spatial profile was 8.8% for the never smokers, rising to 14.2% for the past smokers, and the 18.8% for current smokers (Table 2). The logistic regression output in Table 4 shows that, with

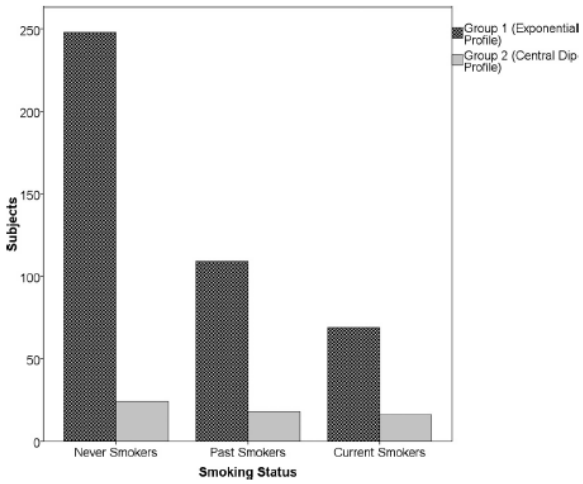

FIGURE 5. MP spatial profile group with respect to smoking status.

TABLE 2. Cross-tabulation of Smoking Status versus MP Profile Group Type

	MP Spatial Profile Type		
	Group 1*	Group 2†	Total
Never smoker	248 (91.2)	24 (8.8)‡	271 (100)
Past smoker	109 (85.8)	18 (14.2)	127 (100)
Current smoker	69 (81.2)	16 (18.8)	85 (100)
Total	426 (88)	58 (12)	484 (100)

Data are expressed as *n* (%).
* Typical exponential MP spatial profile.
† Central dip MP spatial profile.
§ Significantly different from current smokers at the 0.05 level.

adjustment for age, the never smokers were significantly less likely to have a central dip MP spatial profile than were the current smokers ($P = 0.005$) and also that the percentage of central dip MP spatial profiles was lower in the past smokers than in the current smokers, but not significantly so ($P = 0.104$).

MPOD with Respect to Putative Risk Factors for AMD

Sex. There was no statistically significant difference in MPOD (at any of the eccentricities measured), nor in MPOD area, between the male and female subjects ($P > 0.05$, for all). Sex was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.984$; Table 1).

Obesity. There was a weak, but statistically significant, inverse relationship between BMI and MPOD at 0.25°, 0.5°,and 1.0° retinal eccentricity ($r = -0.093$ to -0.131; $P < 0.05$, for all). There was also a weak, but statistically significant, inverse relationship between BMI and MPOD area ($P = 0.047$, $r = -0.091$). BMI was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.390$; Table 1).

Dietary L and Z. Data on dietary intake of L and Z are presented in Table 1. There was no statistically significant relationship between dietary intake of L or Z and MPOD (at any of the eccentricities measured) or MPOD area ($P > 0.05$, for all). Dietary intake of L and Z was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.182$ and 0.983, respectively; Table 1).

Serum L and Z. Data on serum concentrations of L and Z are presented in Table 1. There was a positive and statistically significant relationship of serum concentrations of L and Z to MPOD (at each degree of eccentricity measured) and MPOD area ($P < 0.05$, for all). Serum concentrations of L and Z were unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.629$ and 0.312, respectively; Table 1).

Iris Color. Subjects with dark-colored irises had a significantly higher MPOD at 0.5° and 1.0° than did subjects with light-colored irises ($P = 0.007$ and $P = 0.045$), respectively. Iris color was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.069$; Table 1).

Alcohol Consumption. There were no statistically significant differences between MPOD (at any of the eccentricities measured) and any of the five categories of alcohol consumption (see Table 1; $P > 0.05$, for all). Alcohol consumption was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.922$; Table 1).

Relation of MPOD with Risk Factors for AMD, as Assessed by Multiple Linear Regression

Multiple linear regression analysis, with indicator variables used as categorical variables, was performed to analyze the

TABLE 3. Binary Logistic Regression Analysis for Relationship of MPOD Spatial Profile Type to Known and Putative Risk Factors for AMD

	B	SE	Wald	*df*	Sig.	Exp(B)	95% CI for Exp(B)
Age	0.035	0.012	8.541	1	0.003	1.036	1.012–1.061
Cigarettes			7.731	2	0.021		
Never smoker	−0.996	0.358	7.729	1	0.005	0.369	0.183–0.745
Past smoker	−0.642	0.394	2.65	1	0.104	0.526	0.243–1.14
Constant	−2.885	0.579	24.822	1	0.000	0.056	

The reference group for the cigarettes variable is the current smokers group. 95% CI, 95% confidence interval.

relationship between MPOD at each degree of eccentricity measured and the following known and putative risk factors for AMD: age, cigarette smoking, family history of AMD, sex, BMI, dietary L, dietary Z, serum L, serum Z, iris color, and alcohol consumption.

Variables for all these factors were included initially in a multiple linear regression model, with MPOD as the dependent variable, run separately for each retinal eccentricity measured. Statistically nonsignificant variables were then removed, one by one, using the 5% level of significance as the criterion for removal. The final regression model, for each degree of retinal eccentricity measured and for MPOD area, is presented in Table 4; these models explained 15%, 14%, 10%, and 0.04% of the variance in MPOD at the eccentricities 0.25°, 0.5°, 1.0°, and 1.75°, respectively; Fig 6).

Relation of MPOD Spatial Profile Group with Risk Factors for AMD, as Assessed by Binary Logistic Regression

Binary logistic regression analysis, with indicator variables as the categorical variables, was performed to analyze the relationship between MPOD spatial profile group and the following known and putative risk factors for AMD: age, cigarette smoking, family history of AMD, sex, BMI, dietary L, dietary Z, serum L, serum Z, iris color, and alcohol intake.

Variables for all these factors were included initially in a binary logistic regression model, with MPOD spatial profile group as the dependent variable. Statistically nonsignificant variables were then removed, one by one, with the 5% level of significance as the criterion for removal. The final regression model, for MPOD spatial profile group, is presented in Table 3; this model showed age and cigarette smoking to be independent significant predictors of MP spatial profile group type.

DISCUSSION

Previous studies have shown that healthy subjects (i.e., subjects without AMD) have relatively less MP in the presence of all established,[7] and several putative,[7,24] risk factors for AMD. The purpose of this study was to investigate the association, if any, between the spatial profile of MPOD and these risk factors (i.e., age, family history of AMD, cigarette smoking, sex, obesity, dietary carotenoid intake, and iris color). We collected risk factor data and MP spatial profile data of 484 healthy subjects. The MP spatial profile of each subject was categorized into one of two profile types: the typical exponential profile (group 1) or the central dip profile (group 2).

Analyzing our study population as a whole (n = 484), we found the three established risk factors for AMD—increasing age, cigarette smoking, and family history of the disease—were unrelated to MPOD at any measured degree of retinal eccentricity. When we split our study population by sex, we found a weak, but statistically significant inverse relationship between age and central MPOD (0.25° retinal eccentricity), but in males only. The relationship between increasing age and

TABLE 4. Multiple Linear Regression Analysis with Respect to MPOD at Each Eccentricity Measured and MPOD Area and Known and Putative Risk Factors for AMD

Model	Unstandardized Coefficients		Standardized Coefficients		
	B	SE	B	*t*	Sig.
Dependent variable: MPOD at 0.25° retinal eccentricity					
Constant	0.373	0.019		19.347	0.000
Serum L, μmol/L	0.287	0.038	0.331	7.606	0.000
MPOD profile type	−0.131	0.027	−0.214	−4.921	0.000
Dependent variable: MPOD at 0.5° retinal eccentricity					
Constant	0.251	0.017		14.444	0.000
Serum L, μmol/L	0.27	0.033	0.36	8.222	0.000
Iris color	0.037	0.016	0.103	2.348	0.019
Dependent variable: MPOD at 1.0° retinal eccentricity					
Constant	0.154	0.013		11.736	0.000
Serum L, μmol/L	0.168	0.025	0.303	6.75	0.000
Iris color	0.025	0.012	0.092	2.057	0.040
Dependent variable: MPOD at 1.75° retinal eccentricity					
Constant	0.083	0.01		8.335	0.000
Serum L, μmol/L	0.088	0.02	0.207	4.483	0.000
Dependent variable: MPOD area					
Constant	0.493	0.042	11.649	0.000	
Serum L, μmol/L	0.508	0.084	0.275	6.045	0.000

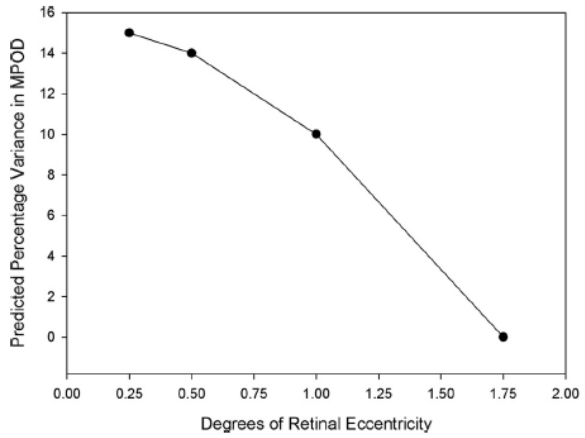

FIGURE 6. Predicted variance in MPOD at each degree of retinal eccentricity measured. The data in this graph are also presented in Table 4.

MPOD has been widely reported in the literature.[7,25,26] The results, however, are somewhat inconsistent.

The absence of this relationship in female subjects is consistent with the findings of the Carotenoids in Age-Related Eye Disease Study (CAREDS), a cross-sectional study of 1698 female subjects, in which MPOD was found to be unrelated to age or smoking (at any measured degree of retinal eccentricity).[27] Sex differences in the metabolism, transport, and accumulation of carotenoids have been documented.[24,28,29] These studies report that female subjects have lower average MPOD, but higher adipose tissue concentrations and higher circulating serum L concentrations than do male subjects. It is possible, therefore, that the L contained in the adipose tissue and circulating in the serum of female subjects may act as a buffer against any decline in MPOD with increasing age.

We report a positive and statistically significant relationship between serum concentrations of L and MPOD (at all degrees of retinal eccentricity measured). This finding is unsurprising, given the exclusive dietary origin of MP and has been reported previously in the literature.[30,31] Also, we found that light iris color (i.e., blue, green, or gray) was associated with having significantly lower levels of MPOD (at 0.5° and 1.0° retinal eccentricity), when compared with that present with dark iris color (i.e., brown or hazel). This finding has also been supported in the literature and may represent a greater transmission of short-wavelength light in eyes with lighter colored irides than in eyes with darker colored irides (given that there was no significant difference between subjects with light and dark irides with respect to age, sex, smoking status, BMI, or alcohol intake), leading to increased free radical production in such individuals and a consequential depletion of their MP.[9]

A key and novel finding of our study was the association between established risk factors for AMD (i.e., age and cigarette smoking) and MPOD spatial profile group type. We found that age was a significant predictor of MP spatial profile group type, with the younger subjects having the typical exponential MP spatial profile (group 1), whereas the older subjects were more likely to have a central dip in their MP spatial profile (group 2), even after adjustment for smoking habits. It should be noted that the subjects in group 1 were significantly younger (5 ± 12 years) than those in group 2. Previous investigators who reported atypical MP spatial profiles, measured using fundus autofluorescence, reported no such association with age.[15,16] It has been suggested that the spatial profile of MP may be affected by unique features of the foveal anatomic architecture.[1] For example, Kirby et al.,[19] using HFP, recently reported an association between a wider foveal depression and the presence of a secondary peak (equivalent to the central dip group defined herein) in the MP spatial profile. However, foveal width was not measured in our study.

Cigarette smoking also emerged from our logistic regression model as a significant predictor of MP spatial profile group type (group 1 or 2). The presence of the typical exponential MP spatial profile was significantly more common in the subjects who never smoked cigarettes when compared with the profile in the past and current cigarette smokers. Conversely, the presence of the central dip MP spatial profile was significantly more common in the current cigarette smokers than in the past or never smokers, even after adjustment for age. Several studies have found cigarette smoking to be a negative predictor of MPOD.[7,32] Although such a finding was not reproduced in this study, we report a novel association between the central dip MP profile group and current cigarette smoking.

To explain our finding, we suggest that the known increased levels of oxidative stress associated with cigarette smoking may have contributed to the association.[32,33] Furthermore, the observation that the central dip MP spatial profile was more common in the current smokers than in the past smokers (albeit to a nonsignificant level) and in comparison to that in the never smokers (to a statistically significant level), suggests that smoking status influences the MP spatial profile centrally. Indeed, a dose–response relationship between cigarette smoking and MPOD levels has been reported in the literature.[7]

Of particular relevance to this study are the recently published findings by Connolly et al.,[34] who reported on serum and MPOD (measured at 0.25°, 0.5°, 1.0°, and 1.75° retinal eccentricity) in response to supplementation with *meso*-Z (7.3 mg; the dominant carotenoid in formulation), L (3.7 mg), and Z (0.8 mg). In their study, 4 of the 10 subjects studied displayed an atypical or central dip (i.e., lower MPOD at 0.25° degrees than at 0.5° degrees of retinal eccentricity) in their MPOD spatial profiles at baseline. However, after just 8 weeks of supplementation, all four subjects displayed the more typical exponential MPOD spatial profile, after augmenting their (0.25°) MPOD centrally. Of note, *meso*-Z (the dominant carotenoid in that study formulation) is the predominant Z-isomer at the foveal center.[20] The presence of *meso*-Z at the macula is attributed to generation from retinal L (as it is not normally found in a typical Western diet).[20] Thus, Connolly et al. speculated that subjects who initially display a central dip MPOD spatial profile are unable to convert L to *meso*-Z at this location. In relation to our finding, it is tempting to hypothesize that increased oxidative stress, a factor common to both older age and cigarette smoking, may prevent the conversion of L to *meso*-Z, in such subjects; however, further study is clearly warranted in this area.

Given the cumulative and chronic nature of AMD, it is reasonable to suggest that any putative protective effect of MP is necessary from a relatively young age and throughout an individual's lifetime. This study was designed to further our understanding of the determinants of MP and its spatial distribution. In conclusion, older subjects and cigarette smokers were more likely to display a central dip in their MP spatial profile. Furthermore, and in light of the established link of age and cigarette smoking with AMD, and consistent with the recent postmortem study by Trieschmann et al.,[20] who reported that AMD subjects were more likely to display low central MPOD than were non-AMD subjects, we speculate that the central dip MPOD spatial profile represents an undesirable distribution of MP.

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The relationship between macular pigment and visual performance ☆

James Loughman [a,1], Mukunda C. Akkali [b], Stephen Beatty [b,2], Grainne Scanlon [a], Peter A. Davison [a], Veronica O'Dwyer [a], Tom Cantwell [c], Philip Major [b], Jim Stack [b], John M. Nolan [b,*,2]

[a] Macular Pigment Research Group, Optometry Department, Dublin Institute of Technology, Dublin, Ireland
[b] Macular Pigment Research Group, Waterford Institute of Technology, Waterford, Ireland
[c] FOCAS Institute, School of Physics, Dublin Institute of Technology, Dublin, Ireland

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ABSTRACT

This study was designed to assess whether macular pigment optical density (MPOD) is associated with visual performance. One hundred and forty-two young healthy subjects were recruited. Macular pigment optical density and visual performance were assessed by psychophysical tests including best corrected visual acuity (BCVA), mesopic and photopic contrast sensitivity, glare sensitivity, photostress recovery time (PRT). Measures of central visual function, including BCVA and contrast sensitivity, were positively associated with MPOD ($p < 0.05$, for all). Photostress recovery and glare sensitivity were unrelated to MPOD ($p > 0.05$). A longitudinal, placebo-controlled and randomized supplementation trial will be required to ascertain whether augmentation of MPOD can influence visual performance.

1. Introduction

The macula is a specialized part of the retina and is responsible for high spatial resolution and color vision (Hirsch & Curcio, 1989). The carotenoids lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (*meso*-Z) accumulate at the macula where they are collectively referred to as macular pigment (MP). (Bone, Landrum, Hime, Cains, & Zamor, 1993) L and Z are of dietary origin, whereas *meso*-Z is not normally found in a conventional diet, and is generated at the retina following L isomerization (Bone et al., 1993; Neuringer, Sandstrom, Johnson, & Snodderly, 2004).

Age-related macular degeneration (AMD) is a disease of the macula and results in the loss of central and color vision. AMD is the most common cause of blindness in the elderly population in the developed world (Congdon et al., 2004). It is now understood that oxidative stress (Beatty, Koh, Henson, & Boulton, 2000; Winkler, Boulton, Gottsch, & Sternberg, 1999), exacerbated in part by cumulative short-wavelength visible light exposure (Algvere, Marshall, & Seregard, 2006; Fletcher et al., 2008), is important in the aetiopathogenesis of AMD. MP is a short-wavelength (blue) light filter (Bone, Landrum, & Cains, 1992) and a powerful antioxidant (Khachik, Bernstein, & Garland, 1997), and is therefore believed to protect against AMD. This hypothesis, referred to as the "protective" hypothesis of MP, has been studied and reported on extensively (Loane, Kelliher, Beatty, & Nolan, 2008).

Beyond its "protective" hypothesis, MP's optical and anatomic properties have prompted the "optical" hypotheses of this pigment. The "optical" hypotheses of MP were originally discussed by Reading and Weale (1974) and later by Nussbaum, Pruett, & Delori (1981) and include MP's putative ability to enhance visual performance and/or comfort by attenuation of the effects of chromatic aberration and light scatter, via its light-filtering properties (Walls & Judd, 1933).

Several studies have evaluated, and reported on, the role of MP in various aspects of visual performance including visual acuity, contrast sensitivity, glare sensitivity, photostress recovery, critical flicker fusion frequency (CFF), and color vision, among others (Bartlett & Eperjesi, 2008; Engles, Wooten, & Hammond, 2007; Hammond & Wooten, 2005; Kvansakul et al., 2006; Rodriguez-Carmona et al., 2006; Stringham, Fuld, & Wenzel, 2004; Stringham & Hammond, 2007; Stringham & Hammond, 2008; Wooten & Hammond, 2002). However, the findings from these studies are inconsistent, which might be explained, at least in part, by methodological differences between studies.

In this manuscript, we present baseline data from the Collaborative Optical Macular Pigment ASsessment Study (COMPASS), and as such represents a cross-sectional evaluation of the relationship between MP optical density (MPOD) and visual performance and comfort across a broad and refined range of functional tests.

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* Corresponding author. Fax: +353 51 302679.
E-mail address: jnolan@wit.ie (J.M. Nolan).
[1] This author does consultancy work for nutraceutical companies in a personal capacity.
[2] These authors do consultancy work for nutraceutical companies, in a personal capacity, and as directors of Nutrasight Consultancy Limited.

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2. Methods

2.1. Subjects

One hundred and forty-two healthy subjects volunteered to participate in this study, which was approved by the research ethics committees at both Waterford Institute of Technology (WIT) and Dublin Institute of Technology (DIT). Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

The study was conducted at WIT and DIT vision science laboratories, located in the southeast and east of the Republic of Ireland, respectively. Self-selected recruitment of subjects (WIT: $n = 61$ and DIT: $n = 81$) was facilitated by poster and newsletter advertisement, and also by word of mouth, in the respective local communities. All subjects were aged between 18 and 41 years, in perfect general (self report) and ocular health, and with visual acuity of at least 20/30 in the study eye. A typical study visit lasted approximately four hours. Those aspects of visual performance assessed, and their sequence, are presented in Table 1.

All subjects recruited into the study could be classed as naïve observers to the tests carried out (with the exception of the visual acuity test, with which all subjects were familiar). To optimize performance, and also to minimize any potential learning effects on performance, all subjects underwent a defined period of pre-test training. This training consisted of careful explanation of the nature of each test, pictorial and/or video demonstration of the test requirements and procedure, and was followed by a defined session of pre-test practice.

2.2. Demographic, medical history, lifestyle and vision case history questionnaires

The following details were recorded for each volunteer by questionnaire: demographics; general health status; smoking habits (never, current or past); alcohol consumption (average unit weekly intake); exercise (minutes per week); body mass index (BMI, kg/m^2); blood pressure; ethnicity; marital status; education; occupation.

Vision case history included: time since last eye examination; spectacles or contact lens use; history of ocular treatment or surgery; history of occlusion therapy or visual training in childhood; family history of eye disease; current problems with vision; asthenopia associated with computer use; history of headaches.

Table 1
Parameters assessed and their sequence for a typical study visit.

Description	Time (min)
Information leaflet discussion and informed consent	10
Collection of blood for serum carotenoid analysis	10
Demographic, medical history, lifestyle and vision case history questionnaires	20
Spectacle refraction, visual acuity, and ocular dominance	25
Color vision	20
Glare sensitivity	10
Visual function questionnaire	10
Contrast sensitivity	25
Break	~30
Macular pigment optical density spatial profile	30
Dietary questionnaire	30
Short wavelength automated perimetry	15
Photostress recovery	15
Fundus and iris photography	10
Total time	260

2.3. Spectacle refraction, visual acuity, and ocular dominance

Each subject underwent precise spectacle refraction by an experienced optometrist to determine refractive error and best corrected visual acuity (BCVA) for each eye. A computer generated LogMAR test chart (Test Chart 2000 Pro; Thomson Software Solutions) was used to determine BCVA at a viewing distance of 4 m, using a Sloan ETDRS letterset. BCVA was determined as the average of three measurements, with letter and line changes facilitated by the software pseudo-randomization feature. Best corrected visual acuity was recorded using a letter-scoring visual acuity rating, with 20/20 visual acuity assigned a value of 100. Best corrected visual acuity was scored relative to this value, with each letter correctly identified assigned a nominal value of one, so that, for example, a BCVA of 20/20^{+1} equated to a score of 101, and 20/20^{-1} to 99. The study eye was selected on the basis of ocular dominance, determined using the Miles Test (Roth, Lora, & Heilman, 2002) with the dominant eye chosen as the study eye, except in cases of observed equidominance, in which case the right eye was selected. All subsequent tests were conducted with the subject's optimal subjective refraction in place.

2.4. Glare sensitivity

Glare sensitivity was assessed using a Functional Vision Analyzer (Hohberger, Laemmer, Adler, Juenemann, & Horn, 2007) (Stereo Optical Co., Inc., Chicago, IL) using the Functional Acuity Contrast Test (FACT) Hitchcock, Dick, & Krieg, 2004; Terzi, Buhren, Wesemann, & Kohnen, 2005) and a customized inbuilt glare source. The test comprised linear, vertically oriented, sine wave gratings presented at five different spatial frequencies including 1.5, 3, 6, 12 and 18 cycles per degree (cpd). Nine circular patches were presented at each spatial frequency, the contrast of each patch decreasing by 0.15 log units from the previous. Gratings were tilted −15°, 0° or +15° with respect to the vertical, to keep them within the orientation bandwidth of the visual channel. The background was tapered into a grey field in order to keep retinal illumination constant and avoid ghost imaging. Baseline contrast sensitivity was determined on the basis of the lowest contrast compatible with accurate determination of patch orientation across all five spatial frequencies for mesopic (3 cd m^{-2}) conditions, initially in the absence of a glare source. Subjects were asked to identify grating orientation, starting with the patch at highest contrast, and continuing until identification was no longer possible due to reducing contrast. Subjects were instructed not to guess, but to respond "don't know" if patch orientation could not be correctly identified.

Glare sensitivity was assessed using a radial glare source consisting of 12 white LED's arranged circumferentially in an oval pattern surrounding the grating charts (ranging from 4.5° to 6° from central fixation). Two customized intensity settings were used to determine the effect of different levels of glare on contrast sensitivity. Glare source settings were set at a medium intensity of 42 Lux and a higher intensity of 84 Lux. All correct responses were entered into the Eyeview software provided, and contrast sensitivity scores for no glare, medium and high glare conditions were determined for the respective spatial frequencies.

2.5. Contrast sensitivity function

A Dell Dimension 9200 computer and a Metropsis Visual Stimulus Generation device (VSG (ViSaGe S/N: 81020197), Cambridge Research Systems Ltd., Cambridge, U.K.) were used to generate and control the stimuli. The VSG provided 14-bit output resolution per phosphor. The stimuli were displayed on a 19″ ViewSonic professional series p227f color CRT flat screen monitor with a frame

rate of 119.98 Hz. The resolution of the monitor was set to 1024 × 769 pixels. Non-linearities in the screen luminance output were eliminated by gamma correction prior to testing using a photometer system (Opti-Cal; Minolta, Japan). The Metropsis software calculated the inverse curves required to correct for the monitor's non-linearities.

The Metropsis contrast sensitivity system generated luminance modulated sine gratings (Gabor patches). The orientation of the stimuli was vertical. The Gabor patches were presented on the CRT monitor and subtended a visual angle of 4.2°. The mean luminance was used as the background luminance. The Gabor had a two-dimensional spatial Gaussian envelope and was radially symmetrical with equal standard deviations, δx and δy.

Contrast sensitivity functions were determined under both mesopic and photopic conditions. Each subject was seated at a fixed viewing distance of 1.5 m from the CRT monitor. Natural pupils were used throughout the experiment. The non-dominant eye was occluded. Testing was carried out in a light free environment. The subject was dark adapted for 5 min and a 5-min training session was given prior to testing under mesopic conditions. Subject responses were recorded using a handheld responder (CR6, Cambridge Research Systems Ltd., Cambridge, UK), which communicated with the VSG device via an infra red link. A four alternate forced choice testing system was used, with four possible target locations. The stimuli were randomly presented at 2° spatial offset from the central cross target. The subject indicated the location of the target in relation to the fixation cross using the appropriate button on the responder box. The subject's contrast sensitivity was determined for five different spatial frequencies (1.0, 4.1, 7.5, 11.8 and 20.7 cpd) under both mesopic and photopic conditions, all at a mean luminance of 3 cd m^{-2} (mesopic) and 100 cd m^{-2} (photopic).

A linear staircase method was used to determine the contrast threshold. The first Gabor at a particular location was presented at an initial contrast level where it was anticipated that the observer would be able to detect the Gabor patch for that particular spatial frequency (initial contrast settings were informed by a brief pilot study involving five young healthy subjects). Subsequently, the contrast of the Gabor patch was varied using an adaptive staircase procedure, which was computer controlled and depended upon the subject's responses. The stimulus contrast was reduced in steps of 0.3 log units until the subject did not detect the Gabor patch (first reversal). The contrast was subsequently increased by 0.15-log unit steps until the subject saw the Gabor patch and responded correctly (second reversal). The Metropsis software calculated the contrast threshold for each location and spatial frequency by taking the mid-point between the mean for peaks and troughs for 12 reversal points. The standard deviation was calculated by taking the deviations of the peak reversals from their peak means and using the average square of these deviations to calculate a peak variance. This method was repeated for the troughs. The square root of both variances were then calculated and averaged to provide the threshold standard deviation.

For each subject, the Metropsis software plotted the inverse of the contrast threshold against the range of spatial frequencies tested to provide a contrast sensitivity function under both mesopic and photopic conditions.

2.6. Photostress recovery

Photostress recovery time (PRT) was calculated using a macular automated photostress (MAP) test. (Dhalla & Fantin, 2005; Dhalla, Fantin, Blinder, & Bakal, 2007) MAP is a novel photostress method for the evaluation of macular function using the Humphrey® field analyzer (Model 745*i* Carl Zeiss Meditec Inc. Dublin, CA, USA). The foveal threshold feature of the field analyzer was used to establish baseline foveal sensitivity as the average of three consecutive foveal sensitivity measurements recorded in decibels (dB), with each dB representing a 0.1 log unit sensitivity variation.

Following baseline foveal sensitivity calculation, the subject was exposed to a photostress stimulus, which consisted of a 5-s exposure to a 300-W, 230 V tungsten lamp head from a viewing distance of one meter. The spectral irradiance in the wavelength range, 300–800 nm, was measured using a Bentham DMc 150 double monochromator scanning spectroradiometer. The input optic consisted of a very high precision cosine response diffuser (f2 error < 1%) and the measurements were performed in 1 nm intervals. Calibration was carried out with reference to a quartz-halogen lamp traceable to the UK National Physical Laboratory. The illuminance at 1 m was obtained by using the photopic weighting function. The spectral irradiance at 1 m fixation distance from the photostress lamp is presented in Fig. 1.

Immediately post-photostress, a continuous and timed cycle of foveal sensitivity measurements were conducted and recorded for each subject. The reduction in foveal sensitivity from baseline, along with the time taken to recover to baseline foveal sensitivity, was recorded.

2.7. Macular pigment optical density

We used the Macular Densitometer™, a device developed and originally described by Wooten, Hammond, Land, and Snodderly (1999) to measure MPOD, including its spatial profile across the retina (i.e. 0.25°, 0.5°, 1.0°, 1.75° and 3° of retinal eccentricity). The Macular Densitometer™ uses heterochromatic flicker photometry (HFP) to obtain a valid measure of MPOD at a given retinal location (Hammond, Wooten, & Smollon, 2005). This method has recently been refined and is now referred to as customized HFP or cHFP. For a detailed description of this protocol please see recent publications by our research group and others (Loane, Stack, Beatty, & Nolan, 2007; Nolan et al., 2009; Stringham et al., 2008). One subject (cwit2553) was excluded from analysis due to inability to use the Densitometer to obtain reliable MPOD data.

2.8. Fundus photography

Fundus photographs were obtained in both eyes using a NIDEK non-mydriatic fundus camera (AFC-230). Fundus photographs were assessed by a qualified optometrist to exclude fundoscopically evident retinal/nerve pathology.

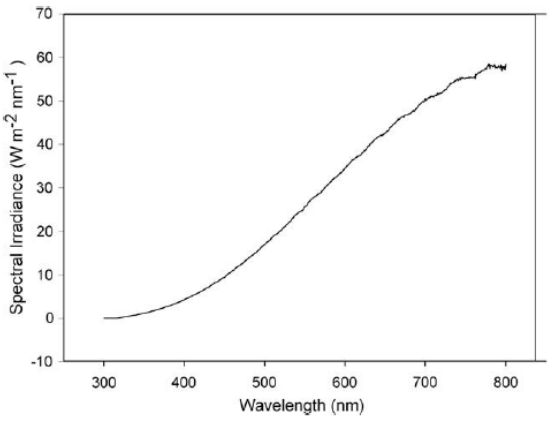

Fig. 1. Spectral Irradiance at 1 m fixation distance from Arri 300 photostress lamp.

2.8.1. Reliability testing of methods

Given that all subjects recruited into the study were classed as "naïve" to the tests carried out (with the exception of the visual acuity test), we conducted a pilot reliability study prior to the study commencing. Following pre-test training (see above), repeat testing on 10 subjects at three separate study visits (over a 10 day period) was conducted. The intraclass correlations (ICC) obtained for all methods were high and are presented in Table 2. In addition, repeat testing of radiance values obtained to compute MPOD values had previously been conducted by our research group. The data from this investigation concluded that the radiance values obtained using the Densitometer were very high (i.e. ICC in the range of 0.93–0.96; see recent publication by (Kirby et al., 2009) In addition, we conducted Bland Altman analyses of differences in MPOD at eccentricities 0.25°, 0.5°, 1° and 1.75°, measured at two separate study visits. The limits of agreement, at all eccentricities, were in the range 0.06–0.07 units away from the mean difference, which seems satisfactory. The coefficient of repeatability ranged from about 6% at the central eccentricities (0.25°, 0.5°), to 19.4% at 1.75°.

Mean differences in MPOD between study visits were 0.02, −0.01, 0.02, and 0.0 at eccentricities 0.25°, 0.5°, 1° and 1.75°, respectively. The first two of these differences were statistically significant, at the 5% level, using the paired *t*-test, suggesting bias; clinically, however, a bias of this very small magnitude is of no practical importance.

2.9. Statistical analysis

The statistical software package SPSS (version 17) was used for analysis. All variables investigated exhibited a typical normal distribution. Mean ± SD's are presented in the text. Pearson correlation coefficients were calculated to investigate bivariate relationships and partial correlation coefficients when controlling for confounding variables. We used the 5% level of significance throughout our analysis. A statistical power analysis determined a minimum sample size of 91 subjects in order to achieve 99% power with a one-tailed 5% test, with an affect size of ρ (rho) = 0.4. The 142 subjects recruited exceed these stringent statistical requirements, but more importantly, allowed for continued follow up (and standard drop-out) as part of the COMPASS lutein interventional study (ISRCTN number = 35481392), which was designed to investigate whether MPOD augmentation, following lutein supplementation, improves visual performance. Of note, this study is currently on-going.

3. Results

The demographic, medical, lifestyle, anthropometric, and vision-related data of the 142 subjects recruited into the study are summarized in Table 3. No subject was excluded from the study on the basis of fundus findings. The mean (±SD) age of the sample was 29 (±6) and ranged from 18 to 41 years. The mean (±SD) BMI was 25 (±4) and ranged from 19 to 43.

3.1. Macular pigment optical density

The mean (±SD) MPOD, at all degrees of retinal eccentricity measured, is summarized in Table 4. MPOD at peak (0.25° eccentricity) was positively and significantly correlated with MPOD at

Table 2
Reproducibility of visual performance tests used in COMPASS, assessed using intraclass correlation coefficient (ICC).

Test	Visit 1	Visit 2	Visit 3	ICC
Mesopic CSF[b] with no glare (cpd)				
1.5	1.55 (±0.21)	1.68 (±0.23)	1.62 (±0.20)	0.683
3	1.67 (±0.27)	1.74 (±0.24)	1.77 (±0.23)	0.852
6	1.51 (±0.58)	1.64 (±0.27)	1.61 (±0.25)	0.682
12	0.78 (±0.61)	0.88 (±0.52)	0.97 (±0.57)	0.867
18	0.56 (±0.45)	0.43 (±0.53)	0.39 (±0.46)	0.843
Mesopic CSF under medium glare lights (cpd)				
1.5	1.47 (±0.20)	1.55 (±0.22)	1.45 (±0.21)	0.626
3	1.31 (±0.54)	1.52 (±0.34)	1.43 (±0.57)	0.533
6	1.03 (±0.77)	1.16 (±0.69)	1.18 (±0.68)	0.893
12	0.49 (±0.59)	0.60 (±0.58)	0.51 (±0.62)	0.770
18	0.19 (±0.37)	0.25 (±0.39)	0.33 (±0.41)	0.767
Mesopic CSF under high glare lights (cpd)				
1.5	1.25 (±0.52)	1.34 (±0.32)	1.28 (±0.52)	0.829
3	1.26 (±0.55)	1.33 (±0.56)	1.30 (±0.51)	0.942
6	1.01 (±0.77)	0.94 (±0.71)	0.98 (±0.74)	0.978
12	0.48 (±0.57)	0.33 (±0.50)	0.36 (±0.55)	0.485
18	0.19 (±0.37)	0.07 (±0.20)	0.13 (±0.27)	0.707
CSF by metropsis mesopic (cpd)				
1	1.54 (±0.10)	1.55 (±0.15)	1.60 (±0.11)	0.432
4.1	1.73 (±0.15)	1.77 (±0.13)	1.77 (±0.17)	0.399
7.5	1.32 (±0.09)	1.31 (±0.15)	1.34 (±0.18)	0.683
11.8	0.83 (±0.14)	0.84 (±0.18)	0.82 (±0.23)	0.732
20.7	0.22 (±0.07)	0.24 (±0.09)	0.25 (±0.09)	0.746
Photopic CSF (cpd)				
1.0	1.60 (±0.17)	1.58 (±0.15)	1.59 (±0.15)	0.645
4.1	1.95 (±0.13)	1.98 (±0.13)	1.97 (±0.13)	0.662
7.5	1.75 (±0.13)	1.75 (±0.17)	1.78 (±0.18)	0.632
11.8	1.29 (±0.21)	1.34 (±0.25)	1.39 (±0.25)	0.727
20.7	0.43 (±0.24)	0.43 (±0.19)	0.41 (±0.20)	0.857
Photostress recovery test	37.41 (±1.30)	38.41 (±1.52)	38.08 (±1.68)	0.560

[a] ICC = intraclass correlation coefficient.
[b] CSF = contrast sensitivity function.

Table 3
Demographic, medical, lifestyle, anthropometric, and ocular related data for the entire study group.

Characteristic	n[a]
Sex	
Male	74
Female	68
Medical history	
Diabetes	1
High blood pressure	4
High cholesterol	6
Angina	0
Stroke	0
Family history of eye diseases	
Unknown	3
AMD	22
Cataract	12
Glaucoma	28
Retinal problem	4
None	82
Smoking habits[b]	
Never smoked	86
Ex-smoker	25
Current smoker	31
Exposed second-hand smoke	17
BMI	
Desirable weight (BMI < 25)	83
Overweight (BMI 25–30)	42
Obese (BMI > 30)	17
Ocular dominance	
Right	86
Left	53
Equidominant	3
BCVA	
<100	1
100–105	3
>105–110	42
>110–115	79
>115–120	17

[a] *n* = sample size.
[b] Smoking habits: ex-smoker = smoked ⩾ 100 cigarettes in lifetime but none in last 12 months; current smoker = smoked ⩾ 100 cigarettes in lifetime and at least 1 cigarette per week in last 12 months; exposed second-hand smoke = commonly exposed to second-hand smoke at home or in the work place.

Table 4
MPOD at all measured degrees of retinal eccentricity, for the entire study group.

Retinal eccentricity[a] (°)	MPOD[b]
0.25	0.48 (±0.19)
0.5	0.39 (±0.17)
1	0.21 (±0.12)
1.75	0.09 (±0.09)
3	0.09 (±0.07)
Average	0.25 (±0.12)

n = 141.
[a] Degrees retinal eccentricity.
[b] MPOD = mean (±SD) macular pigment optical density.

all other degrees of retinal eccentricity ($r = 0.472–0.919$, $p < 0.01$ for all).

3.2. MPOD and its relationship with BCVA

The mean (±SD) BCVA of the study group was 112 (±3). There was a positive and statistically significant relationship between MPOD at each eccentricity measured and BCVA ($r = 0.237–0.308$, $p < 0.01$ for all). The relationship between MPOD at 0.25° of eccentricity and BCVA is presented in Fig. 2.

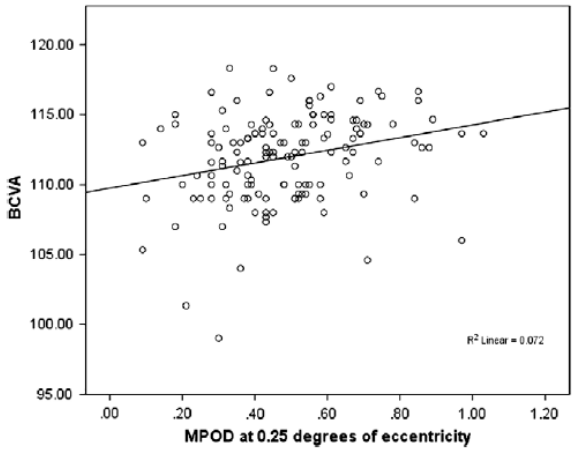

Fig. 2. The relationship between MPOD at 0.25° and BCVA.

3.3. MPOD and its relationship with contrast sensitivity

The relationships between MPOD at each eccentricity measured and log mesopic and photopic contrast sensitivity at different spatial frequencies are presented in Table 5.

The strongest relationship was seen between MPOD at 0.25° and log contrast sensitivity at 7.5 cpd for mesopic conditions ($r = 0.22$, $p < 0.01$) (Fig. 3).

3.4. MPOD and its relationship with glare sensitivity

There was no statistically significant relationship between MPOD, at any of the eccentricities measured, and mesopic contrast sensitivity observed under medium or high glare conditions for any spatial frequency ($p > 0.05$, for all), with the exception of the negative and statistically significant relationship between peripheral MPOD (at 1.0°, 1.75° and 3.0°) and mesopic contrast sensitivity under medium glare conditions ($r = -0.178$ to -0.213, $p < 0.05$).

3.5. MPOD and its relationship with PRT

The mean (±SD) foveal sensitivity of the study group was 38.1 (±1.4) dB. The mean (±SD) sensitivity post-photostress was 27.7 (±2.9) dB, representing a mean sensitivity reduction of 27.3% from baseline, across the entire study group. The mean (±SD) PRT

Table 5
The relationships between MPOD and mesopic and photopic contrast sensitivity at different spatial frequencies.

Spatial frequency	MPOD 0.25°	MPOD 0.50°	MPOD 1.0°	MPOD 1.75°	MPOD 3.0°
Mesopic					
1	−0.019	−0.034	−0.120	−0.200*	−0.097
4.1	0.065	0.016	−0.046	−0.080	−0.093
7.5	0.220**	0.192*	0.138	0.102	0.111
11.8	0.184*	0.183*	0.122	0.084	0.031
20.7	0.139	0.113	0.028	0.089	0.024
Photopic					
1.0	0.210*	0.159	0.108	0.160	0.081
4.1	0.124	0.100	0.007	0.067	0.053
7.5	0.176*	0.167*	0.115	0.133	0.101
11.8	0.193*	0.187*	0.135	0.131	0.114
20.7	0.153	0.153	0.082	0.132	0.117

* $p < 0.05$.
** $p < 0.01$.

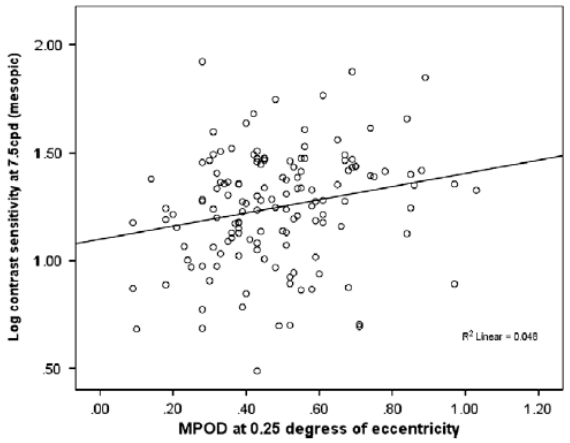

Fig. 3. The relationship between MPOD at 0.25° and log contrast sensitivity at 7.5 cpd for mesopic conditions.

(recorded as the time taken for foveal sensitivity to recover to 95%, or typically to within 2 dB, of the baseline value) was 135.8 (±63.9) s. There was no statistical relationship between MPOD at any of the eccentricities measured and either foveal sensitivity reduction (%) caused by photostress ($p > 0.05$, for all), or PRT ($p > 0.05$, for all).

4. Discussion

Given the central and pre-receptoral location (Snodderly, Auran, & Delori, 1984; Trieschmann et al., 2008) and the optical properties of MP (Bone et al., 1992), it is reasonable to hypothesize that MP would impact on visual performance, via its potential to attenuate chromatic aberration and light scatter (Nussbaum et al., 1981; Reading & Weale, 1974; Walls & Judd, 1933; Wooten & Hammond, 2002). In this study, we investigated the relationship between MPOD at various degrees of eccentricity (i.e. at 0.25°, 0.5°, 1.0°, 1.75° and 3° of retinal eccentricity) and clinically important parameters of central visual performance including BCVA, contrast sensitivity, glare sensitivity, and photostress recovery.

We report that MP (at each degree of eccentricity) is positively associated with BCVA in our study population, which suggests that MP may play a role in the optimization of visual acuity under photopic conditions; however, it is important to note that the *r* values ranged from 0.237 to 0.308 and the observed relationships can therefore only explain 5.6–9.5% of the variability. This finding is all the more provocative given that subjects in the current study were young, free from ocular pathology, and uniformly demonstrated high visual acuity. Indeed, It is somewhat intriguing to note that this statistically significant relationship was detected in a population sample where the majority of participants exhibited average to high levels of MP (at 0.25° of eccentricity). Indeed, only a very small number of subjects (~13.4%) had central MPOD of less than 0.3 in the current study. It has been previously suggested that levels above 0.3 might be superfluous to visual performance, due to the non linear nature of the effect of MP on vision (Reading & Weale, 1974).

It is important to point out that extensive efforts were made by the COMPASS study investigators to probe the limits of visual acuity, so that even the most subtle contributions of MP to visual performance might be detected. This was facilitated by customization of the vision test charts (i.e. inclusion of additional letter sizes to allow testing to a limit equivalent to 20/8) and recruitment of experienced optometrists to perform functional evaluations at both study sites (WIT and DIT). Best corrected visual acuity among the study participants ranged from a minimum of 99 ($20/20^{-1}$) to a maximum of 118 ($20/8^{-2}$). MP, it appears, could account for the theoretical refinement of acuity by up to 0.1 log units in the study sample here. This represents a substantial contribution and might be equated to the elimination of up to 0.25 dioptres of optical defocus, and appears to be consistent with previously reported limiting effects of chromatic aberration on the spatial modulation transfer function (Thibos, Bradley, & Zhang, 1991).

This finding is, however, somewhat at odds with previously reported investigations of the "acuity hypothesis" Engles et al. (2007) explored the relationship between MPOD and both gap and vernier acuity under "photopic" conditions (Engles et al., 2007). They reported that neither gap acuity nor vernier acuity was significantly related to MPOD. Their findings however are not directly comparable to the results described here, and for a number of reasons. Specifically, their adopted background luminance levels were in the low photopic range (i.e. 17 cd m^{-2} for the achromatic condition, and 15.7 cd m^{-2} for the chromatic condition. Also, gap, vernier and recognition acuity measures are not directly interchangeable, so it is entirely plausible that findings with relation to the acuity hypothesis might differ when different visual attributes are assessed. Despite the aforementioned methodological differences, the conflicting outcomes do serve to emphasize the challenges inherent in the evaluation of the role of MP on visual performance, particularly by associative means.

We also report that central MPOD (i.e. at 0.25° and at 0.5° of eccentricity) is positively and significantly related to both mesopic and photopic contrast sensitivity at intermediate spatial frequencies (i.e. 7.5 and 11.8 cpd). Central MP appears to influence sensitivity at spatial frequencies to which the visual system is highly tuned (Campbell & Robson, 1968). However, and similar to the association between MP and BCVA, it is important to note that the *r* values for MP's association with contrast sensitivity ranged from 0.167 to 0.220 and therefore the observed relationships can only explain 2.8–4.8% of the variability.

For photopic conditions, this finding might be attributable to the attenuation of the effects of chromatic aberration and light scatter, whereby image refinement potentially cause lateral inhibitory surround responses to be dampened, and the resultant ganglion cell response optimized (Kuffler, 1953). Under mesopic conditions, it is more likely that enhanced visual performance is a consequence of the selective diminution of rod mediated signals. While rod and cone photoreceptors operate interactively in the high mesopic conditions employed here (Kuffler, 1953), rods remain optimally sensitive to shorter wavelengths than cones (explaining the Purkinje shift in peak retinal spectral sensitivity towards blue under mesopic conditions). The pre-receptoral absorption of short-wavelength light by MP might, therefore, serve to attenuate rod activity and allow cone-mediated vision (which typically exhibits better contrast sensitivity (Puell, Palomo, Sanchez-Ramos, & Villena, 2004), to dominate further into the mesopic range. This theory is supported by the limited nature of the relationship observed between MP and contrast sensitivity, confined to the most central anatomic locations where MP is highest and cone activity predominates.

Of note, this is the first study to report on the association between MP and contrast sensitivity in a young healthy population (not confounded by dietary supplementation or ocular pathology). Our findings are consistent with those of Kvansakul et al. (2006) who reported that MP augmentation, via supplementation, enhances contrast acuity thresholds under mesopic conditions.

Finally, we found that MPOD was not related to either glare sensitivity or photostress recovery, as assessed here. At first glance, these findings might appear to conflict directly with a number of

recent studies, which have reported positive and statistically significant associations between MP and several parameters of visual performance including: visual discomfort (Stringham, Fuld, & Wenzel, 2003), photophobia (Wenzel, Fuld, Stringham, & Curran-Celentano, 2006), veiling glare (Stringham & Hammond, 2007) and photostress recovery (Stringham & Hammond, 2007; Stringham & Hammond, 2008). The cited series of experimental analyses are consistent with the rationale whereby MP attenuates the effects of blue light, which is both valid and important. Fundamental methodological differences may, however, explain the differences between those reports and our observations.

Firstly, all the above studies employed a Maxwellian-view optical system to generate and present stimuli. While the rationale for doing so remains sound, in that it eliminates pupil diameter and pupil responses as a potential confounding factor, it is difficult to extrapolate their findings into a natural environment, outside of the laboratory, where changes in pupil diameter for example, are a natural consequence of the luminance changes typically observed on a daily basis, and may confer some level of protection against the deleterious effects of glare and excessive light stimulation. However, adoption of a natural pupil introduces other difficulties. Most importantly, the individual variation in pupil size, and the consequential variation in retinal illuminance, clouds the interpretation of MP's contribution to visual performance under glare conditions. It should therefore be conceded, that for a cross-sectional evaluation, the natural pupil is less appropriate for a comprehensive evaluation of the role of MP, if any, in terms of its contribution to visual comfort and glare attenuation.

Secondly, the studies cited above invariably employed stimuli containing a strong short-wavelength blue light component. Again, there is an obvious rationale for doing so, as MP predominantly absorbs blue light. However, the concept of the environmental validity of such stimuli must again be questioned. Specifically, the most common light sources employed in industrial, commercial and home lighting systems typically contain significantly less blue light than those employed in cited studies. Tungsten and tungsten–halogen filament lighting systems, in fact, contain a minimal blue light component (see Fig. 1). The absence of a strong blue light component in the photostress lamp, employed here, may partially explain the absence of any association between MP on PRT observed in our study. Our findings, therefore, in fact corroborate and extend the findings of Stringham et al. (2004) and Stringham and Hammond (2007) in that the associations between MP and glare are strongly wavelength dependent, and the influence of MP on glare disability is critically dependent on the spectral output of the source. It is worth noting, however, that the current trend for change to compact fluorescent and light emitting diode installations, which typically emit significantly more blue light (unpublished data from our laboratory suggests a twofold increase in blue light irradiance for compact fluorescent bulbs compared to tungsten), may render the role of MP for visual performance, if any, ever more important.

In conclusion, visual performance, as assessed by visual acuity and contrast sensitivity measures, appear to be weakly associated with MPOD. However, photostress recovery and visual performance under glare conditions were unrelated to this pigment. The lack of consistency between our findings and those of others possibly reflects the difficulties inherent in investigating the role of MP with respect to visual performance using a study of cross-sectional design. Fundamental experimental design issues for visual performance evaluation must also be considered. There are no gold standard techniques, no means to accurately simulate the broad range of environmental conditions experienced on a daily basis, so the selection of individual test parameters will influence both the results of the investigation, and any subsequent comparison with previous experimental results. The results of the current investigation should be interpreted with full appreciation of its design limitations, and conclusions should therefore be restricted to the specific testing conditions employed herein.

Visual acuity has been shown to relate to quality of life (Datta et al., 2008) and is important in our highly visual society, where the demands for high quality visual resolution are constant. Contrast sensitivity correlates with various functional vision tasks such as mobility orientation, balance control, driving, face perception and reading performance (Owsley & Sloane, 1987; Owsley et al., 2002), and has been established as an important measure of visual function, which is related to quality of life (Owsley & Sloane, 1987). These associations between MP and visual performance are likely to apply equally and possibly more substantially, in an older population, where, for example, the incidence of driving accidents and falls directly relate to visual performance (Owsley et al., 2002).

In summary, a placebo-controlled, randomized, L-based supplementation trial, designed to investigate if augmentation of MPOD enhances visual performance and/or comfort, is required to more adequately address this critical research question, and fully explore the proposed "optical" hypotheses of MP.

Disclosure

None.

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REVIEW

Macular pigment and its contribution to visual performance and experience

James Loughman[a,*], Peter A. Davison[a], John M. Nolan[b], Mukunda C. Akkali[b], Stephen Beatty[b]

[a]*Optometry Department, Dublin Institute of Technology, Dublin, Ireland*
[b]*Macular Pigment Research Group, Waterford Institute of Technology, Waterford, Ireland*

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KEYWORDS
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Abstract
There is now a consensus, based on histological, biochemical and spectral absorption data, that the yellow colour observed at the macula lutea is a consequence of the selective accumulation of dietary xanthophylls in the central retina of the living eye. Scientific research continues to explore the function(s) of MP in the human retina, with two main hypotheses premised on its putative capacity to (1) protect the retina from (photo)-oxidative damage by means of its optical filtration and/or antioxidant properties, the so-called protective hypothesis and (2) influence the quality of visual performance by means of selective short wavelength light absorption prior to photoreceptor light capture, thereby attenuating the effects of chromatic aberration and light scatter, the so-called acuity and visibility hypotheses. The current epidemic of age-related macular degeneration has directed researchers to investigate the protective hypothesis of MP, while there has been a conspicuous lack of work designed to investigate the role of MP in visual performance. The aim of this review is to present and critically appraise the current literature germane to the contribution of MP, if any, to visual performance and experience.

PALABRAS CLAVE
Pigmento macular;
Rendimiento visual;
Hipótesis óptica;
Degeneración macular relacionada con la edad;

El pigmento macular y su contribución al rendimiento y experiencia visuales

Resumen
En la actualidad, en función de los datos histológicos, bioquímicos y de la absorción espectral, se ha alcanzado un consenso, de que el color amarillo observado en la mácula lútea es consecuencia de la acumulación selectiva de xantófilos dietéticos en la retina central del ojo vivo. La investigación científica continúa examinando las funciones del pigmento macular en la retina humana, con dos hipótesis principales formuladas sobre su supuesta capacidad para: 1) proteger la retina frente a la lesión (foto)oxidativa por medio de sus propiedades de filtración óptica y/o antioxidantes,

*Corresponding author. Optometry Department, DIT, Kevin Street, Dublin 8, Dublin, Ireland. Tel.: +353 1 4022841; Fax: +353 1 4024915
E-mail address: james.loughman@dit.ie (J. Loughman).

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Longitud de onda corta

la llamada hipótesis protectora e 2) influir en la calidad del rendimiento visual por medio de la absorción selectiva de luz de longitud de onda corta antes de su captura por parte de los fotorreceptores, lo que atenúa los efectos de la aberración cromática y dispersión de la luz, la llamada hipótesis de la agudeza y la visibilidad. La epidemia actual de degeneración macular relacionada con la edad ha dirigido a los investigadores a examinar la hipótesis protectora del pigmento macular, mientras que es evidente la falta de investigación destinada a investigar el papel del pigmento en el rendimiento visual. El objetivo de la presente revisión es describir y valorar de forma crítica los estudios publicados actuales pertinentes a la contribución del pigmento macular, si desempeña algún papel, en el rendimiento y experiencia visual.

Introduction

Vision, and how we perceive the world, involves the complex interaction of physical, physiological and psychological processes, which ultimately provide the final sensation of seeing. "Visual performance", as discussed here, describes the sensitivity of the eye where limits of vision are quantified using established clinical and laboratory techniques. Such techniques cannot readily account for variable and highly individual experiences and interactions in the real world. "Visual experience" incorporates subjective experience, which may, for example, explain inconsistencies between patient's symptoms and measured functional vision. Any influence of macular pigment (MP) on vision needs to be assessed therefore, in terms of both measured performance and reported experience.

Macular pigment was first observed by Buzzi[1] in 1782, and speculation persists as to its role in the visual system. Indeed, at first there were conflicting views as to the very existence of this pigment in the living eye, with numerous authors, including Home[2] and Gullstrand[3] believing it to be a post-mortem artifact.

It has long been recognised that MP preferentially absorbs short wavelength light prior to photoreceptor stimulation, and the hypothesis that filtering such defocused short wavelength light could enhance visual performance by reducing the effects of chromatic aberration goes back as far as Schültze[4] in 1866. This hypothesis, especially in relation to MP, remains unproven and poorly investigated. In this review, we explore the contribution of MP to visual performance and experience, and report and critically appraise the evidence in support of the notion that MP is important for vision.

The selective accumulation at the macula of only three dietary carotenoids, to the exclusion of the other forty dietary carotenoids, suggests an exquisite biological selectivity for lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (*meso*-Z) at the site of maximum visual acuity in the human retina, and also suggests a specific role for these carotenoids which is uniquely suited to this anatomic location. Given that Darwinian natural selection is based on the premise that phenotypic expression of genetic background confers advantage before and until the period of procreation, it is reasonable to infer that the biological selectivity of MP's accumulation in the retina is advantageous in young and middle age.

MP may protect against the development of age-related macular degeneration (AMD) by defending the retina against cumulative and chronic (photo)-oxidative damage. It is likely, however, that the primary role of MP rests on its contribution to visual performance and experience, although the pigment may also longitudinally contribute to the preservation of macular function by preventing or delaying the onset of retinal disease such as AMD through its protection against chronic (photo)-oxidative damage. In other words, and in theory at least, MP's putative contribution to visual performance rests on its optical properties, whereas the putative protective effect of this pigment for AMD rests on its optical and/or its biochemical properties.

MP alters the spectral composition of the light incident upon macular photoreceptors, but whether such short wavelength absorption influences the quality of the visual experience, and whether the magnitude of any such effect correlates with MP optical density (MPOD), are questions that remain unanswered. Meaningful comment on the contribution of MP, if any, to visual performance must (1) consider the primary factors that affect visual performance, (2) outline the properties of MP that make it potentially important for visual performance in light of any such limiting factors, (3) critically appraise the current literature germane to the role of MP in visual performance and experience and (4) suggest experimental strategies designed to investigate whether MP is important for visual performance and experience.

Visual performance

Current and unifying concepts

Snellen was the first to standardise the measurement of visual acuity with his letter chart, a chart design, which despite numerous limitations, remains the most widely used means of quantifying visual performance in the clinical setting. There remains, however, a myriad of other independent and/or overlapping techniques by which one can measure visual performance and experience across a range of functional levels.

Vision includes the capacity to detect objects against a contrasting background, to detect gaps between objects, to perceive subtle vernier offsets (which provides one example of hyperacuity), to recognise and identify objects, to perceive colour, to detect movement, and to perceive depth, amongst other faculties. It is important to note that the capacity to recognise a small distant object bears little relation to the

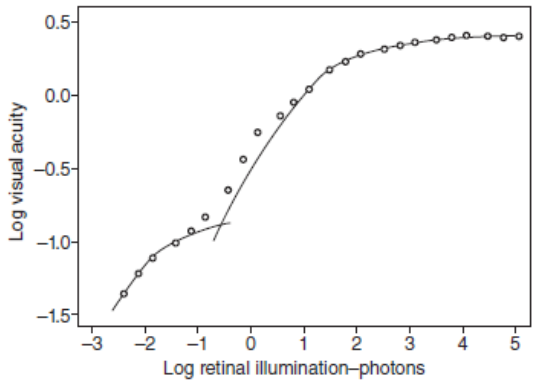

Figure 1 Relationship between visual performance (as log visual acuity) and retinal illuminance. As retinal illuminance increases, visual acuity increases by up to 2 log units (cone-mediated improvements account for the most significant improvements from approximately 6/60 to 6/3 Snellen equivalent-see upper portion of curve). Shlaer[5].

capacity to differentiate colours, or to detect a potential threat such as an oncoming vehicle in the peripheral field of view.

Visual performance is critically dependent on illumination, and the range of illumination we experience in the course of a typical day is vast. The visual system copes with such changes in illumination by adapting to the prevailing conditions, and can function through an approximate 8 log unit luminance range. Although adaptation facilitates performance over a wide range of ambient illumination levels, it does not follow that we see equally well at all levels. Under dim conditions, for example, the visual system is very sensitive and can detect subtle changes in luminance, but acuity for pattern details and colour discrimination is poor.

Shlaer[5] has explored the relationship between illumination and visual acuity (Figure 1). Converting his findings to Snellen equivalent, daylight (photopic) performance of 20/10 reduces to 20/600 under dim conditions, a 60-fold reduction. Threshold visibility, colour appearance and visual acuity all vary dramatically with illumination, and these visual parameters change over the time-course of light and dark adaptation. Therefore, and by definition, no single test or testing condition can be used to investigate visual performance, and no single test can predict performance on other tests.

Further, any discussion of the visual processes must include those mechanisms contributing to perception. The visual system employs numerous anatomic and physiological strategies, including lateral interactions between cells, specific receptive field organisation, spatial retinotopic organisation in retinal and non-retinal areas of the pathway, colour opponency and parallel visual pathways, amongst others, in order to achieve an instantaneous, coherent and highly detailed perception of the outside world and our position within it. Such image processing is not exclusive to the brain, but extends throughout the visual pathway beginning at the retina.

The eyes and brain are thus inextricably linked with the visual universe. The eyes actively record the form, colour and movements of the world, and the brain moulds these raw perceptions into recognisable patterns. The retina essentially acts as a spatial, temporal and spectral filter of patterns of light striking its surface. Its anatomic structure and the functional properties of individual cells determine the type of information extracted from a visual scene and delivered to the brain.

Specialisation of the maculas

The macula, which comprises less than 4% of the total retinal area, subserves almost all of our useful photopic vision. Several distinctive anatomic and neural adaptations facilitate such a high level of visual performance. These include:

1. Cone density peaks at the centre of the macula (fovea), which intersects the line of sight. Cones here are smaller, more densely packed and more numerous than elsewhere in the retina, thus extending the limits of spatial acuity. Cone density exceeds rod density only at the lower part of the foveal slope, reaching a maximum at the base of the fovea (foveola) where cone density is over three times that observed at the foot of the foveal slope.[6] Rods, ganglion cells and all inner nuclear layer neurons are absent from the foveola, so that only here is light directly incident on photoreceptors (elsewhere light must traverse the various retinal cells and layers to reach photoreceptors). It is also worth noting that short wavelength sensitive cones are absent at the foveola.
2. Midget pathways arising from these foveal cones dominate. Such parvocellular midget pathways are tuned to high spatial frequencies and also exhibit colour opponency.
3. Such midget pathways are distinctive because of the absence of convergence of photoreceptor signals onto bipolar and ganglion cells. Absent or reduced convergence of information preserves the data gathered at the fovea for delivery to the visual cortex. Such differences between foveal and extra-foveal pathways generate a hierarchy in the processing of information gathered by the retina.

Retinal hierarchy

Anatomic and physiological observations, such as the differential light sensitivity of photoreceptors, the variable density and distribution of photoreceptors and ganglion cells across the retina and the convergence of information from the extra-foveal retina, means that a hierarchy exists in the architecture of retinal processing, where foveal information is given higher priority. This hierarchy is preserved to the striate cortex, where a high percentage of cortical cells are dedicated to information of foveal origin. The central retinal pathways have by far the greatest proportion of representation (estimates range from 25% of the cortex devoted to the central 5 degrees, 37% devoted to the central 15 degrees[7] and 87% of the cortex devoted to the central 30 degrees of visual field[8]).

Having outlined those anatomic and neural factors central to primates' capacity for high acuity vision, it is now important to consider the potential role of MP in visual performance. In order to do so, it is essential to characterise (a) the optical limitations that might restrict visual performance (in particular chromatic aberration and light scatter) and (b) the properties of MP that might serve to

lessen the effect of such limitations, and thereby facilitate optimal visual performance.

Optical limitations of the eye

Monochromatic aberrations and diffraction limit the image quality produced by the eye, so that the image is not always a high quality representation of the object. While there is significant ocular and neural correction for, and adaptation to, such image defects, MP most likely has no role in altering their effects (although Kvansakul et al.[9] have noted some surprising observations of a trend towards lower root mean square wavefront aberrations in a small group of subjects following supplementation with L and Z, which, they postulate, may be as a result of the as yet unknown effects of carotenoid intake on crystalline lens function).

Chromatic aberration

Chromatic aberration, comprising both longitudinal (LCA) and transverse (TCA) components, has been cited as possibly the most significant aberration affecting visual quality.[10] Indeed, LCA creates up to two dioptres of wavelength-dependent optical defocus. Campbell and Gubbisch[11] have demonstrated improvements in contrast thresholds of up to 65% at intermediate spatial frequencies once monochromatic yellow light is employed in place of spectrally broadband white light. Although Bradley[12] later modelled the effects of chromatic aberration, and concluded that the effect of chromatic aberration on the modulation transfer function was small, and equivalent to approximately 0.15D of defocus, upper resolution limits of the visual system however, are most likely defined by the effects of chromatic aberration.[13]

The effect of LCA across wavelength, in terms of blur, is non-linear, as shorter wavelengths are significantly more defocused than longer wavelengths. For example, an eye focussed at 550 nm, light at 460 nm suffers 1.2D myopic defocus, while the equivalent long wavelength of 640 nm is only 0.50D out of focus.[10] This serves to create a purple blur circle haze around the focussed "green" component. Figure 2 demonstrates the non-linearity of defocus and the relative luminance profile across wavelength. As the spectral extremities have less luminosity, the effects of chromatic aberration on image focus are mitigated in terms of the effects on vision. Mitigation is potentially further aided by the fact that blue light is selectively absorbed by MP.

Light scatter

If one looks up to the sky on a bright, cloudless sunny day, one could be fooled into thinking that the sun's rays traverse an unobstructed path to the eye. Furthermore, one could certainly not imagine that the quality of the light visible was being degraded as it traversed the seemingly clear sky, even in the most remote countryside locations far from the smog-filled cityscapes, on its way to the eye. The fact that the sky is blue is testament to the impact of the process of light scatter, whereby particle matter abstracts and re-radiates energy from light incident upon it.

A multitude of visible and non-visible particles, varying in size from atmospheric oxygen and nitrogen, to haze aerosols, to larger complexes such as fog, cloud and rain, all contribute to such scatter. Wooten & Hammond,[14] in an excellent review of the importance of light scatter to the "visibility" of objects, eloquently describe why light scatter, especially that induced by haze aerosols "critically determines how far one can see and how well details can be resolved", so that, aside from the optical and neural limits, "scatter in the aerosol haze is the primary determinant of visual discrimination and range in the outdoors".

The question therefore arises, what effect does light scatter have on visual performance? And it is a good question. On a clear day one can see for miles despite the effects of scatter. Wooten & Hammond,[14] however, propose a model whereby compensation for the effects of light scatter, such as could reasonably be achieved by increasing the optical density of MP, would increase the visibility and discriminability of targets in natural settings. In their model, a 1 log unit increase in MPOD attenuates the veiling luminance of the short-wave dominant background by 26% (or 17% for a more

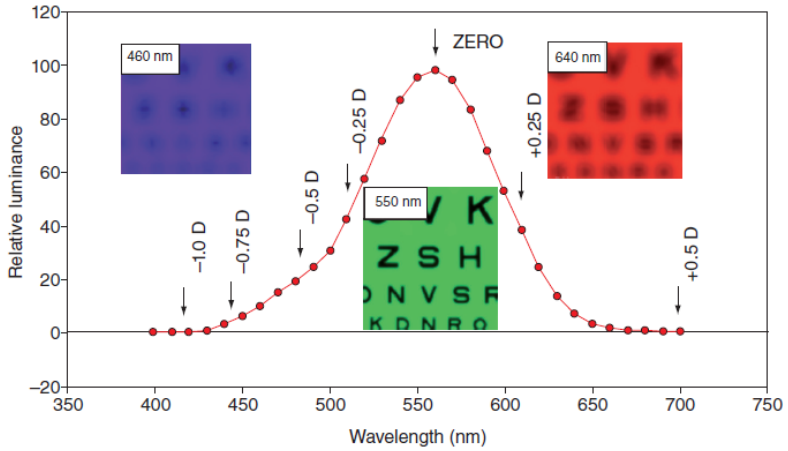

Figure 2 Illustration of the relative luminance profile and the effect of chromatic aberration across wavelengths. The relative blur is more pronounced at the blue end of the spectrum such that, for example, the short wave 460 nm text is significantly more difficult to recognise than the long wave 640 nm text for the above scenario where the optimal focus is between 540-560 nm.

practical 0.5 log unit increase in MPOD), while having minimal effect on the short wave deficient distant target. The attenuation of the effects of light scatter is thereby observed to enhance target detection and discrimination capacity, and extend the visual range by up to 18.6%.

Tackling the question from another perspective, the problems caused by scatter, while not consciously experienced by most people, do become a significant symptom of which many patients complain in the form of discomfort and disability glare. Aside from patients without detectable ocular abnormality, typical patients with such symptoms include those with cataract, corneal abnormalities, intraocular inflammation, and following laser refractive surgery, amongst others. Therefore, scatter does have an adverse effect on the visual experience of normal subjects and on those with ocular pathology, and any means of alleviating such effects would be of clinical importance.

The possible effect of aberrations such as LCA, and also of short wavelength light scatter, is that capacity limits are somewhat reduced so that the anatomic limits of acuity based on foveal cone diameter (30 seconds arc — equivalent to 6/3) are seldom achieved, even in healthy normal individuals, with the exception of hyperacuity tasks which have different underlying neural bases.

So the question arises, what are the properties of MP that might allow it to improve visual performance in light of the limiting factors outlined above?

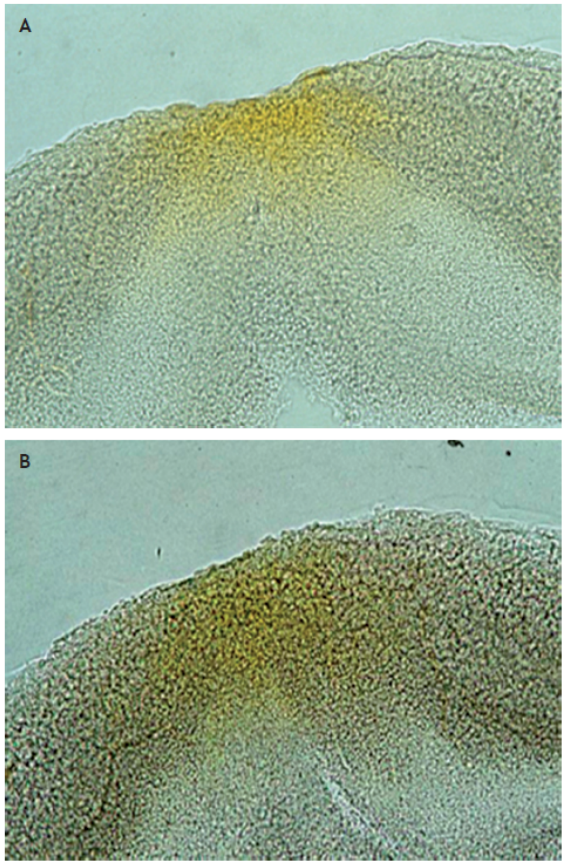

Figure 3 Histological section illustrating the spatial profile and pre-receptorial location of MP, the main location of macular pigment was in the layer of the fibres of Henle in the fovea (a) and in the inner nuclear layer at the parafoveal site (b). Reprinted with permission: Trieschmann et al., 2008[78]

Optical and anatomic properties of MP

MP's optical and anatomic properties have prompted the "optical" hypothesis of this pigment, which has been discussed in detail by Reading & Weale[15] and later by Nussbaum et al.[16] The optical effect of MP is somewhat evidenced by two entoptic phenomena known to exist which are specific to the macula, namely Maxwell's spot and Haidinger's brushes.[16] The former, first described in 1844, is attributed directly to the deposition of pigments at the macula and results in a dark red spot being visible around the fixation point if a brightly illuminated white surface is viewed alternately through purple and neutral filters. Magnussen et al.[17] have shown that the absence of short-wave-sensitive cones in the human foveola, which normally goes unnoticed unless a subject's field of view is restricted to the foveola, producing the artificial colour vision defect of foveal tritanopia,[18,19] results in a blue scotoma which can be visualised as the negative afterimage of a short-wavelength adapting field on a larger white background. The afterimage has an annular shape with a lighter inner region that corresponds to Maxwell's spot, and a small bright spot in the centre, corresponding to the foveal blue scotoma. The MP distribution measured for the same observers closely corresponded to the lighter annular region of the afterimage.

Haidinger's brushes, first reported in 1844, refers to a propeller-shaped image which is seen most clearly through a rotating filter producing plane-polarised light. It is known that lutein has dichroic properties[20,21] and it has been shown that bovine lutein and zeaxanthin bind to bovine retinal tubulin.[22] It is thus possible that dichroic macular pigments are laid down in a highly organised manner following the radial arrangement of Henle's fibres at the macula, thus explaining the shape and brush-like appearance of the propeller-like images.[23]

However, it should be noted that neither of these entoptic phenomena is visible in normal viewing conditions, probably because of adaptatory effects, particularly at the level of the visual cortex. It is uncertain whether the concentration of MP has any significant influence on vision under such conditions.

MP may be important for visual performance and/or experience however by at least one of the following mechanisms (summarised by Walls & Judd[24]): MP may enhance visual acuity by reducing chromatic aberration (effects); MP may reduce visual discomfort by attenuation of glare and dazzle; MP may facilitate enhancement of detail and visual contrast by the absorption of "blue haze". MP has the capacity to achieve the above optical effects because of its optical properties and because of its location within the retina.

The term macula lutea is actually attributable to the presence of the xanthophyll pigments, L, Z, and *meso*-Z at the central region of the retina, which give rise to the appearance of a yellow spot (macula lutea) when viewed under red-free light (Figure 3). The yellow coloration of MP is such that it selectively absorbs blue-green incident light,

with maximum absorption circa 460 nm and little or no absorption above 530 nm.[25] Given that (1) the peak retinal spectral sensitivity lies at 555 nm, (2) the proportion of blue (short wavelength sensitive) cones in the central macula is far lower than that of red (long wavelength sensitive) and green (medium wavelength sensitive) cones and (3) the region of maximal visual performance, the foveola, is essentially devoid of short wavelength sensitive cones, it would appear that the optical properties of MP are such that it attenuates the component of light that is least beneficial, and most deleterious, with respect to visual performance and experience. As Wald[26] summarised, the various adaptive mechanisms in the human eye serve to "withdraw vision from the blue" end of the spectrum.

Two aspects of MP's location within the retina are also central to the hypothesis that it has a role to play in visual performance. Firstly, although MP is found throughout the retina and other ocular structures,[27] it reaches its greatest concentration at the macula, and remains optically undetectable elsewhere. Secondly, and importantly, MP is located at a pre-receptoral level, so that absorption of short wavelength light occurs prior to stimulation of the underlying photoreceptors, thereby altering the spectral distribution of light incident on such photoreceptors in a favourable way (Figure 3).

Short wavelength light absorption attenuates the more disadvantageous component of LCA. Retinal image quality is thereby improved, and visual performance across the full contrast range is theoretically more refined. As MP absorption overlaps with that of rhodopsin, MP may reduce rod signal effectiveness in the mesopic range, and thus extend the usefulness of cone-mediated vision into the mesopic range.[9] In addition, short wavelength light absorption has the benefit of improving target contrast by selectively reducing the scattered short wavelength light in the background. Reduced LCA and reduced scatter effects, resulting from MP's absorptive characteristics, have the potential to improve visual acuity and target visibility, and perhaps in an interactively additive fashion.[14]

The higher energy and retinal irradiance associated with shorter wavelengths (International Commission on Non-Ionizing Radiation Protection, 1997[28]) also merits consideration. Bright light, which interferes with the quality of visual perception, is termed glare, of which there are numerous types. In high luminance or high contrast situations, where glare and dazzle are maximal, MP absorption of short wavelength light attenuates the highest energy light component, and reduces retinal irradiance, and therefore may minimise the impact of glare on performance, and increase the threshold for photophobia under normal viewing conditions. Because of their linear structure, L, Z, and *meso*-Z also exhibit dichroic properties,[29] which facilitate glare reduction by preferential absorption of polarised light. Glare symptoms remain a common and important clinical entity in optometric and ophthalmological practice, and very troublesome for those who experience it.[30] Furthermore, symptoms of glare remain difficult to quantify and treat. Interestingly, difficulty with glare is often one of the earliest manifestations of AMD.

It should now be clear, because visual performance is a complex subject, which is difficult to quantify, and dependent on numerous independent and overlapping variables, that to investigate the contribution of any one factor (such as MP) presents numerous challenges. It is with this thought in mind that currently available evidence on the impact of MP on visual performance and experience will now be explored.

Evidence that MP plays a role in visual performance and experience

Background

The evidence in relation to a role for MP in visual performance is sparse and is largely associative. To our knowledge, there are no published studies which have satisfactorily investigated the hypothesis that MP influences visual performance and experience. However there are numerous and conflicting reports on the effect of yellow filters on visual performance,[31] but none of these have included measures of MPOD. Failure to do so confounds any reasonable interpretation of short wavelength light absorption effects on visual performance, as variations in MPOD between and within study populations could account for the reported observations.

There are thus two strategies to investigate the impact of MP on visual performance. The first is to quantify performance using a range of functional tests, and to correlate the results with measures of MPOD. Given the other variables involved in vision, the true effect of MP would, in our opinion, prove difficult to isolate with such a paradigm. The alternative and most appropriate means to investigate the effect of MP is to measure baseline visual performance, as above, and to record baseline MPOD, and then repeat functional vision tests during an extended period of supplementation with MP xanthophylls. If MP influences visual performance it must do so either as (1) a filter or (2) through some biological mechanism. With respect to the former (1), any effects on visual performance should follow the known absorbance characteristics of the pigments. Hence, the visual stimuli to be used to investigate the role of MP should have significant amounts of short wave energy, in order to replicate the effects of ecologically valid stimuli (e.g. the sun) which have lots of short wave energy. Biological effects (2) would likely be based on either enhanced protection (healthier retinas and crystalline lenses would lead to better vision, especially in the elderly) or effects throughout the visual system. If MP has a role, and its contribution is related to either its optical density and spectral absorbance characteristics, or to possible biological effects on retinal, crystalline lens and visual system health, then increasing MPOD through supplementation should result in improved performance and experience. The key then is to accurately detect and quantify any such changes through a comprehensive battery of appropriate tests that analyse vision on a number of functional levels, including basic acuity, contrast sensitivity across illumination levels, colour perception, and glare sensitivity, amongst others.

Those studies that have addressed visual performance are largely confined to populations with established eye disease (summarised in Table 1), and therefore the results should be interpreted with full appreciation of the fact that the findings

Table 1 Publications exploring the relationship between macular pigment and visual performance and experience in subjects with ocular disease

Study (author, year)	Subjects (n)	Supplement (dose per/day & time)
CROSS SECTIONAL STUDIES		
Hammond et al., 1997	Cataract	None
Brown et al, 1999	Cataract	None
Chasan-Taber et al., 1999	Cataract	None
Schupp et al., 2004	Cystic fibrosis (10)	None
INTERVENTION (SUPPLEMENTATION) STUDIES		
Andreani & Volpi, 1956[a]	Retinitis pigmentosa (8)	Lutein dipalmitate
Mosci, 1956[a]	Retinitis pigmentosa	Lutein dipalmitate
Cuccagna, 1956[a]	Myopia & RP	Lutein dipalmitate
Pfeiffer, 1957[a]	Abnormal dark adaptation (13)	Lutein dipalmitate
Hayano, 1959[a]	Retinitis pigmentosa	Lutein dipalmitate
Muller-Limroth & Kuper, 1961[a]	Retinitis pigmentosa (18)	Lutein dipalmitate
Asciano & Bellizzi, 1974[a]	Progressive myopia with chorio-retinal atrophy (50)	Lutein dipalmitate
Richer, 1999	AMD (14)	10mg lutein (5 ounces spinach 4 times per week)
Dagnelie et al., 2000	Retinitis pigmentosa (16)	40 mg lutein (2 months) followed by 20 mg (4 months)
Aleman et al., 2001	Retinitis pigmentosa (47) & Usher syndrome (11)	20 mg lutein (6 months)
Duncan et al., 2002	Choroideremia (13)	20 mg lutein (6 months)
Falsini et al., 2003	AMD (30)	17 subjects took 15 mg/L + 20 mg vitamin E + 18 mg nicotinamide (6 months); 13 subjects had no supplementation
Olmedilla et al., 2003	Cataract (17)	15 mg lutein or 100 mg α-tocopherol or placebo 3 times per week
Richer et al., 2004	AMD (90)	10 mg/L or 10mg/L + antioxidants or placebo (1 year)
Bartlett & Eperjesi, 2007	ARM & AMD (25)	6 mg lutein + vitamins A,C + E + zinc + copper
Aleman et al., 2007	Stargardts' disease or cone-rod dystrophy (17)	20 mg/L (6 months)
Parisi et al., 2008	Early AMD	Vitamin C & E, Zinc, Copper, 10 mg lutein, 1 mg zeaxanthin, 4 mg Astaxanthin (12 months)

AMD indicates age-related macular degeneration; ARM, age-related maculopathy; MPOD, macular pigment optical density.
[a]Data from Nussbaum, 1981.

do not necessarily hold true for subjects without retinal pathology. Studies involving normal subjects will therefore be reviewed separately here (summarised in Table 2).

Studies in subjects with retinal pathology

Hereditary retinal degenerations

Abnormal light sensitivity, difficulty associated with glare, loss of contrast and slow dark adaptation are symptoms commonly reported by patients with hereditary retinal degenerations. It is possible that such symptoms could be attributable, at least in part, to the failure of MP to absorb scattered light, resulting in reduced contrast and definition along with excessive photoreceptor pigment bleaching by short wavelength light components.

The antioxidant and absorptive properties of MP would therefore suggest a potentially useful role for the macular carotenoids in retinal degenerations, where the clinical aim includes optimisation of current visual status in the short term and preservation of macular vision in the long term. Indeed, it is noteworthy that there have been reports (some dating back > 50 years) suggesting that patients with retinitis pigmentosa (RP) demonstrated improvements in visual performance following supplementation with lutein-containing compounds (reviewed elsewhere[16]).

Dagnelie et al.[32] assessed the effect of L supplementation in patients with RP, and reported moderate visual improvements following short-term supplementation with L. Mean visual acuity improved by 0.7 dB and mean visual-field area by 0.35 dB, although the largest gains

Outcome measure	Findings
Crystalline lens transparency versus MPOD	Higher MPOD correlated with a more transparent crystalline lens
Incidence of cataract versus MPOD	Higher MPOD correlates with decreased cataract formation
Incidence of cataract versus MPOD	Higher MPOD correlates with decreased cataract formation
Contrast sensitivity, colour discrimination & erg amplitude	No statistical difference between CF and normals although normals had marginally better performance
Dark adaptation	Primary & secondary portions of DA curve improved
Light sensitivity	Sensitivity improved
Dark adaptation	DA improved
Dark adaptation	Only marginal improvements observed, but used smaller doses than others
Dark adaptation	DA improved proportional to the increase in blood lutein
ERG potentials	No change
Light & chromatic sensitivity	Sensitivity on both measures improved
Contrast sensitivity	92% showed improvements in contrast sensitivity
Visual acuity and visual field	VA improved 0.7 dB, visual field area increased by 0.35 dB, largest gains in blue eyes
Foveal sensitivity	No improvement - lower dose than Dagnelie study, MP density may be affected by stage of retinal disease
Foveal sensitivity (dark adapted)	No improvement
Focal Electroretinogram (ERG) amplitude	Significant improvement, MPOD not recorded
Visual acuity & glare sensitivity	Improvements in both measures, no change in placebo group or α-tocopherol group
Visual Acuity & CSF & Amsler	Significant improvement in both groups L = 5.4 letter increase, L + antioxidants = 3.5 letter increase; no effect on contrast sensitivity; improved performance on amsler grid
Contrast sensitivity	No improvement in performance
Visual acuity and foveal sensitivity	No improvement with increased L, MPOD was inversely related to stage of disease
Multifocal ERG Response Amplitude Density (RAD)	Central (5 deg) RAD reduced at baseline in AMD compared with healthy controls, Central (5 deg) RAD improved significantly in the supplemented group, MPOD not recorded

were observed in blue-eyed participants. Aleman et al.[33] explored the relationship between visual function and L supplementation in RP patients over a six month period, and despite increases in MPOD, could find no significant improvement in visual performance (measured as absolute foveal sensitivity). The dosage used in this latter study was lower than that in the Dagnelie report, which may explain the discrepancy in the findings of these two studies. Neither study, however, analysed visual function in sufficient detail or followed patients for sufficient time to make meaningful comment on whether the natural history of RP is modified following supplementation with L.

Duncan et al.[34] analysed MP levels and macular function in choroideremia (a progressive degeneration of photoreceptors, RPE and choroid). Once again, and in spite of augmented MPOD following supplementation, no improvement in retinal sensitivity was observed.

Aleman et al.[35] measured MPOD in patients with Stargardt's disease or cone-rod dystrophy with known or suspected disease-causing mutations in the *ABCA4* gene, and investigated response to supplemental L in terms of changes in MPOD and central visual function. They reported that MPOD is inversely related to the stage of *ABCA4* disease at baseline, and could be augmented by supplemental L in about two thirds of patients. However, measures of visual function, including visual acuity and foveal sensitivity, exhibited no discernable improvement after 6 months of supplementation. They concluded that the long-term influences of L supplementation on the natural history of such macular degenerations require further study.

Table 2 Publications exploring the relationship between macular pigment and visual performance and experience in normal subjects

Study (author, year)	Subjects (n)	Supplement (dose & time)
CROSS SECTIONAL STUDIES		
Hammond et al., 1998	Normals	None
Stringham et al., 2003	Normals	None
Stringham et al., 2003	Young normals (16)	None
Stringham & Hammond, 2007	Normals (36)	None
Engles et al., 2007	Normals (80)	None
INTERVENTION (SUPPLEMENTATION) STUDIES		
Monje, 1948[a]	Normals (14)	Lutein dipalmitate (2-6 months)
Wustenberg, 1951[a]	Normals (7)	Lutein dipalmitate
Klaes & Riegel, 1951[a]	Normals	Lutein dipalmitate
Andreani & Volpi, 1956	Normals (10)	Lutein dipalmitate
Mosci, 1956[a]	Normals	Lutein dipalmitate
Hayano, 1959[a]	Normals	Lutein dipalmitate
Wenzel et al., 2006	Normals: No supplement (6); supplement (4)	30 mg lutein + 2.7 mg zeaxanthin (12 weeks)
Rodriguez-Carmona et al., 2006	Normal trichromats (24)	10 mg (6 months) + 20 mg (6 months) of lutein or zeaxanthin, 10 mg lutein + 10 mg zeaxanthin or placebo
Kvansakul et al., 2006	Normals (34)	10 mg lutein, 10 mg zeaxanthin, 10 + 10 mg combination or placebo (6 months)
Bartlett & Eperjesi, 2008	Normals (46)	6 mg lutein + vitamins A, C, E + zinc + copper
Stringham & Hammond, 2008	Normals (40)	10 mg lutein + 2 mg zeaxanthin (6 months)

MPOD indicates macular pigment optical density.
[a]Data from Nussbaum, 1981.

Age-related macular degeneration

AMD, as the leading cause of blindness in the western world, is the most commonly investigated retinal condition with respect to the potential benefits of supplemental L, Z, or *meso*-Z. Observations, including relative preservation of short wave sensitive cones centrally when compared to the perifoveal region[36] and the initiation of geographic atrophy in the perifovea, where MPOD is lowest, are consistent with the view that MP protects against AMD and against psychophysical changes known to precede this condition. Since publication of the findings of the Eye Disease Case-Control Study Group, where a 60% risk reduction for AMD in association with a high dietary intake of L and Z was reported,[37] numerous investigators have further explored the relationship between dietary and serum levels of MP's constituent carotenoids and risk for AMD.[38] With a couple of exceptions (outlined below), studies investigating serum levels of, dietary intake of, or supplementation with, L and/or Z with respect to risk for AMD and/or its progression have (understandably) considered preservation, rather than enhancement, of visual performance, to represent the most appropriate outcome measure (reviewed elsewhere[39]).

Richer[40] evaluated the effect of dietary modification on visual performance for patients with atrophic AMD. Fourteen male patients (70 ± 9 years), receiving 0.73 ± 0.45 portions of dark-green, leafy vegetables/day base intake, were placed on an additional portion of 5 ounces sautéed spinach 4 to 7 times per week or lutein-based antioxidant (3 subjects). Patients demonstrated short-term enhancement of visual function in one or both eyes in terms of amsler grid testing, Snellen acuity, contrast sensitivity, glare recovery, and subjectively on the Activities of Daily Vision Subscale. The authors concluded that the effect of dietary modification on the natural course of atrophic AMD warranted investigation in the context of a randomised, controlled trial.

Such an evaluation was conducted in the LAST (Lutein Antioxidant Supplementation Trial) study. Richer et al.[41] evaluated the effect of supplementation on visual performance in atrophic AMD on 90 subjects in a double blind, placebo controlled trial. Average MPOD increased by 0.09 log units (or 50%) after 12 months, in the L and L plus antioxidant groups. The investigators observed concurrent and statistically significant improvements in contrast sensitivity, visual acuity and subjective measures of glare recovery in both treatment groups, but not in the control group. Snellen-equivalent acuity improved by 5.4 letters in patients supplemented with L, and by 3.5 letters in patients supplemented with L plus antioxidants, whereas improvements in contrast sensitivity were significantly better in the L plus antioxidant group than in the L group.

Falsini et al.[42] studied the effect of supplemental L on central retinal function, assessed electrophysiologically, in patients with early AMD, and reported a significant

Outcome measure	Findings
Scotopic sensitivity & short wave sensitivity	Higher MPOD associated sensitivities equivalent to younger observers
Photophobia	Higher MPOD correlated with less photophobia
Short wave increment thresholds	No correlation with MPOD
Photostress recovery and grating visibility	Higher MPOD relates to shorter recovery times and improved sensitivity
Gap acuity and vernier acuity	No correlation with MPOD
Dark adaptation & scotopic visual acuity	Both dark adaptation and scotopic visual acuity showed transient improvements
Dark adaptation	No improvement but experimental error has been suggested
Dark adaptation	Dark adaptation improvement lasting up to 4 months
Dark adaptation	Primary & secondary portions of dark adaptation curve improved
Light sensitivity	Sensitivity improved
Dark adaptation	Dark adaptation improvements proportional to blood lutein increase
Photophobia	MPOD correlated with baseline sensitivity and improved with supplementation
Blue/yellow colour discrimination	No effect of supplementation on colour discrimination
Mesopic contrast acuity	Supplementation improved performance with lutein, zeaxanthin or combination, no improvement with placebo
Visual acuity (near + distance), contrast sensitivity and photostress recovery	No performance improvement over 9 months or 18 months
Photostress recovery and grating visibility	Increased MPOD led to improved performance and faster recovery

improvement in focal ERG amplitude after six months of supplementation, and this was followed by regression back to baseline values following discontinuation of the supplement. Unfortunately, the investigators did not measure MPOD, and therefore conclusions must be interpreted with full appreciation of this limitation.

Bartlett and Eperjesi[43] undertook a prospective, 9-month, double-masked randomised controlled trial of the effect of supplementation with lutein combined with vitamins and minerals on contrast sensitivity among participants with age related maculopathy and atrophic AMD. Contrast sensitivity was assessed using a Pelli-Robson chart and participants were randomised into active and placebo treatment groups. The authors report no significant improvement in contrast sensitivity among either group and suggest that supplementation with 6 mg/L and other antioxidant vitamins and minerals has no tangible benefit to this group (although one could argue that preservation rather than enhancement of performance might be a more suitable outcome measure for AMD patients) and further, that determination of optimum dosage levels requires further work. Their findings are naturally confined to the somewhat limited measure of contrast sensitivity with a Pelli-Robson chart that may not be best equipped to detect subtle changes in performance. Failure to record MPOD at baseline, and the low dosage of supplemental L, represent design flaws in that study, and limit the scope for meaningful comment. Parisi et al.[44] have also recently explored the influence of short-term carotenoid and antioxidant supplementation on electrophysiologically assessed retinal function in early AMD. Of the 27 early AMD patients enrolled in their study, 15 had daily oral supplementation of vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg) for 12 months, while the remaining 12 had no dietary supplementation during the same period. Fifteen age-similar healthy controls were also assessed at baseline and followed-up for the duration of the study period without supplementation. Multifocal electroretinograms, in response to 61 M-stimuli presented to the central 20 degrees of the visual field (averaged across 5 retinal eccentricity areas between the fovea and mid-periphery: 0 degrees to 2.5 degrees, 2.5 degrees to 5 degrees, 5 degrees to 10 degrees, 10 degrees to 15 degrees, and 15 degrees to 20 degrees) were assessed at baseline in controls and in early AMD patients, and again at 6 months and 12 months. At baseline, they observed highly significant reductions of N1-P1 response amplitude densities (RADs) for the central five degrees surrounding the fovea in AMD patients when compared with healthy controls. For more peripheral retinal eccentricities, RADs were not significantly different from controls. After 6 and 12 months of treatment, the treated group showed highly significant increases in N1-P1 RADs for the two most central retinal areas, but not for more peripheral eccentricities beyond 5 degrees. The

non-treated control group exhibited no significant RAD changes at any eccentricity. These findings suggest that in early AMD eyes, central retinal function (0 degrees -5 degrees) can be improved by supplementation with carotenoids and co-antioxidants. The study design, however, does not clarify whether such improvements in retinal function have a measurable impact on visual performance and experience, and the failure to measure and record MPOD somewhat limits the interpretation of these potentially important findings.

Cataract

Olmedilla et al.[45] investigated whether supplemental L influences visual function in patients with age-related cataract, where visual performance was evaluated by measures of visual acuity and glare sensitivity. This randomised, placebo-controlled trial revealed significant improvements in visual acuity and glare sensitivity following supplemental L, and the observed improvements were related to changes in serum levels of L, whereas no such improvements were observed in patients supplemented with placebo or with α-tocopherol. While contrast sensitivity was not recorded at baseline or during the supplementation phase, it is interesting to note that in cataract patients supplemented with lutein, contrast sensitivity at the end of the supplementation period was similar to or even better than that expected for control subjects of a similar age. The authors postulated that the observed improvements in the outcome measures were not the result of any change in the crystalline lens, but more likely to be the result of improved retinal function.

Studies in normal populations

Photophobia and glare

Photophobia is a phenomenon experienced by all persons when illumination is suddenly and dramatically changed from dark to light, and is typified by the experience of switching on a bedroom light at night time. However, under normal daylight conditions, the experience of photophobia is somewhat more variable. Numerous clinical conditions (e.g. RP & AMD) are associated with photophobia, and, even in the absence of detectable disease, clinicians are often presented with patients whose primary complaint is of periodic or persistent sensitivity to bright light (but at levels which do not similarly affect colleagues/friends/family). Given its absorption characteristics, the optical density of MP may be important in determining an individual's threshold for the subjective complaint of photophobia.

Stringham et al.[46] explored the effect of the spectral composition of a target on visual discomfort, using electromyography and a rating scale to determine photophobia thresholds. They showed that, while there was a positive relationship between wavelength and the energy needed to produce photophobia for wavelengths between 520 and 640 nm, at shorter wavelengths there was a notch centred at 460 nm, the trough and shape of which resembled the log transmittance spectrum of MP. Their findings led the authors to suggest that MP may attenuate photophobia or visual discomfort induced by short wavelength sources.

These observations prompted a subsequent study investigating the relationship, if any, between MP and photophobia.[47] This two-part experiment explored the relationship between baseline MPOD levels and photophobia thresholds, as well as the effect of augmenting MPOD on such thresholds. Four subjects were supplemented with 30mg/L and 2.7 mg Z daily for 12 weeks. Peak MPOD was observed to increase from 0.452 ($\pm$ 0.11) at baseline to 0.536 ($\pm$ 0.11) at the end of the period of supplementation. A significant and inverse relationship between baseline MPOD and threshold for photophobia was observed, such that individuals with higher MPOD had higher tolerance for short wavelength light. Furthermore, increasing MPOD over a 12-week period appeared to increase the threshold for photophobia for all subjects for short wavelength sources.

Recently, Stringham & Hammond have explored the influence of glare on visual performance, and how MPOD might influence any observed relationships. They first looked at baseline visual performance under glare conditions by evaluating photostress recovery (a sensitive indicator of macular function) and grating visibility.[48] The effect of veiling glare on grating visibility was explored using a five cycles per degree contrast grating stimulus, surrounded by a concentric annulus of adjustable intensity. For the photostress recovery test, the same stimulus was viewed following photostress with a 5 degree xenon white disc providing 5.5 log Trolands of retinal illuminance over 5 seconds' duration. MPOD was a significant determinant of the deleterious effects of glare, with visual thresholds and photostress recovery times significantly and inversely related to MPOD. Further, high MPOD was associated with better visual performance in a way that was consistent with its spectral absorbance and spatial profile.

These observations prompted the same investigators to design and execute a trial of supplemental L (10 mg per day) and Z (2 mg per day), using the same testing conditions, but on this occasion looking for changes in performance associated with augmentation of MPOD. In this instance, they found that, following six months of supplementation, and an average increase in MPOD from 0.41 to 0.57, most subjects exhibited improved photostress recovery and glare tolerance in association with an increase in MPOD. More specifically, a 39% increase in MPOD enhanced tolerance of intense glaring light by up to 58% and reduced photostress recovery times by 14%.[49]

Although the authors wisely suggest a cautionary approach to the interpretation of their data and the wider implications of such findings, their conclusion that the results are "both large enough and sufficiently general to be meaningful in real life", and that "supplementing L and Z could indeed be palliative for those suffering the consequences of glare", is important and warrants further investigation in the form of a randomised clinical trial.

Spatial vision

Engles et al.[50] have investigated the "acuity hypothesis", exploring the relationship between MPOD and gap acuity and vernier acuity under "photopic" conditions. They report that neither gap acuity nor vernier acuity were significantly related with MPOD, and concluded that their "data suggest that the predictions of the acuity hypothesis do not hold". While the authors qualify their findings as appropriate to their specific testing conditions alone, several study

limitations (other than those recognised by the authors) warrant brief discussion.

Firstly, although the authors report that their conclusions are relevant for photopic conditions, their adopted background luminance levels are in the low photopic range at best (17 cd/m^2 for the achromatic condition, and 15.7 cd/m^2 for the chromatic condition), and certainly not appropriate for evaluation of photopic visual function. Indeed, given the subtle nature of any performance changes likely to be facilitated by MP, the background luminance difference (≈8%) between the two testing conditions is also a potentially confounding factor.

Secondly, while all subjects were corrected to 6/6, it is plausible, indeed probable, that the actual acuity limits of their study population ranged widely between the 6/6 level employed up to a likely 6/3 limit for a young healthy subject. This potential two-fold range in acuity, subserved by individual optical, anatomic and neural architectures, would have a strong influence on both gap and vernier acuity tasks, almost certainly more powerful than MPOD. Also, by adopting a 6/6 limit, the investigators most likely failed to correct for potentially significant amounts of uncorrected axial astigmatism in some subjects, which could significantly influence performance on both of the chosen tasks (testing of vernier and gap acuity limits). While the authors could argue that any such variables remained consistent between testing conditions, we believe it would be more appropriate to eliminate sources of variability such as residual refractive error, so that all subjects operate at their limits of acuity.

The adoption of a single spatial frequency and contrast setting further limits the conclusions that can be drawn from this paper. The effect of MP, for example, might differ significantly under different spatial frequency and or contrast ranges. Assessment of visual performance across the full contrast sensitivity function might represent a more thorough and rigorous assessment of MP's capacity to affect visual performance through attenuation of the effects of chromatic aberration and light scatter.

Finally, the subjects employed in the Engles study typically exhibited average to high MP levels, with few subjects exhibiting MP levels below 0.20. Reading and Weale[15] previously modelled the potential effect on MP in terms of attenuation of the effects of chromatic aberration, and suggested that, due to the non-linear nature of the effect, MPOD levels above 0.30 were probably superfluous. Based on the assumptions of this model, a study on the effect of MP on visual performance might require the inclusion of relatively more subjects that exhibit low MPOD levels in order to demonstrate an effect.

These limitations of the cited study serve to emphasize the challenges inherent in investigating the role of MP in visual performance and experience, which rest on the need (insofar as is possible) to disentangle the influence of MP from the often unquantifiable and variable influences of individual optical and neural architectures.

Loughman et al.,[51] in a cross sectional analysis involving some 142 young healthy subjects, observed statistically significant relationships between MPOD and best corrected visual acuity, and also with photopic and mesopic contrast sensitivity at intermediate spatial frequencies. MP appeared to contribute to up to a 0.1 log unit refinement of high contrast visual acuity (equivalent to one extra line on the acuity chart, or the effective correction of up to 0.25D or residual blur). The correlations between MPOD and visual acuity and contrast sensitivity however, although statistically significant, account for only a small percentage of the potential variability (r^2 values < 10%), so should be interpreted cautiously with respect to its clinical relevance in the absence of a more rigorous placebo controlled supplementation study.

Bartlett and Eperjesi[52] set out to explore the effect of L supplementation on visual performance among healthy observers. Similar to their AMD trial (2007), the authors report no effect of supplementation on performance measures ranging from distance and near visual acuity, contrast sensitivity and photostress recovery. The results are somewhat unsurprising however given (a) the low dose, 6 mg/L supplement used, (b) the basic nature of the series of tests employed to evaluate visual performance, and (c) the small number of subjects tracked over 9 months (n = 46) and 18 months (n = 29) across such a broad age range (22-73 years). Once again, their failure to record MPOD or serum L and Z levels means that only qualified comment can be made as to the significance of the reported findings.

Armstrong et al., in a primitively designed pilot study (involving only one subject) presented at a recent conference (ARVO 2008, Poster # 4964/D984), evaluated macular function on a serial basis throughout a 4-month period of supplementation with L and Z. Looking at a series of psychophysical and electrophysiological outcome measures, they evaluated the effect of supplementation on dark-adapted thresholds and recovery kinetics, pattern visual evoked potentials (PVEPs) [before and after photostress], and PERG amplitude. An MPOD increase of approximately 33% was accompanied by a 23% improvement in 650nm dark adapted thresholds (from 30dB to 37dB) and by an increase in PERG amplitude, but not by a change in cone recovery kinetics or photostress PVEP recovery. Although these findings should be interpreted with caution, particularly given that only one subject was tested, they are again suggestive of an improvement in macular function following augmentation of MPOD in young healthy subjects.

The inconsistencies in spatial vision data with respect to MPOD reflect the difficulty inherent in isolating performance tasks which may be influenced by MP. Furthermore, the wide inter-individual variability of MPOD[53] renders the interpretation of such studies all the more challenging, particularly where such investigations depend on cross-sectional rather than longitudinal data. It would however seem to be the case that, as far as spatial vision is concerned, the effect, if any, of MP on performance appears small, at least for individuals with average to high MPOD.

Colour vision

Since the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 460 nm,[54] it would seem likely that these pigments influence colour vision through selective absorption of short wavelengths, thereby influencing the short-wave sensitive (SWS) cones and the blue-yellow opponent-colour channel. Moreland and Dain[55] (1995) reported that hue discrimination, measured using the Farnsworth-Munsell 100-Hue test (FM100), is indeed adversely affected (primarily) for blue wavelengths, by simulation of high MPOD using liquid notch filters containing

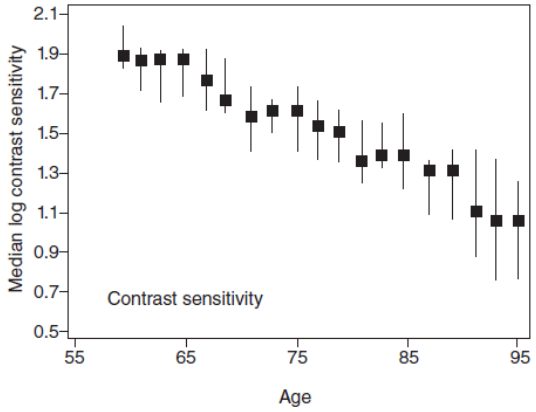

Figure 4 Effect of normal aging on contrast sensitivity. Experimental data show a 1-log unit sensitivity decrease from age 60 to 95. Reprinted with permission: Haegerstrom-Portnoy, et al. 2007;79.

carotene in a benzene solution. Comparing the results with those obtained with a neutral filter, they concluded that this effect was not simply the result of reduced retinal illuminance. Further evidence supporting an effect of MPOD on short wavelength vision has been obtained from studies of SWS cone sensitivity.[56,57] It has also been shown that colour discrimination measured by a colour matching technique is influenced by MPOD.[58,59]

However, two recent studies using alternative methods, produced conclusions differing from those of the above mentioned studies. Firstly, a study of the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP (measured by heterochromatic flicker photometry) and red-green (RG) or yellow-blue (YB) colour discrimination thresholds, though it was reported that RG vision was improved following supplementation.[60] Secondly, RG cancellation profiles have been reported to be highly correlated with MPOD, while profiles for YB were independent of both eccentricity and MPOD.[61] Further support comes from a study of anomaloscope Moreland match midpoint data, in which no difference was reported between post-cataract patients with blue-absorbing intra-ocular lenses (IOLs) and those with clear IOLs.[62]

Thus, the influence, if any, of MP on colour vision remains uncertain at the present time. However, It is possible that an artificial filter creates short-term changes in colour vision and that an autoregulatory process adjusts retinal and/or cortical colour mechanisms on a long-term basis in response to an individual's naturally occurring MPOD.[61] This hypothesis is supported by data showing a consistent shift in achromatic locus over a three month period for post-surgical cataract patients,[63] and by evidence of plasticity of adult neural colour mechanisms.[64]

Preservation of 'youthful' vision into old age

In the elderly, pre-retinal image degradation and slower encoding results in featurally-compromised representation of spatially-extended search arrays. Even with appropriate optical correction, older adults therefore do not possess the spatial resolving power of the young adult. Such losses are not confined to high spatial frequencies, but contrast sensitivity losses are observed across a range of intermediate frequencies.[65] Indeed, many changes in both structure and function of the visual system, such as pupillary miosis and loss of crystalline lens transparency,[66] accompany the aging process (summarised elsewhere[67]). The consequence of such changes is a reduction in retinal illuminance, such that equiluminant stimuli do not result in equal retinal illuminance for different age groups. Human visual performance therefore tends to decrease with age (Figure 4). Such effects are to some extent unavoidable, and a natural consequence of aging.

The most significant role of MP in vision, however, may rest on the potential of L, Z and *meso*-Z to retard the aging process through their antioxidant properties. It is important to note that MP acts, uniquely, as an antioxidant, both passively and actively, the former mechanisms being dependent on its ability to limit photo-oxidative damage by filtering short wavelength light at a pre-receptorial level and the latter mechanism attributable to its capacity to quench reactive oxygen intermediates.

The inter-individual variability in MPOD, consistently observed in cross-sectional studies, may have important implications for the long term health and viability of the central retina. In subjects with little MP, the cumulative and chronic effects of increased exposure of photoreceptors to short wavelength light, coupled with a weaker local capacity to quench free radicals, could, in theory at least, accelerate the onset of physiological and pathological aging of the retina.

In support of such a notion, Hammond et al.[56] have shown that high MPOD was associated with the retention of youthful scotopic and short wave sensitivity and suggested that MP may retard an age-related visual decline. The potential benefits of increased MPOD appear not to be confined to the retina. Hammond et al.[68] reported a positive and significant association between crystalline lens transparency and MPOD, and speculated that high concentrations of the macular carotenoids in the lens probably accompany high concentrations at the macula, and protect against the effects of oxidation in the lens (thereby maintaining transparency). Indeed, other studies have shown an association between a high dietary intake of L and Z with decreased incidence of cataract formation.[69,70]

Werner and Steele[71] demonstrated age-related sensitivity losses of foveal colour mechanisms across all three cone types, although the sensitivity loss for short wavelength sensitive cones (S-cone) was lower (at 0.08 log units per decade), when compared to 0.11 log units loss per decade for both medium (M-cone) and long wave (L-cone) cones. Werner et al.[57] later explored the senescence of foveal and parafoveal cone sensitivities and their relation to MPOD. Again, they report age-related decline of foveal and parafoveal increment thresholds. Interestingly however, and consistent with the hypothesis that the MP protects the photoreceptors from senescent losses in sensitivity, a significant and positive correlation was found between foveal MPOD and differential S-cone log sensitivity losses at the fovea and at the parafovea, but not with differential M- and L- cone log sensitivity losses at the retinal loci. This finding, however, was independent of age, prompting the authors to postulate that it was due to local gain changes,

resulting from differential filtering of incident light by the MP between the fovea and the parafovea.

Haegerstrom-Portnoy[72] also examined S-cone versus L-cone sensitivity in a group of young and older adults to determine whether MP protects the human fovea from retinal neural damage caused by visible-light exposure over a lifetime. While there was no difference observed for L-cone sensitivity between groups, the older group showed a significant differential loss of S-cone sensitivity across the retina compared with the younger group, with greater loss of sensitivity at non-foveal locations than at the fovea. This observation is again suggestive of a protective effect of MP on foveal function.

Schupp et al.[73] endeavored to explore the hypothesis from a different perspective, postulating that if high levels of MP might forestall the effects of normal aging, then low levels of MP might accelerate the normal aging process. Cystic fibrosis (CF) is a condition associated with defective gastrointestinal absorption of carotenoids as a result of pancreatic insufficiency. Low serum concentrations of carotenoids, including the constituents of MP, are invariably reported in CF patients. Given the repeatedly observed positive and significant relationship between MPOD and serum concentrations of its constituent carotenoids (reviewed elsewhere[74]), it can be reasoned that patients with CF would have low MPOD. Schupp et al.[73] assessed visual performance in ten cystic fibrosis patients, in whom serum concentrations of L and Z and MPOD were predictably and significantly lower than control subjects, and typically less than 50% of the values observed amongst control subjects. However, visual performance (contrast sensitivity, colour discrimination and multifocal ERG amplitudes) were statistically similar for CF patients and control subjects.

While the basic rationale of this study is provocative, there are however a number of concerns with the methodology. With six of the ten CF subjects aged between 21-27 years, it is unlikely that such a youthful population sample would demonstrate accelerated aging effects on visual function (even in the presence of chronically low MPOD levels). In any case, given the theoretical possibility that higher levels of MP might be associated with enhanced visual performance, it is unclear from this publication as to how functional differences, which might have been observed between the CF and control groups, could be attributable to age effects rather than simply to differences in MPOD. The authors conceded that a longitudinal assessment of an older CF population is required to address the hypothesis more appropriately.

Hammond and Wooten[75] investigated the relationship between MP, critical flicker fusion frequency (CFF) and age, citing CFF as a general measure of visual health. They found a significant decline in CFF values with age. There was a significant and positive relationship however between MPOD and CFF values that was independent of age. The authors conclude that these results are consistent with a protective effect of MP on visual health across the lifespan. While such investigations appear to be at a very early stage, preliminary results suggest a role for MP in temporal vision and, specifically, that high MPOD may protect the retina and defer some typical age-related changes in temporal vision.

Conclusions

Visual performance in the normal human is less than ideal, and it has been shown that visual performance improves once chromatic and monochromatic aberrations are removed.[76] As a consequence, numerous interventions which attenuate these aberrations have been developed in an attempt to optimise and/or enhance visual performance, Wavefront-guided laser refractive eye surgery, wavefront-guided spectacle lenses, short wavelength-filtering intraocular lens implants, short wavelength-filtering contact lenses and short wavelength filtering spectacle lenses are all directed towards improving or optimising visual performance. These techniques, however, are primarily intended for persons with pre-existing ocular abnormality or disease, and there has been a conspicuous lack of concerted effort to improve (or maintain) visual performance in subjects without demonstrable ocular pathology. Augmentation of MPOD by means of supplementation remains a plausible and realistic means (in theory at least) of optimising and/or enhancing visual performance in a normal population.

Future studies should address the issue of whether variations in MPOD relate to visual performance, and whether high MP levels can preserve or prolong optimal central visual function into old age. Indeed, some studies have reported that high levels of MP are associated with preservation of retinal sensitivity in the elderly.

MP has ideal properties, in terms of location and spectral absorbance, to be beneficial for visual performance and experience. Longer life expectancy, increased exposure to short wavelength light (ancestors had little or no short wavelength light exposure after dark), increased effects of scatter from expanding smog and haze, modern visual requirements and the ever-increasing incidence of AMD heightens the importance of both optimising (and possibly enhancing) visual performance in the working population, and preserving such performance into old age. Robust evidence, in support of the psychophysically plausible rationale, that MP contributes to visual performance and experience in a favourable way is, however, still lacking. The findings of the studies cited above, whether demonstrating a benefit of MP to visual performance and experience or not, should be interpreted with full appreciation of their design limitations, and it should be understood that a cross-sectional study represents an inappropriate design to investigate fully any contribution that MP makes to visual performance. It is unwise to assume that the role of MP in visual performance, if any, can be easily studied, given the multitude of typically individual and occasionally enigmatic factors that influence our visual experience.

Given the numerous optical and neural factors that influence and dictate visual performance, and the consequential and associated difficulties in isolating improvements in visual performance, any study designed to investigate the influence of MP in this regard should include questionnaire-based analyses of subject perceptions of personal visual experience. Such an approach will facilitate investigation of the potential role of MP in visual performance in the real world, in a natural and ever-changing environment, which is often poorly reflected in our current

and limited arsenal of testing modalities. None of the studies which reported a beneficial effect of MP augmentation adequately address the question of (1) whether such increases in MPOD and the observed psychophysical functional improvements translate into tangible improvements in visual experience outside the laboratory or (2) whether such improvements can be longitudinally maintained to preserve functional performance and experience into old age.

Because of the inter-individual variability in MPOD and psychophysical visual function, a study designed to investigate the contribution of MP to visual performance and experience should be able to study the relationship between changes in these parameters within subjects over time, and only a study where MP is augmented by supplementation and/or dietary modification can meet this essential criterion. Interestingly, of the studies cited in this review, there appears to be one reasonably consistent finding, despite varied design limitations, studies involving supplementation among normal and diseased eyes typically report measurable benefits in terms of visual performance, in terms of photophobia thresholds, glare sensitivity, dark adapted thresholds, PERG amplitudes and mesopic contrast sensitivity among others.

Thus far, there appears to be little or no evidence of any adverse effect of higher levels of MP on visual performance. In a study designed to determine the influence of macular pigment absorption on blue-on-yellow perimetry, Wild and Hudson[77] found that the net effect of ocular media and MP absorption relative to 460 nm was to attenuate the blue-on-yellow visual field at the fovea by approximately 0.80 log units and elsewhere by 0.40 log units, the difference being attributable to MP. Unpublished results from our own laboratory suggest no association between MPOD and colour matching or colour discrimination ability, although we have observed a non-significant inverse association between central short wavelength sensitivity and MPOD (data on file). The possibility of an adverse effect of MP augmentation on colour vision, short wavelength sensitivity and other functional measures does merit future investigation.

The optical, physiological and neurological interactions that contribute to vision suggest that the optimal level of MPOD, from a performance perspective, may be personal to an individual eye. In other words, and for example, even if MP is found to be important for visual performance and experience, exceeding a particular optical density of the pigment may yield no further measurable or appreciable advantage, and this level may vary substantially from one individual to the next. It is also important to note that testing conditions are often incapable of reflecting more natural environments, and any observed absence or presence of MP's contribution to visual performance and experience may not necessarily hold true in a natural environment (for example, against the background of a bright blue sky).

Although it remains difficult to draw firm conclusions regarding the relationship between MP and visual performance, certain patterns do appear to exist. In normal observers, the effect on spatial and colour vision appears small in comparison to the observed effects on photophobia and glare sensitivity, while, in subjects with established eye disease, there appears a relatively consistent beneficial effect of MP supplementation on visual performance. Any effects observed, whether through optical or biological mechanisms, may also be magnified when increased emphasis is afforded to those with chronically low MPOD levels. We need and should support an appropriately powered, randomised, controlled trial, which is designed to further evaluate whether visual performance and experience can be optimised or enhanced, or indeed adversely affected, with supplemental macular carotenoids.

Conflict of interest

The authors state they have no conflict of interest.

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Clinical

Macular supplements

Edward Loane, Dr John Nolan, Peter Baranyovits, Mukunda Akkali and **Stephen Beatty** present the latest results from the Waterford Macular Pigment Research Group

This is a retrospective, observational non-comparative report of two cases. The first patient had late age-related macular degeneration (AMD) and the second early AMD. Following diagnosis, both patients were supplemented with all three macular carotenoids in the form of a commercially available product. Both were observed with fundus photography and Snellen visual acuity (VA) measurement. Case 2 also had her macular pigment (MP) optical density measured.

Case 1 showed resolution of central macular drusen and improvement in Snellen VA from 6/9 to 6/6+4 OS following supplementation with all three macular carotenoids. Case 2 showed an increase in foveal MP optical density from 0.4 to 0.79 following supplementation with all three macular carotenoids. Supplementation with lutein, zeaxanthin and meso-zeaxanthin in patients with AMD may result in resolution of central macular drusen, improvement in VA, and augmentation of foveal MP.

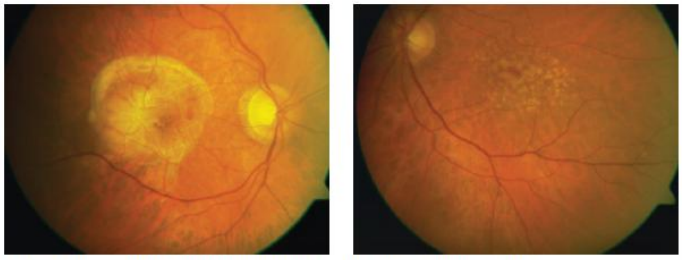

Figure 1 Neovascular AMD in the right eye and macular drusen in the left

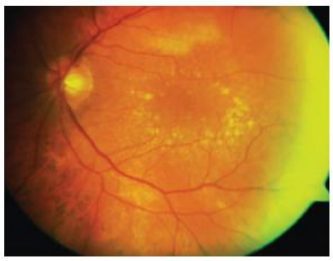

Figure 2 Resolution of drusen centrally in the left eye

Macular degeneration

AMD is the leading cause of blind registration in people over 50 years of age in the developed world.[1] Late AMD results in loss of central and colour vision. It is estimated that late AMD affects more than 1.75 million individuals in the US, and this figure is expected to rise to almost 3 million by 2020.[2]

Early AMD is characterised by drusen and/or retinal pigment epithelium (RPE) hyper- or hypo-pigmentation.[3] Drusen are whitish-yellow spots external to the neurosensory retina or the RPE. Histologically, drusen are focal deposits of heterogeneous debris external to the RPE basal lamina and internal to the inner collagenous layer of Bruch's membrane. Drusen have been found to contain acute-phase reactants, markers of inflammation, complement proteins, lipoproteins, neutral lipids, and oxidative protein modifications.[4,5] These findings contribute to growing evidence implicating oxidative injury and inflammation in the pathogenesis of drusen formation and AMD.[6] Late AMD is characterised by either geographic atrophy (any sharply delineated area of hypopigmentation, or depigmentation, or apparent absence of the RPE, in which the choroidal vasculature is more visible than in the surrounding area; the area of atrophy must be ≥175µm in diameter), or choroidal neovascularisation.

Macular pigment (MP) is a yellow pigment, entirely of dietary origin that accumulates at the macula. There is a growing body of evidence suggesting that MP protects against AMD.[7] MP is composed of the carotenoids: lutein (L); zeaxanthin (Z); and meso-zeaxanthin (meso-Z). L and Z are found in a conventional western diet in the yolk of eggs, leafy green vegetables, and in yellow and orange coloured fruits and vegetables.[8] Meso-Z is not found in a conventional western diet, but may be found in less commonly eaten types of fish and shell-fish.[9] However, meso-Z is also formed in the retina by isomerisation of L.[10,11] MP is understood to protect the macular photoreceptors by acting as a filter of actinic short-wavelength (blue) light and/or by its innate antioxidant activity.

Methods

Fundus photography and Snellen VA measurement were performed. Both patients received supplementation with all three macular carotenoids in the form of a commercially available product (Macushield, Macuvision Europe, UK; containing: L 10mg, Z 2mg, meso-Z 10mg). The second patient had her MP optical density measured by heterochromatic flicker photometry using a macular densitometer.[12]

Case reports

Case 1

A 73-year-old male emmetropic patient was diagnosed with neovascular AMD in April 2001 in his right eye, and with macular drusen in his left eye (Figure 1). Visual acuity (VA) at this time was 6/12 L and 6/6 R. He is a non-smoker with a past medical history of a myocardial infarct in 1988, following which he has been on treatment with aspirin, antihypertensives, and a statin. His family history of AMD consists of a sister, aged 72, who has recently been diagnosed with geographic atrophy.

VA rapidly decreased to counting fingers R, and between 2005 and 2007 VA L decreased from 6/6 to 6/9. He also volunteered a history consistent with an increased photostress recovery time and poor colour vision. In April 2007 he was commenced on supplemental L, Z and meso-Z (Macushield) once daily. He does not report any other significant changes in his diet or lifestyle since commencing this supplement. Over the succeeding months, he reported subjective improvement in vision. Examination in March 2008 revealed an improvement in VA to 6/6+4 L and resolution of macular drusen centrally (Figure 2).

Case 2

A 63-year-old female emmetropic patient was diagnosed with bilateral early AMD in 2004. She has smoked two cigarettes per day for the past 25 years. She has a past medical history of non-metastatic breast cancer, osteoporosis and hypertension, for which she is treated with aspirin and antihypertensives. She has no known family history of AMD, but reports a low intake of fruits and vegetables during her lifetime.

VA is currently 6/6 OU. Examination in March 2008 showed a MP optical density of 0.4 at 0.25° retinal eccentricity, and 0.64 at 0.5° retinal eccentricity, measured by heterochromatic flicker photometry, before commencing Macushield. Prior to this, she had been taking a supplement containing L and Z only. Follow-up assessment in June 2008 revealed an increase in MP optical density at 0.25° retinal eccentricity to 0.79, and an increase at 0.5° retinal eccentricity to 0.72 (Figure 3). Fundal appearance and VA remained stable (Figure 4).

Conclusion

The concentration of MP peaks at the foveal centre and declines with increasing retinal eccentricity. Within the layer structure of the retina, MP reaches its peak concentration in the photoreceptor axon layer and the inner plexiform layer. Interestingly, meso-Z followed by Z are the dominant carotenoids centrally, whereas L is found in higher concentrations parafoveally. Supplementation studies in humans have shown that MP levels increase following supplementation with the macular carotenoids.

Spontaneous resolution of drusen has been previously described in 16 (34 per cent) of 47 participants in the Waterman study, after five years of follow-up.[13] However, spontaneous resolution of drusen is understood to be associated with atrophy of the overlying RPE and subsequent photoreceptor atrophy leading to vision loss. In Case 1, the resolution of drusen centrally was not accompanied by RPE atrophy, but rather an apparent re-establishment of normal anatomy and an improvement in vision. In Case 2, the MP optical density showed a dramatic increase centrally following supplementation with all three macular carotenoids, despite the fact that this patient had previously been supplementing with a product containing L and Z, but not meso-Z. This observation suggests that supplemental meso-Z was required to maximise this patient's MP levels centrally, and is consistent with the resolution of centrally located macular drusen observed in Case 1, perhaps resulting from the more potent anti-oxidant activity of meso-Z when compared with that of either L or Z. Despite the fact that meso-Z can be formed by isomerisation of L, certain individuals may lack the ability to effect this conversion in the retina, and may therefore benefit from direct supplementation with meso-Z to maximally augment their MP levels.

The two cases presented here suggest that the resolution of drusen centrally may be attributable to the capacity of supplements containing all three macular carotenoids to augment MP levels centrally. The mechanism for this remains unclear, but may relate to the potent antioxidant capacity of meso-Z and the observation that oxidative protein modifications play a critical role in drusen formation. The antioxidant properties of the macular carotenoids, particularly meso-Z, may thus promote a return to normal photoreceptor-RPE-Bruch's membrane function.

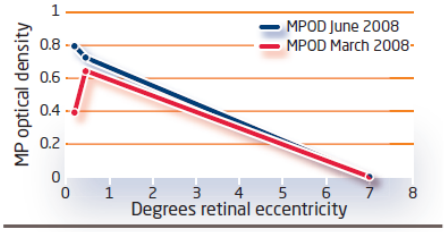

Figure 3 Change in MP optical density (MPOD) following supplementation with Macushield

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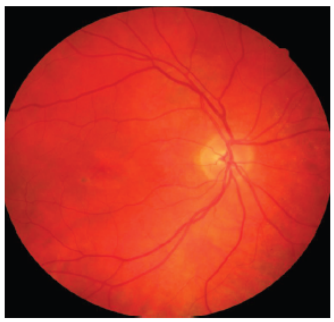
● **Edward Loane, Dr John Nolan, Mukunda C Akkali** and **Stephen Beatty** are based at the Macular Pigment Research Group, Waterford Institute of Technology, Ireland. **Peter Baranyovits** works in the Department of Ophthalmology, Kettering General Hospital, Northamptonshire



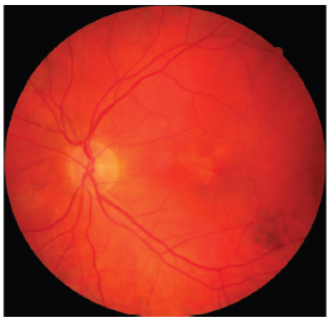
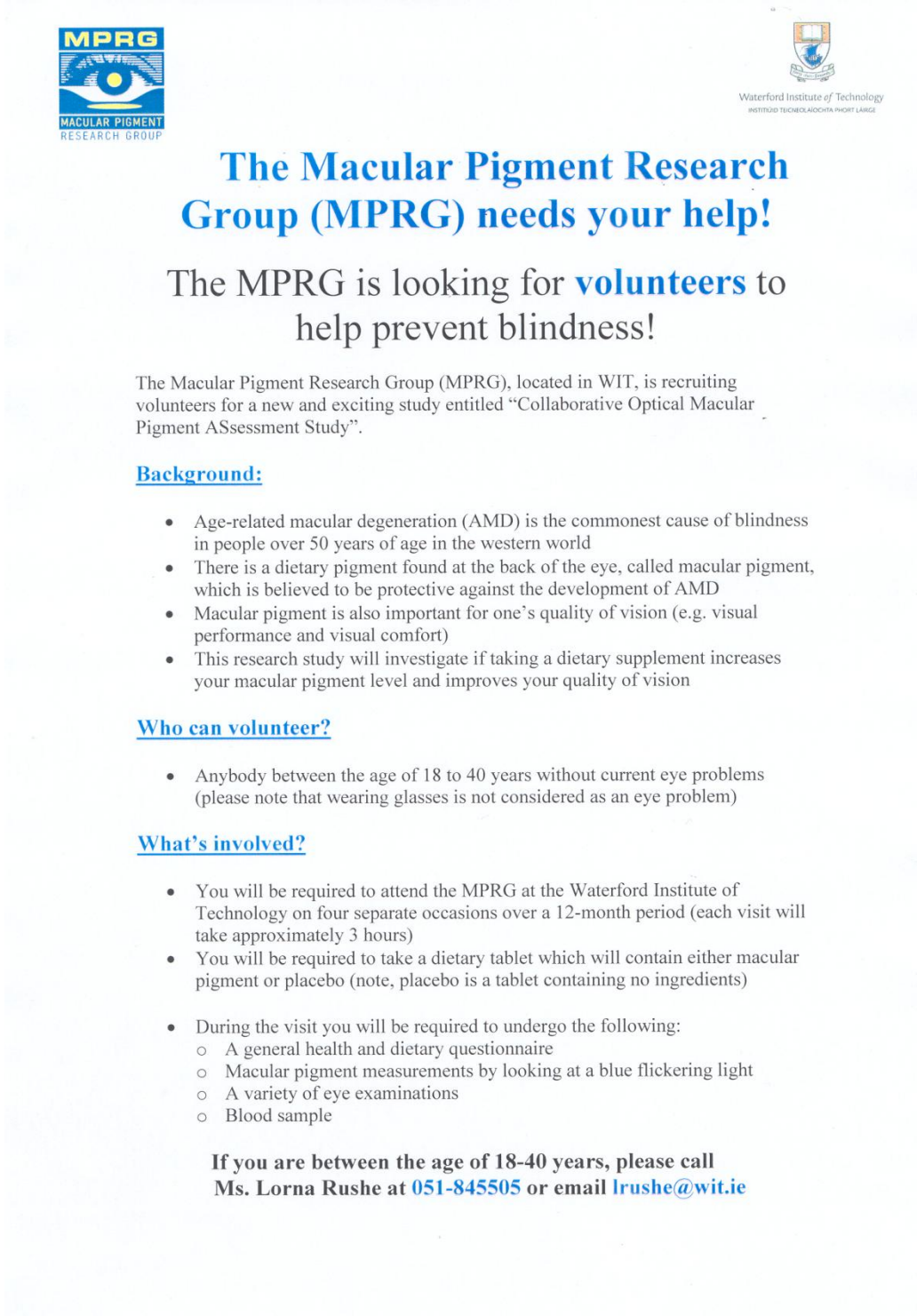


Figure 4 Bilateral early AMD

APPENDICES

7. APPENDICES

APPENDIX 7.1: SAMPLE RECRUITMENT POSTER



MPRG
MACULAR PIGMENT
RESEARCH GROUP

Waterford Institute of Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

The Macular Pigment Research Group (MPRG) needs your help!

The MPRG is looking for **volunteers** to help prevent blindness!

The Macular Pigment Research Group (MPRG), located in WIT, is recruiting volunteers for a new and exciting study entitled "Collaborative Optical Macular Pigment ASsessment Study".

Background:

- Age-related macular degeneration (AMD) is the commonest cause of blindness in people over 50 years of age in the western world
- There is a dietary pigment found at the back of the eye, called macular pigment, which is believed to be protective against the development of AMD
- Macular pigment is also important for one's quality of vision (e.g. visual performance and visual comfort)
- This research study will investigate if taking a dietary supplement increases your macular pigment level and improves your quality of vision

Who can volunteer?

- Anybody between the age of 18 to 40 years without current eye problems (please note that wearing glasses is not considered as an eye problem)

What's involved?

- You will be required to attend the MPRG at the Waterford Institute of Technology on four separate occasions over a 12-month period (each visit will take approximately 3 hours)
- You will be required to take a dietary tablet which will contain either macular pigment or placebo (note, placebo is a tablet containing no ingredients)

- During the visit you will be required to undergo the following:
  - A general health and dietary questionnaire
  - Macular pigment measurements by looking at a blue flickering light
  - A variety of eye examinations
  - Blood sample

If you are between the age of 18-40 years, please call Ms. Lorna Rushe at 051-845505 or email lrushe@wit.ie

APPENDIX 7.2: STUDY INFORMATION LEAFLET

Collaborative Optical Macular Pigment ASsessment Study (COMPASS)

Patient Information Sheet

Invitation to participate:

You are invited to participate in a research study designed to measure your macular pigment level, and various measures of visual performance and visual comfort. This will allow us to identify any relationship(s), which may exist between macular pigment and visual performance and/or visual comfort.

Background Information

There is a yellow pigment at the back of the eye (retina) called macular pigment which is believed may be important for improving visual performance and visual comfort. Macular pigment is of dietary origin i.e. we are not born with macular pigment but we accumulate it from eating certain fruits and vegetables.

Study Design

This study aims to recruit 120 healthy volunteers between the ages of 18-40 years. Each volunteer will attend the Waterford Institute of Technology (WIT) or Dublin Institute of Technology (DIT) on five separate occasions (at baseline [first visit], after three months, after six months, and after 12 months). Each study visit will last approximately three hours. Volunteers will be given a dietary supplement (containing the components of the macular pigment) or placebo to be taken daily for the duration of the study. A placebo is a preparation which does not contain the active ingredients under investigation (e.g. the macular pigments) but which may have a medical effect based solely on the power of suggestion, a response known as the placebo effect or placebo response. This study will be conducted in a double blind placebo controlled randomised fashion. This means that 50% of the people who enrol in the study will be given the supplement and 50% will be given the placebo. The dietary supplement will be provided free of charge by the study investigators.

The following will take place during a study visit:

- You will be asked to sign an informed consent document which states that you are happy to participate in the study and that all aspects of the study have been explained to you by the study investigator.

- A blood sample will be taken to measure macular pigment levels in your blood.

- You will be asked to complete a brief questionnaire to gather information that might be important to the status of your retina, and/or the macular pigment within your retina. This questionnaire covers the following areas: contact details; lifestyle details; personal medical history etc.

- We will measure the quality of your vision using specialised vision testing techniques.

- We will take a picture of the back and front of your eye using a specialised camera.

- We will measure the quality of your colour vision using specialised testing equipment.

- You will be asked to fill in a dietary questionnaire which is an important measure of eye health.

- You will be asked to look into an optical system. You will see circles that will be flickering. You will be asked to adjust a knob to regulate a flickering light to make the flicker stop. This will measure your macular pigment.

- We will measure and record your spectacle prescription.

- Your vision will be assessed using a range of standard vision tests (as used by Opticians/Optometrists in routine practice) that will determine the

quality of your central vision (used for reading and watching television), the quality of your peripheral vision and your sensitivity to glare from bright light sources.

- You will also be asked to complete a short visual function questionnaire that will allow us to compare your perceptions about how good your vision is to the results of the tests described above.

Subject Payment

This study is entirely voluntary. You will not be paid for your participation in this study. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

Risks and/or Discomforts

We foresee no risks to the subjects participating in this research.

Benefits

You will gain knowledge of your macular pigment level. It has been suggested that a person's macular pigment level is a good indicator of overall eye health. You will also be informed on the quality of numerous aspects of your vision such as your glare sensitivity and your central, peripheral and colour vision.

Also, it is possible that society may gain from the results of this study.

Difference of Research Study to Clinical Practice

Your involvement in this study is for research purposes only. This is not a medical examination for your benefit.

Data Confidentially

All the data collected in this study will be treated as strictly confidential and will be obtained and processed in keeping with the Data Protection Act 1988 and the amended Data Protection Act of 2003. All data will be analysed collectively as a group and coded by data link to ensure subjects confidentiality.

Compensation

The study investigators are covered by an insurance, which protects you in case of problems caused by this study.

Organisers & Sponsors

The Macular Pigment Research Group (MPRG) at WIT and the Optometry Research Group at DIT), both well-known and highly established research groups, will be conducting and overseeing this study. This study is sponsored by Bausch & Lomb, a global healthcare company and a leader in eye care products and Enterprise Ireland who are an Irish state development agency focused on transforming Irish industry.

Questions

The MPRG project manager, Ms. Eithne Connolly, will answer any further questions you may have concerning the study, the procedures, and any outcomes that may appear to be related to the research. Eithne can be contacted at 051 845505.

We hope that this information has answered most of your questions. Should you have further questions or do not fully understand the information given, please feel free to ask us.

The doctors and researchers at WIT and DIT, who are carrying out this research, would like to thank you for taking the time to read this information

APPENDIX 7.3: CONSENT FORM

Collaborative Optical Macular Pigment ASsessment Study

Date:_____ Subject Number: _____

1. I confirm I have read and understand the Information Leaflet regarding this study. I further attest that the relevant information has been discussed fully in non-technical terms, and all my questions have been replied to with full satisfaction.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without my medical care or legal rights being affected.
3. I understand that my data concerning this study will be entered on a computer in order to be analysed together with the data obtained from other patients. My identity will always be protected. I give permission for this analysis.
4. I understand that responsible authorities within the Macular Pigment Research Group may look at my data collected for this study where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.
5. I agree to take part in the above study and hereby give my consent to have a blood sample collected for serum and/or DNA analysis.

_____	_____	_____
Name of Volunteer	Date	Signature of Volunteer
_____	_____	_____
Name of Witness	Date	Signature of Witness

☐ **I have no objection to being contacted by the Macular Pigment Research Group about future research studies.**

☐ **I give permission for any data collected today to be used for other research studies carried out by the Macular Pigment Research Group.**

Tel: 00 353 51 845505 Fax: 00 353 51 302697 Email: mprgresearch@wit.ie Web: www.wit.ie/mprg
Macular Pigment Research Group • Chemical and Life Sciences Department • Waterford Institute of Technology • Cork Road • Waterford

APPENDIX 7.4: ADVERSE EVENT FORM

Adverse Event form

Patient No: _____ Patient Initials:_____ Visit_____ Exam Date: _____

Please circle the appropriate statements:

Severity	Action taken with study supplement	Relationship to study supplement	Possible supplement explanation
1-Mild	1-None	1-Definitely unrelated	1-Condition being treated
2-Moderate	2-Reduced frequency of admin	2-Unlikely	2-Intercurrent illness
3-Severe	3-Discontinued temporarily	3-Possible	3-Study supplement
4-Life threatening	4-Discontinued permanently	4-Probable	4-Non Study Medication
	5-Other (comment below)	5-Definitely related	5-Drug interaction
			6- Idiosyncratic effect
			7-Other (Specify)

Date of Onset: **Date Resolved:**

Description of Event:

Signature: _____ **Date:_____**

APPENDIX 7.5: CASE REPORT FORM

COMPASS

Collaborative **O**ptical **M**acular **P**igment

ASsessment **S**tudy

Case Report Form

Investigator Parties:

1. Macular Pigment Research Group
   Waterford Institute of Technology

2. Optometry Department
   Dublin Institute of Technology

3. Bausch & Lomb

Study procedures (Phase 2)

DESCRIPTION	EST. TIME MINS
A. Information leaflet discussion and informed consent	5 mins
B. Demographic, medical history, lifestyle and vision case history questionnaires	20 mins
C. Collection of blood for serum carotenoid analysis	5 mins
D. High and low contrast Visual Acuity (VA), and refraction	15 mins
E. Glare tests (Functional Vision Analyzer™)	10 mins
F. Visual performance questionnaire	10 mins
G. Contrast sensitivity (Metropsis)	15 mins
BREAK	**~30 mins**
H. Macular Pigment Optical Density (MPOD) spatial profile measurement	35 mins
I. Dietary questionnaire	30 mins
J. Photostress recovery test	10 mins
K. Fundus and iris photographs	5 mins

A. Informed consent (consent for all 4 visits).

Was the patient given a copy of his/her consent? yes ☐ no ☐

If yes,

Date of informed consent: _____ Obtained by: _____

(DD/MM/YYYY)

Signature of person obtaining consent: _____

Subject number_____ Subject initials _____ Date _____

Phase 2 (baseline visit) **Date: _____**

(DD/MM/YYYY)

B. Demographic, medical history, lifestyle and vision case history questionnaires

Forename:_____ Surname: _____

Address: _____

_____

Contact No(s): _____

Email: _____

Date of birth: _____ Age: _____

(DD/MM/YYYY)

Please circle number corresponding to correct answer. All questions must be answered unless otherwise specified.

1. Sex

Male ……. 1

Female ……. 2

2. Race

White ……. 1

Black ……. 2

Asian ……. 3

Spanish or Hispanic 4

Mixed race…… 5

3. Marital status

Are you now:

Married (or cohabiting)…… 1

Widowed ……... 2

Single ……... 3

Divorced or separated..... 4

4. Education

Briefly describe your educational background:

_____

_____

_____

5. Occupation

Briefly describe your occupation:

_____

_____

_____

6. Medical History

Have you any of the following medical conditions?	Yes	No
Diabetes…….	1	2
High blood pressure…….	1	2
High cholesterol……	1	2
Angina…….....	1	2
Stroke…….	1	2

If yes for any of the above please give details in the space provided below (e.g. year it occurred, treatment, medication etc.)

_____

_____

7. History of Eye Disease

	Yes	No
Have you ever been told by a doctor that you have Cataract?	1	2
Have you had an operation for Cataract?	1	2
Have you ever been told by a doctor that you have Macular Degeneration?	1	2
Have you ever been told by a doctor that you have Glaucoma?	1	2
Other?	1	2

If yes for any of the above please give details in the space provided below (e.g. year it was diagnosed, doctor etc.)

_____

_____

_____

_____

	Yes	No
Have you a family history of any of the above eye diseases?	1	2

(e.g. age-related macular degeneration, glaucoma etc.)

If a family member, what is their relation to you, and what eye disease do/did they have?

_____

8. Vision Case History

Approximately how long since your last eye examination?

	Yes	No
Do you currently wear spectacles and/or contact lenses?	1	2

If yes – for what?

_____

since when?

_____

any problems with?

_____

Have you ever undergone any ocular treatment or surgery (including Laser eye surgery)?

	Yes	No
	1	2

If yes – for what? _____

when? _____

any complications? _____

Were you required to wear an eye patch as a child?

	Yes	No
	1	2

If yes, at what age? _____

or how long? _____

which eye? _____

Do you have any current problems with your vision?

	Yes	No
	1	2

If yes, please describe in the space provided

_____

Do you use a computer?

	Yes	No
	1	2

If yes,

Do you ever suffer eyestrain associated with using the visual display unit (VDU)? Yes No

1 2

If yes,

Is your VDU task difficult (e.g. lots of glare from windows, very small print, use of coloured print/backgrounds, lack of regular breaks from VDU etc)?

Yes No

1 2

Do you ever suffer headaches? Yes No

1 2

If yes, please give details on the following: frequency, onset, location, duration, associated factors, relieving factors, medical history etc.

_____

Additional Information (please add any other details, if appropriate)

_____

_____

9. Smoking

a) Which best describes your smoking habits (whether cigarette, cigar, pipe etc.)?

Never smoker (smoked < 100 cigs in lifetime).....1

Ex-smoker (smoked ≥ 100 cigs in lifetime and none in past year)….....2

Current smoker (smoked ≥ 100 cigs in lifetime and at least 1 cig in last year)...3

b) Have you smoked at least 100 cigarettes in your life? Yes No

☐ ☐

If no skip to question f

c) How long has it been since you last smoked?

Less than 1 day ☐

Less than 7 days ☐

Less than 1 month ☐

Less than 3 months ☐

Less than 6 months ☐

6 months to a year ☐

Greater than 1 year ☐

d) What is the average number of cigarettes you smoke (or smoked) on a daily basis? _____

e) For how many years have you smoked (or did you smoke)? _____

f) Are you commonly exposed to second-hand smoke at home or in the work place? Yes ☐ No ☐

10. Alcohol

a) Regarding alcohol, which of the following statements best describes the way you drink?

I never drink…….. 1

I drink only on special occasions……... 2

I drink once or twice a month…….. 3

I drink once or twice a week…… 4

I drink three to four times a week…… 5

I drink every day…… 6

I drink twice a day or more…… 7

b) What is your average alcohol consumption on a weekly basis?

0 units a week……..... 0

1 unit a week…… 1

2-5 units a week…….. 2

6-10 units a week…… 3

> 10 units a week…… 4

11. Exercise

Do you perform any of the following physical activities?

	Yes	No
Walking……	1	2
Running…….	1	2
Cycling……..	1	2
Swimming…….	1	2
Gym-based work-outs..……..	1	2
Team sport……	1	2
Other…….	1	2

If ‘Team sport’ or ‘Other’, please describe in the space provided

How many times a week do you carry out the above exercise? time(s)/week ☐

In a typical week what is the total time you would spend performing the above activities?

☐ minutes

12. Body Mass Index (BMI)

Please record the subject's weight and height in the spaces provided

Weight**……** ☐ Kg

Height…….. ☐ M

BMI…….. ☐ Kg/M^2

13. Blood pressure

Please record the subject's blood pressure level in the space provided mmHg ☐

C. Blood extraction record sheet

Was a blood sample taken from the subject (2 x 5 mL yellow top vacuette)?

Yes ☐ No ☐

If yes,

Time of blood extraction: _____

Time of subject's last meal: _____

Was this sample centrifuged, the serum extracted and stored in duplicate at -70^{o}C? Yes ☐ No ☐

If yes,

Time of centrifugation: _____

Name of person obtaining blood: _____

Signature of person obtaining blood: _____

D. High contrast visual acuity and refractive error

1: High Contrast Visual Acuity (HCVA) using Logarithm of the Minimum Angle of Resolution (LogMAR) chart

Please record the subject's unaided VA and aided VA (own spectacles/contact lenses if appropriate) in the spaces provided:

Current Rx Focimetry ☐

Unaided VA…… R ☐ L ☐

Habitual VA (own Rx)…… R ☐ L ☐

2: Refractive Error

Please record the subject's refractive error for both eyes:

R _____ Best Corrected HCVA ☐

L _____ Best Corrected HCVA ☐

3: Ocular Dominance

Please record which eye is dominant:

R ☐ L ☐ Equidominant ☐

4: Study Eye

Please indicate which eye will be used for the current study:

Note: The study eye is the dominant eye.

R ☐ L ☐

5: Best corrected HCVA of study eye (average of 3 measures)

R ☐ L ☐

6: Best corrected LCVA of study eye

R ☐ L ☐

H. Glare / Photosensitivity

Functional Acuity Contrast Test (FACT) - Optec 6500 Vision Tester

Mesopic Contrast Sensitivity With/Without Glare

Attach Graph

I. Visual Function in Normals questionnaire (VFNq30)

Colour Discrimination

(a) Situational Analysis / 100

(b) Comparative Analysis / 100

(c) Subject Satisfaction Score / 100

Glare Disability

(a) Situational Analysis / 100

(b) Comparative Analysis / 100

(c) Subject Satisfaction Score / 100

Acuity / Spatial Vision

(a) Situational Analysis / 100

(b) Comparative Analysis / 100

(c) Subject Satisfaction Score / 100

Light / Dark Adaptation

(a) Situational Analysis / 100

(b) Comparative Analysis / 100

(c) Subject Satisfaction Score / 100

Daily Visual Tasks

(a) Situational Analysis / 100

(b) Quantitative/Comparative Analysis / 100

(c) Subject Satisfaction Score / 100

J. Contrast Sensitivity Function (CSF)

Mesopic CSF

Attach CSF plot below

Attach data sheet with minimum contrast threshold defined for all spatial frequencies

Photopic CSF

Attach CSF plot below

Attach data sheet with minimum contrast threshold defined for all spatial frequencies

K. Macular Pigment Optical Density Spatial Profile

Record the Critical Flicker Fusion Frequency (CFF) values and calculate the Optimal Flicker Fusion Frequency (OFF) values as per COMPASS Densitometer Standard Operating Procedure (SOP)

CFF obtained approaching from lower frequency (10 Hz)

Average:

Use below calculation to calculate the OFF and report below.

Location	**Calculation**	**Predicted OFF**
0.25^0	CFF-8	
0.5^0	CFF-7	
1^0	CFF-7	
1.75^0	CFF-7	
3^0	CFF-9	
7^0	CFF-14	

	Predicted CFF	Radiance		
	0.25°			
Predicted CFF				
Actual CFF				MPOD =
	0.5°			
Predicted CFF				
Actual CFF				MPOD =
	1°			
Predicted CFF				
Actual CFF				MPOD =
	1.75°			
Predicted CFF				
Actual CFF				MPOD =
	3°			
Predicted CFF				
Actual CFF				MPOD =
	7°			
Predicted CFF				
Actual CFF				
				MPOD =

Note: Please attach graph.

L. Diet Questionnaire

Note: Please attach complete dietary questionnaire.

M. Photostress Recovery

Pupil Size [ ] Bleaching Exposure Distance = 1metre

Baseline: Sensitivity (Decibels (dB)) [ ] Time [ ]

Post Bleaching:

Number	Sensitivity (dB)	Time (seconds)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

Number	Sensitivity (dB)	Time (seconds)
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		
20.		

Percentage of sensitivity reduction following post-bleaching [ ]

Time taken to return to the 95% of the baseline foveal threshold (seconds) [ ]

Time taken to return to the 100% (maximum) of the baseline foveal threshold (seconds) [ ]

O. Fundus and Iris photographs

Fundus images taken from:

Right eye	Yes	No
	1	2
Left eye	Yes	No
	1	2

Iris image taken from:

Right eye	Yes	No
	1	2
Left eye	Yes	No
	1	2

Subject and Investigator agree that the subject's iris colour is? _____

_____

P. Oculus HMC Anomaloscope Results

Moreland Mix

Range [ ] Mid-point [ ] AQ_{min} [ ] AQ_{max} [ ]

Insert Graph here

Q. Farnsworth-Munsell 100 hue test (FM100) Results

Colour Type: Normal [ ] Protan [ ] Deutan [ ] Tritan [ ]

Total Error Score [ ]

Colour Discrimination Rank: Low [ ] Average [ ] Superior [ ]

Error score (with correction for minimum possible score) for each colour quadrant

Quadrant 1 [ ]

Quadrant 2 [ ]

Quadrant 3 [ ]

Quadrant 4 [ ]

% Partial error score (50-68 caps) [ ]

% Partial error score (36-54 caps) [ ]

Insert polar co-ordinate error chart.

R. Short Wavelength Automated Perimetry

Reliability Indices Pass ☐ Fail ☐

Attach reliable field plot printout below:

Mean Central Sensitivity (dB)

1° ☐

2° ☐

3° ☐

4° ☐

5° ☐

Overall ☐

Comments

_____

_____

_____

_____

APPENDIX 7.6: VISUAL FUNCTION IN NORMALS QUESTIONNAIRE

G. Visual Function in Normals questionnaire (VFNq30) (Collaborative Optical Macular Pigment ASsessment Study (COMPASS) baseline visit)

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Please circle the number which corresponds with the correct answer. All questions must be answered.

Colour Discrimination

Situational Analysis

1: I have difficulty distinguishing between colours:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

2: I tend to confuse colours:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

3: The colour names that I use disagree with those that other people use:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate the quality of my colour vision as:

Significantly better than others ……. 5

Marginally better than others …….. 4

Equivalent to others ……... 3

Marginally worse than others …….. 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your colour vision, on a scale where zero equates to no colour perception and ten equates to best possible colour perception?

Ten (best) ……. 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three……..… 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Glare Disability

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

4: I have problems with lights around me causing glare when I'm trying to see something:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

5: I have trouble driving when there are headlights from oncoming cars in my field of view:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

6: My eyes are sensitive to bright sunny conditions:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

7: When driving at night in the rain, I have difficulty seeing the road because of headlights from oncoming cars:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

8: During the course of an eye examination I find the lights used to be excessively bright:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

9: My eyes become tired or sensitive when working under artificial light conditions:

Never…… 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

10: I need to adjust the brightness intensity of my computer screen to a low setting for comfortable use:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my tolerance to glare as:

Significantly better than others …….. 5

Marginally better than others ……. 4

Equivalent to others …… 3

Marginally worse than others …… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your tolerance to glare, on a scale where zero equates to a complete inability to cope with glare and ten equates to absolutely no difficulty?

Ten (best) …….. 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three……..… 3

Two ……..…. 2

One…… 1

Zero (worst)…….. 0

Acuity / Spatial Vision

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

11: I have problems reading small print (for example, labels on medicine bottles, phone books, glossy colour magazines, buy and sell magazine etc):

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

12: I have trouble reading the menu in a dimly lit restaurant:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

13: I have difficulty recognising people from long distance:

Never…….	5
Rarely……	4
Sometimes……	3
Often.....	2
Always.....	1
N/A.....	0

14: I find it difficult to recognise the bus number until the bus gets close:

Never…….	5
Rarely……	4
Sometimes……	3
Often.....	2
Always.....	1
N/A.....	0

15: I have difficulty reading teletext /small print (such as match scores/time elapsed) on TV:

Never…….	5
Rarely……	4
Sometimes……	3
Often.....	2
Always.....	1
N/A.....	0

16: When driving, I struggle to read distant registration plates or signposts:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

17: I have difficulty performing fine handwork, such as threading a needle:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

18: I have problems carrying out activities that require a lot of visual concentration and attention:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my ability to see fine detail as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others ……...… 3

Marginally worse than others …….…….…. 2

Significantly worse than others…….….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your ability to see fine detail, on a scale where zero equates to a complete inability to perform fine tasks and ten equates to no difficulty with any type of visual task?

Ten (best) …….. 10

Nine……..… 9

Eight ……...… 8

Seven……. 7

Six……...… 6

Five ……... 5

Four…….….. 4

Three…….…. 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Light / Dark Adaptation

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

19: I have problems adjusting to bright room lighting, after room lighting has been rather dim:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

20: It takes me a long time to adjust to darkness after being in bright light:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

21: It takes me a long time to adjust to bright sunshine after I have been inside a building for a lengthy period of time:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

22: I have trouble adjusting from bright to dim lighting, such as when going from daylight into a cinema:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

23: I have trouble driving at twilight / dusk:

Never…… 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my capacity to cope with changes in illumination as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others ……...…….. 3

Marginally worse than others ……..…… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your ability to continue to see effectively, despite changes in illumination, on a scale where zero equates to a complete inability to continue to function visually and ten equates to no difficulty continuing with any type of visual task?

Ten (best) …….. 10

Nine……..… 9

Eight ……...… 8

Seven……. 7

Six……...… 6

Five ……... 5

Four…….….. 4

Three……..… 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Daily Visual Tasks
All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

24: I have trouble finding a specific item on a crowded supermarket shelf:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

25: I have difficulty noticing when the car in front of me is speeding up or slowing down:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

26: I misjudge the position of steps / curbs when walking:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

27: I have problems locating something when it's surrounded by a lot of other things (e.g. car keys on your desk):

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

28: I have problems carrying out activities that require a lot of visual concentration and attention:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

29: I have trouble noticing things in my peripheral vision:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

30: I have difficulty driving on poorly lit back-roads:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my visual performance for daily visual tasks as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others……..... 3

Marginally worse than others ……..…… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate your satisfaction with the overall quality of your vision in general, on a scale where zero equates to complete dissatisfaction and ten equates to complete satisfaction with every aspect of your vision?

Ten (best) …….. 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three…….… 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Scoring the VFNq30:

The purpose of the VFNq30 is to generate a composite score for each visual function area, which summarises the subject's responses to the items addressing that visual function. To score an individual item, the following scale is used:

Never = 5, Rarely = 4, Sometimes = 3, Often = 2, Always = 1

If any questions are irrelevant to an individual, they are marked N/A and scored as '0'.

(a) The mean composite score for each functional section should be multiplied by 20, to give a numerical index of functional capacity scored out of 100 (where 100 = perfect visual function).

(b) The comparative analysis section should be scored separately from the functional sections and be used to produce an additional index of performance using the same multiplier as above.

(c) The 'Subject Satisfaction' question should be scored out of 100 also by multiplying the chosen number by 10 for each functional section.

G. Visual Function in Normals questionnaire (COMPASS three, six and final visits)

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Please circle the number which corresponds with the correct answer. All questions must be answered.

Colour Discrimination

Situational Analysis

1: I have difficulty distinguishing between colours:

Never……	5
Rarely……	4
Sometimes……	3
Often.....	2
Always.....	1
N/A.....	0

2: I tend to confuse colours:

Never……	5
Rarely……	4
Sometimes……	3
Often.....	2
Always.....	1
N/A.....	0

3: The colour names that I use disagree with those that other people use:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate the quality of my colour vision as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others ……... 3

Marginally worse than others …….. 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your colour vision, on a scale where zero equates to no colour perception and ten equates to best possible colour perception?

Ten (best) …….. 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three…….…. 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Change Analysis

Do you think your colour vision has changed since commencing supplementation…… Yes No

If yes, has it: Improved Disimproved

1 2

If yes, what percentage change would you estimate? _____%

Glare Disability

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

4: I have problems with lights around me causing glare when I'm trying to see something:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

5: I have trouble driving when there are headlights from oncoming cars in my field of view:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

6: My eyes are sensitive to bright sunny conditions:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

7: When driving at night in the rain, I have difficulty seeing the road because of headlights from oncoming cars:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

8: During the course of an eye examination I find the lights used to be excessively bright:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

9: My eyes become tired or sensitive when working under artificial light conditions:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

10: I need to adjust the brightness intensity of my computer screen to a low setting for comfortable use:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my tolerance to glare as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others …… 3

Marginally worse than others …… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your tolerance to glare, on a scale where zero equates to a complete inability to cope with glare and ten equates to absolutely no difficulty?

Ten (best) ……. 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three……..… 3

Two ……..…. 2

One…… 1

Zero (worst)…….. 0

Change Analysis

Do you think your tolerance for glare has changed since commencing supplementation……..…Yes No

If yes, has it: Improved Disimproved

1 2

If yes, what percentage change would you estimate? _____%

Acuity / Spatial Vision

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

11: I have problems reading small print (for example, labels on medicine bottles, phone books, glossy colour magazines, buy and sell magazine etc):

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

12: I have trouble reading the menu in a dimly lit restaurant:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

13: I have difficulty recognising people from long distance:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

14: I find it difficult to recognise the bus number until the bus gets close:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

15: I have difficulty reading teletext /small print (such as match scores/time elapsed) on TV:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

16: When driving, I struggle to read distant registration plates or signposts:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

17: I have difficulty performing fine handwork, such as threading a needle:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

18: I have problems carrying out activities that require a lot of visual concentration and attention:

Never…… 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my ability to see fine detail as:

Significantly better than others ……. 5

Marginally better than others ……. 4

Equivalent to others ……...…….. 3

Marginally worse than others …….…….. 2

Significantly worse than other .……..….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your ability to see fine detail, on a scale where zero equates to a complete inability to perform fine tasks and ten equates to no difficulty with any type of visual task?

Ten (best) …….. 10

Nine……..… 9

Eight ……...… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three…….…. 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Change Analysis

Do you think your quality of vision has changed since commencing supplementation…… Yes No

If yes, has it: Improved Disimproved

1 2

If yes, what percentage change would you estimate? _____%

Light / Dark Adaptation

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

19: I have problems adjusting to bright room lighting, after room lighting has been rather dim:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

20: It takes me a long time to adjust to darkness after being in bright light:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

21: It takes me a long time to adjust to bright sunshine after I have been inside a building for a lengthy period of time:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

22: I have trouble adjusting from bright to dim lighting, such as when going from daylight into a Cinema.

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

23: I have trouble driving at twilight / dusk:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my capacity to cope with changes in illumination as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others ……...… 3

Marginally worse than others ……..…… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate the overall quality of your ability to continue to see effectively, despite changes in illumination, on a scale where zero equates to a complete inability to continue to function visually and ten equates to no difficulty continuing with any type of visual task?

Ten (best) …….. 10

Nine……..… 9

Eight ……...… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three…….… 3

Two …….…. 2

One…… 1

Zero (worst)…….. 0

Change Analysis

Do you think your visual performance as affected by variations in illumination has changed since commencing supplementation…… Yes No

If yes, has it: Improved Disimproved

1 2

If yes, what percentage change would you estimate? _____%

Daily Visual Tasks

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

Situational Analysis

24: I have trouble finding a specific item on a crowded supermarket shelf:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

25: I have difficulty noticing when the car in front of me is speeding up or slowing down:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

26: I misjudge the position of steps / curbs when walking:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

27: I have problems locating something when it's surrounded by a lot of other things (e.g. car keys on your desk):

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

28: I have problems carrying out activities that require a lot of visual concentration and attention:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

29: I have trouble noticing things in my peripheral vision:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

30: I have difficulty driving on poorly lit back-roads:

Never……. 5

Rarely…… 4

Sometimes…… 3

Often..... 2

Always..... 1

N/A..... 0

Comparative Analysis

In comparison to my friends/family, I would rate my visual performance for daily visual tasks as:

Significantly better than others …….. 5

Marginally better than others …….. 4

Equivalent to others ……..…… 3

Marginally worse than others ……..…… 2

Significantly worse than others…….. 1

Subjective Satisfaction Score

How would you rate your satisfaction with the overall quality of your vision in general, on a scale where zero equates to complete dissatisfaction and ten equates to complete satisfaction with every aspect of your vision?

Ten (best) …… 10

Nine……..… 9

Eight ……..… 8

Seven……. 7

Six……..… 6

Five ……... 5

Four…….….. 4

Three……..… 3

Two …….…. 2

One…… 1

Zero (worst)……. 0

Change Analysis

Do you think your everyday vision for routine visual tasks such as those described above has changed since commencing supplementation…… Yes No

If yes, has it: Improved Disimproved

1 2

If yes, what percentage change would you estimate? _____%

APPENDIX 7.7: FOOD FREQUENCY QUESTIONNAIRE

Subject Code | | | | | | |

Scottish Collaborative Group Food Frequency Questionnaire version 6.5

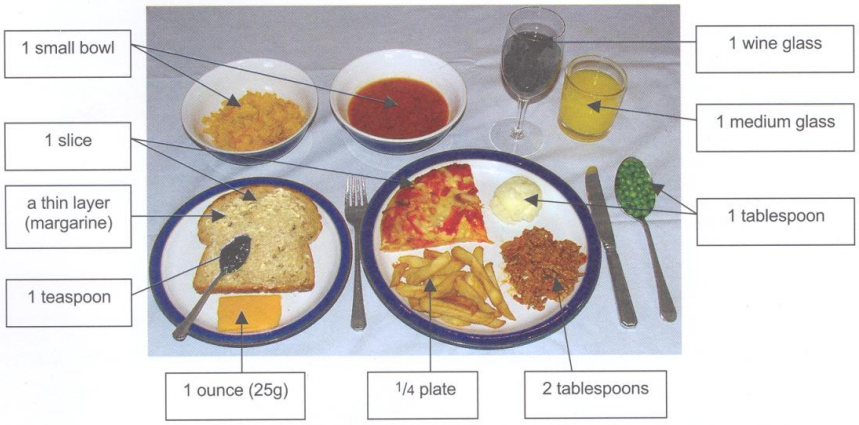
Diet Questionnaire

Thank-you for agreeing to complete this questionnaire.
It should take 20-30 minutes to complete.

Please take a few minutes to read the instructions carefully.

We would like you to describe your usual diet over the last 2-3 months. This should include all your main meals, snacks and drinks which you had at home or away from home e.g. at work, at restaurants or cafes and with friends and family.

The questionnaire lists 170 foods and drinks, and for each one a measure is given to help you estimate how much you usually have. The photograph below shows examples of some of these measures:



Please use **black or blue pen** to complete the questionnaire: do not use pencil.

How to complete the questionnaire

For **every line** in the questionnaire, we would like you to answer two things.

- **how much** of the food you had in a day you ate the food, and
- **how many** days a week you had the food.

To estimate **how much** of the food you had, you should circle a number under 'Measures per day'. Each food is described in common measures such as slices, glasses or tablespoons as illustrated in the photograph. *Please note that the measures are designed to be quite small, so your usual portion may easily be 2 or more measures.*

To estimate **how many** days a week you had the food, you should circle a letter or number under 'Number of days per week'.

- If you had the food less than once a month, you should circle **R** (for **R**arely or never). *For these foods you do not need to fill in the number of measures per day.*
- If you had the food more than once a month but less than once a week, you should circle **M** (for **M**onth).
- If you had the food on average 1-6 days a week, you should circle 1-6 as appropriate.
- If you had the food every day, you should circle 7.

The example below shows the answers for someone who had 4 slices of bread every day, 1 apple 5 days a week, 1/2 a plate of chips (i.e. two 1/4 plates) once or twice a month but rarely or never had tomato juice:

	Measure	Measures per day	Number of days per week
a) Bread (including toast & sandwiches)	1 medium slice	1 2 3 **(4)** 5+	R M 1 2 3 4 5 6 **(7)**
b) Apples	1 medium apple	**(1)** 2 3 4 5+	R M 1 2 3 4 **(5)** 6 7
c) Chips from a chip shop or restaurant	1/4 plate	~~1~~ **(2)** 3 4 5+	R **(M)** 1 2 3 4 5 6 7
d) Tomato juice	1/2 medium glass	1 2 3 4 5+	**(R)** M 1 2 3 4 5 6 7

If you want to change an answer, please put a **cross** through the wrong answer and circle the new answer (see example above).

If there are any foods or drinks that you eat regularly which do not appear on the questionnaire, please list them in section 20 ('other foods and drinks').

It is very important that you give an answer for every line.

If you rarely or never have a food, please make sure that you circle R.

1. Breads

	Measure	Measures per day	Number of days per week
a) Bread (including toast & sandwiches)	1 medium slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b) Bread roll or bun	1 roll or bun	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c) Croissants, butteries or garlic bread	1 roll or 2 pieces	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d) Other breads (pitta, naan, soft tortillas)	1 pitta or 1/2 naan	1 2 3 4 5+	R M 1 2 3 4 5 6 7

e) Which type(s) of bread do you usually eat? **Please tick one or more boxes.** White ☐ Brown / granary ☐ Wholemeal ☐

2. Breakfast Cereals

	Measure	Measures per day	Number of days per week
a) Cornflakes, Special K, Rice Krispies etc.	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b) Bran Flakes, Sultana Bran, All Bran etc.	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c) Shredded Wheat, Weetabix etc.	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d) Coco Pops, Frosties, Sugar Puffs, Crunchy Nut Cornflakes etc.	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e) Muesli (all types)	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f) Porridge or Ready Brek	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7

3. Milk *(including milk on cereals and in drinks, but not in cooked foods)*

	Measure	Measures per day	Number of days per week
a) Full fat milk	1/4 pint	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b) Semi-skimmed milk	1/4 pint	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c) Skimmed milk	1/4 pint	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d) Soya milk	1/4 pint	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e) Dried milk or creamer	1 teaspoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

4. Cream and Yogurt

	Measure	Measures per day	Number of days per week
a) Low fat yogurt (plain or fruit)	1 pot (125 ml)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b) Full fat yogurt (e.g. Greek)	1 pot (125 ml)	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

		Measure	Measures per day	Number of days per week
c)	Low calorie yogurt (plain or fruit)	1 pot (125 ml)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Fromage frais (plain or fruit)	1 pot (125 ml)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Cream (all types)	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

5. Cheese

		Measure	Measures per day	Number of days per week
a)	Full fat hard cheese (e.g. Cheddar, Gruyere, Wensleydale, Gouda)	1 oz. (25g) or 1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Medium fat cheese (e.g. (Edam, Brie, Camembert, Feta, cheese spreads)	1 oz. (25g) or 1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Full fat cream cheese (e.g. Philadelphia, Boursin, Danish Blue)	1 oz. (25g) or 1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Low fat cheese (e.g. low fat cream cheese, low fat hard cheese)	1 oz. (25g) or 1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Cottage cheese (all types)	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

6. Eggs

		Measure	Measures per day	Number of days per week
a)	Boiled or poached eggs	1 egg	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Fried eggs	1 egg	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Scrambled eggs or omelette	1 egg	1 2 3 4 5+	R M 1 2 3 4 5 6 7

7. Meats *(Meat substitutes e.g. Quorn or soya are listed in section 10)*

		Measure	Measures per day	Number of days per week
a)	Mince or meat sauce (e.g. bolognese)	2 tablespoons	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Sausages (pork, beef or frankfurters)	1 sausage	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Burgers (beef, lamb, chicken or turkey)	1 burger	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Beef (roast, grilled, casseroled or fried)	2 tablespoons, 2 slices or 1 steak	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Pork or lamb (roast, grilled, casseroled or fried)	2 tablespoons, 2 slices or 1 chop	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

		Measure	Measures per day	Number of days per week
f)	Chicken or turkey (roast, grilled, casseroled or fried)	1 wing or thigh, 1/2 breast or 2 slices	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Bacon or gammon	1 medium slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Liver, liver sausage or liver pate	1 serving	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Haggis or black pudding	2 tablespoons or 1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
j)	Meat or chicken pies, pasties or sausage roll	1 individual pie or 1 roll	1 2 3 4 5+	R M 1 2 3 4 5 6 7
k)	Cold meats (e.g. ham, corned beef, chicken roll)	1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
l)	Salami or continental sausage	1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7

8. *Fish*

		Measure	Measures per day	Number of days per week
a)	Fish fingers	1 finger	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	White fish (e.g. haddock, cod, plaice or scampi) fried or cooked in batter	1 small fillet or 1 serving	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Grilled, poached or baked white fish	1 small fillet	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Smoked white fish	1 small fillet	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Fish cakes, fish pie	1 cake or 2 tablespoons	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Fried oily fish (e.g. salmon, herring, fresh tuna or mackerel)	1 small fillet	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Grilled, poached or baked oily fish	1 small fillet	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Smoked oily fish (kipper, mackerel or salmon)	1 small fillet or 1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Tinned salmon	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
j)	Tinned tuna	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
k)	Sardines, pilchards or rollmop herrings	2 small fish or 1 large fish	1 2 3 4 5+	R M 1 2 3 4 5 6 7
l)	Prawns, crab etc.	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
m)	Mussels, oysters, cockles, scallops	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

9. Potatoes, Rice and Pasta

		Measure	Measures per day	Number of days per week
a)	Boiled or baked potatoes	1 medium or 1/2 large	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Mashed potatoes	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Oven chips or potato waffles	1/4 plate or 1 waffle	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Home-cooked chips	1/4 plate	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Chips from a chip shop or restaurant	1/4 plate	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Roast or fried potatoes	1/4 plate	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	White rice	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Brown rice	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Pasta (all types) or couscous	1/4 plate	1 2 3 4 5+	R M 1 2 3 4 5 6 7
j)	Noodles (all types)	1/4 plate or 1 pot	1 2 3 4 5+	R M 1 2 3 4 5 6 7

10. Savoury foods, Soups and Sauces

		Measure	Measures per day	Number of days per week
a)	Pizza	1 slice or 1/2 a small pizza	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Quiche or savoury flan	1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Savoury pancakes	1 pancake	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Baked beans	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Nut roast, nut burgers or vegetable burgers	1 slice or burger	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Quorn products (all types)	1 tablespoon, slice or sausage	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Soya beans, TVP, Tofu or soya meat substitute	1 tablespoon or 1 sausage	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Other beans (kidney, butter, chick peas)	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Lentils (excluding soup)	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
j)	Soups (home-made)	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
k)	Soups (tinned)	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
l)	Soups (dried or instant)	1 small bowl or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7
m)	Gravy	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

		Measure	Measures per day					Number of days per week							
n)	Tomato -based sauces (e.g. for pasta)	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
o)	Other savoury sauces (white, cheese etc.)	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
p)	Bottled sauces (e.g. ketchup)	1/2 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
q)	Mayonnaise or salad cream	1 teaspoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
r)	Oil & vinegar dressing	1 teaspoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
s)	Pickled vegetables or chutneys	1 teaspoon or 1 pickle	1	2	3	4	5+	R	M	1	2	3	4	5	6	7

11. ***Vegetables*** *(including fresh, frozen and tinned vegetables)*

		Measure	Measures per day					Number of days per week							
a)	Mixed vegetable dishes (e.g. stir-fry, curry or bake)	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
b)	Tinned vegetables (all kinds)	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
c)	Peas or green beans	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
d)	Carrots	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
e)	Cabbage (all kinds)	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
f)	Brussels sprouts	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
g)	Broccoli	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
h)	Spinach or spring greens	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
i)	Leeks or courgettes	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
j)	Cauliflower, swede (neeps) or turnip	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
k)	Sweetcorn	1 tablespoon or 1 piece	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
l)	Onions	1 tablespoon or 1/2 onion	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
m)	Tomatoes	1/2 medium or 2 small	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
n)	Sweet peppers	1/4 pepper	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
o)	Other salad vegetables (lettuce, cucumber etc)	2 leaves or 4 slices	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
p)	Potato salad	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
q)	Coleslaw or other veg. salads in dressing	1 tablespoon	1	2	3	4	5+	R	M	1	2	3	4	5	6	7

Please make sure you have given an answer for every line before leaving this page

12. *Fruit* (including fresh, cooked, frozen and tinned fruits)

		Measure	Measures per day	Number of days per week
a)	Fresh fruit salad	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Tinned fruit (all kinds)	1 tablespoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Apples	1 fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Bananas	1 fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Oranges, satsumas or grapefruit	1 small or 1/2 large fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Pears	1 fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Peaches or nectarines	1 fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Kiwi fruit	1 fruit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Dried fruit (e.g. raisins, dates or figs)	1 tablespoon or 1 oz (25g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
j)	All other fruits (grapes, strawberries, melon etc)	1 tablespoon or 1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7

13. *Puddings*

		Measure	Measures per day	Number of days per week
a)	Milk-based puddings (e.g. rice, semolina)	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Sponge puddings (e.g. (steamed, syrup, jam)	1 small bowl	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Gateau or cheesecake	1 slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Fruit-based puddings (e.g. pie, tart, crumble)	1 pie, 1 slice or 2 tablespoons	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Mousse, blancmange, trifle, meringue	2 tablespoons or 1 meringue	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Custard or other sweet sauces	2 tablespoons	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Wrapped ice creams (Cornetto, Solero, Magnum etc.)	1 ice cream	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Other ice cream (all flavours)	1 scoop or small tub	1 2 3 4 5+	R M 1 2 3 4 5 6 7

14. *Chocolates, Sweets, Nuts and Crisps*

		Measure	Measures per day	Number of days per week
a)	Chocolate bars (e.g. Mars, Dairy Milk)	1 bar or 2 oz. (50g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Chocolate sweets, toffees or fudge	2 sweets	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

		Measure	Measures per day	Number of days per week
c)	Boiled sweets, mints	2 sweets	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Fruit gums, pastilles, jellies or chewy sweets	2 sweets	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Salted nuts (peanuts, cashews etc.)	1 small packet or 1 oz. (25g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Unsalted nuts	1 small packet or 1 oz. (25g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Crisps	1 small bag (25g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
h)	Reduced fat crisps	1 small bag (25g)	1 2 3 4 5+	R M 1 2 3 4 5 6 7
i)	Other savoury snacks (Quavers, tortilla chips, popcorn etc.)	1 small bag	1 2 3 4 5+	R M 1 2 3 4 5 6 7

15. Biscuits

		Measure	Measures per day	Number of days per week
a)	Plain (e.g. Rich Tea, digestive)	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Sweet (e.g. ginger, custard creams)	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Shortbread	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Chocolate coated biscuits	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Savoury biscuits, (crackers, crispbreads)	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
f)	Oatcakes	1 biscuit	1 2 3 4 5+	R M 1 2 3 4 5 6 7
g)	Cereal bars, flapjacks	1 bar or slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7

16. Cakes

		Measure	Measures per day	Number of days per week
a)	Plain cakes (sponge, madeira, ginger etc.)	1 medium slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Sponge cakes with jam, cream or icing	1 medium slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Fruit cakes (all kinds)	1 medium slice	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Pastries, doughnuts or muffins	1 piece	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Pancakes or scones	1 pancake or scone	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

17. Spreads and Sugar

		Measure	Measures per day	Number of days per week
a)	Jam, honey, or marmalade	1 teaspoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Yeast or meat extract (Marmite, Bovril etc.)	1/2 teaspoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Peanut butter or chocolate spread	1 teaspoon	1 2 3 4 5+	R M 1 2 3 4 5 6 7

d) How many teaspoons of table sugar did you use each day in drinks and on cereals or deserts? (If you did not use any table sugar, please enter 0). ☐☐

e) Did you use any butter, margarine or other fat spread or oil on bread? Yes ☐ No ☐

If yes, please give full details of the one or two types you used most (e.g. Asda Sunflower buttery spread).
If you did not spread any fat or oil on bread, please go straight on to question g.

_____ Office Code ☐☐

_____ Office Code ☐☐

f) How much did you normally spread on one slice of bread? (**Please tick one answer).**
(an example of a thin layer is shown in the photograph on the front cover).

a scrape ☐ a thin layer ☐ a thick layer ☐

g) Did you use any fat or oil for home frying or cooking? Yes ☐ No ☐

If yes, please give full details of the one or two types you used most (e.g. Tesco Pure Vegetable Oil).
If you did not use any fat or oil for home frying or cooking, please go straight on to section 18.

_____ Office Code ☐☐

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18. Beverages and Soft Drinks

		Measure	Measures per day	Number of days per week
a)	Tea (regular)	1 cup or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b)	Herbal, fruit or decaffeinated tea	1 cup or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c)	Instant coffee (regular)	1 cup or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d)	Decaffeinated coffee	1 cup or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7
e)	Filter, espresso or cappuccino coffee	1 cup or mug	1 2 3 4 5+	R M 1 2 3 4 5 6 7

Please make sure you have given an answer for every line before leaving this page

		Measure	Measures per day					Number of days per week							
f)	Pure fruit juice (orange, apple, etc.)	1/2 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
g)	Tomato juice	1/2 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
h)	Blackcurrant squash (e.g. Ribena)	1 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
i)	Other fruit squash	1 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
j)	Diet fizzy drinks (Cola, lemonade etc.)	1 can	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
k)	Regular fizzy drinks	1 can	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
l)	Mineral water	1 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
m)	Tap water (not in other drinks)	1 medium glass	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
n)	Hot chocolate	1 cup or mug	1	2	3	4	5+	R	M	1	2	3	4	5	6	7
o)	Horlicks or Ovaltine	1 cup or mug	1	2	3	4	5+	R	M	1	2	3	4	5	6	7

19. Alcoholic Drinks

Please estimate your average intake of alcohol over the last 2-3 months. If your intake varied from week to week, please try to give an overall estimate which allows for weeks with high or low intake. If you had less than one measure a week on average, please circle 0.

	Drink	Measure	Number of measures per week								
a)	Low alcohol lager or beer	1/2 pint	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
b)	Dark beer (Export, bitter or stout)	1/2 pint	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
c)	Light beer (lager or continental beers)	1/2 pint	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
d)	White wine	1 wine glass	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
e)	Red wine	1 wine glass	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
f)	Sherry, port etc.	1 sherry glass	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
g)	Spirits or liqueurs	1 pub measure	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
h)	Alcopops (e.g. Bacardi Breezer)	1 bottle	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+
i)	Cider	1 bottle or 1/2 pint	0	1-2	3-4	5-9	10-14	15-19	20-29	30-39	40+

Please make sure you have given an answer for every line before leaving this page

20. Other Foods and Drinks

Please enter details of any foods or drinks which you had **more than once a week** in the last 2-3 months which you have not included in the questionnaire above. If you do not want to add any foods, please leave this section blank and go to section 21.

	Food description	Measure	Measures per day	Number of days per week
a)	_____	_____	1 2 3 4 5+	1 2 3 4 5 6 7
b)	_____	_____	1 2 3 4 5+	1 2 3 4 5 6 7
c)	_____	_____	1 2 3 4 5+	1 2 3 4 5 6 7
d)	_____	_____	1 2 3 4 5+	1 2 3 4 5 6 7

21. Vitamin, Mineral and Food Supplements

Please give details and brand name of any supplements (e.g. multivitamins, iron tablets, cod liver oil, evening primrose oil, Complan, wheatgerm, bran) which you took in the last 2-3 months.

	Supplement type	Measure	Measures per day	Number of days per week
a)	_____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
	Brand name and details_____			
b)	_____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
	Brand name and details_____			
c)	_____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
	Brand name and details_____			
d)	_____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
	Brand name and details_____			

22. Other Information

Any other information or comments on your diet in the last 2-3 months

Date of completing the questionnaire _____

Thank-you very much for completing this questionnaire.

Please return it to the investigators as requested.

APPENDIX 7.8: STANDARD OPERATING PROCEDURE FOR FOOD FREQUENCY QUESTIONNAIRE

Scottish Collaborative Group
Food Frequency Questionnaire

Version 6.5

University of Aberdeen

Standard Operating Procedure 1:

Checking completed questionnaires

1. Questionnaires should be checked as soon as possible after completion, ideally in the presence of the subject (e.g. at a clinic visit). For postal questionnaires, if ethical permission permits, the subject should be contacted by telephone or mail if questions arise.

   If the subject cannot be contacted, their answers should be left unchanged: intended responses should never be assumed.

2. The 'subject code' should be clearly marked on the front cover of the questionnaire and any loose pages.

3. Check there is an answer to every question (section) and sub-question (line) of the questionnaire.
   - For rarely eaten foods (i.e. the subject has recorded 'R' under days per week) no answer is required under measures per day
   - For all other foods, there should be one answer under measures per day and one answer under measures per week.

- Where there are no answers the subject should be asked to provide the missing answer.
- Where there are two or more answers, the subject should be asked to select one.

4. Double check specific lines and sections for completeness.
   - 'Bread type' (line 1 e)
   - 'Sugar consumption' (line 17 d)
   - 'Fat spread and oil type' (lines 17 e and g): full brand name and descriptions should be provided.
   - 'Other foods' (section 20): full description and measures outlined
   - Supplements (section 21): full brand name(s); supplement strength and measures should be specified.

Scottish Collaborative Group
Food Frequency Questionnaire

Version 6.5

University of Aberdeen

Standard Operating Procedure 2:

Preparation of questionnaires for data entry

1. Selection and entry of 'official codes' for fats and spreads (line 17e) should be undertaken using the attached Scottish Collaborative Group (SCG) fat coding sheet.

2. 'Other foods' (section 20) should be checked in collaboration with nutritionist or dietician. If you do not have easy access to nutritionist or dietician, please contact us for further advice.

- Foods containing low energy or few nutrients of interest (e.g. sugar-free jelly, raw mushrooms, garlic, water chestnuts) can be ignored.
- Food nutritionally similar to foods already listed on the questionnaire can be added to the appropriate line. The 'measures per day' and 'number of days per week' should be edited to reflect reported total intake.

> e.g. A subject recorded 2 measures per day, 2 days per week, under 'other breads' (line 1d), then also reported - in the 'other foods' (section 20) - eating 1 measure per day, 2 days per week of ciabatta bread. In this case line 1d should be updated to the 3 measures per day, while days per weeks should remain unchanged.

- Any changes to reflect addition of ‘other foods’ should be made using different coloured ink to that originally used by the subject.
- A list of suggested lines on the questionnaire for other foods is attached.
- Foods which cannot be entered to existing lines should be given a food code and measure weight by a dietician / nutritionist. The measure weight per day should be calculated as:

  (measure* weight x measures per day x days per week) / 7 *
  measure provided by subject

The nutrient content of the calculated daily consumption of the food should be derived, by entering the food code and weight per day into a nutrient analysis package e.g. Windiet or Foodbase.

Please note the nutrient content calculated for ‘other foods’ not incorporated into appropriate lines of the questionnaire, should be added to the questionnaires nutrient analysis output file, before undertaking analysis and interpretation of the complete Food Frequency Questionnaire (FFQ) nutrient data.

Scottish Collaborative Group
Food Frequency Questionnaire

Version 6.5

University of Aberdeen

Standard Operating Procedure 3:

Food Frequency Questionnaire data entry into Micro Soft Access file

1. Before entering the completed questionnaires ensure you are familiar with the MS Access entry package provided.
2. Open the MS Access file provided, from the 'Nutrition Questionnaire Menu' select the 'form input' button leading to the 'Nutrition questionnaire – check input' window.
3. Create a study data file by entering and saving your pre-determined 'study reference' (study file name) into the 'select or enter study reference' section and press your tab button.
4. Insert the subject code of the completed and checked questionnaire into the 'subject code' box. The 'form reference' should confirm the study file into which the FFQ data is to be stored.
    - If the study file quoted in 'form reference' is not the correct file - return to the 'select or enter study reference' section and choose the correct file.
5. The subjects reported intake 'measures per day' (measure) and number of days per week' (frequency) can now be entered by selecting appropriate 'number of measures per day' and 'days per week' from the drop down boxes. The question layout of the entry package mirrors FFQ layout, therefore individual responses can be entered as reported.
    - Information entered at this stage is automatically saved.

6. When the questionnaire has been entered, select 'new record' (►*) from the status bar on the bottom left-hand corner of the screen. Before entering the next questionnaire as described in points 4-6.

    - Do not enter the new subject code into the 'subject code' box unless this box is empty. If the previous subject's data record is still on screen it will be over-written!

7. When all completed questionnaires have been entered into the MS Access entry file, we recommend double-checking your data entry. Typically, a random sample of 10% questionnaires should be double-checked by someone not involved with the original data entry.

8. Before returning the FFQ data for nutrient analysis, a 'text file' should be created.

    - From the 'Nutrition Questionnaire Menu' select 'create text file for analysis' leading to 'Export Data To Text File' screen.
    - Select the appropriate 'study reference' from the drop down box, and then select the first and last subject data records to be exported.
    - Check the number of subject records selected for export matches the number of questionnaires to be analysed.
    - Enter the name of the export file to be created in the 'output file' box
        - For example: If you type export in the 'output file' box on the 12th of January 2005, the export file would appear as export_12_jan_2005.txt in the same directory as the database.
    - Select the 'Export to text file' button, to create the new export file.
        - If the export file has been successfully generated an 'Export Complete' message will pop-up.
        - The new export file will automatically be saved in the same directory as the study data file (database).

- If you wish to create second export file, remember to name the file differently from the first. Otherwise the first export file will be over-written!

9. The export file (text file) can now to be returned to for nutrient analysis and can be returned to **j.kyle@abdn.ac.uk**

APPENDIX 7.9: FOOD CODES FOR FOOD FREQUENCY QUESTIONNAIRE

Scottish Collaborative Group
Food Frequency Questionnaire

Coding sheet for spreads and oils

February 2005

- Enter one or two codes for butter/margarine and oils/cooking fats, using the alphabetic listing attached.

- If the spread does not appear on the alphabetical list but the spread or oil can be found in local shops, send information on the total, saturated, monounsaturated and polyunsaturated fat content (g/100g) to Aberdeen for coding.

- If no information on fatty acid composition is obtainable, leave the coding boxes blank. In this case the main nutrient output (including total fat and fat soluble vitamins) will be calculated using code 7, but no fatty acid output will be generated for the subject.

- If the subject reports not using any butter, margarine, or other spread or oil on bread (or has left both 20a1 and 20a2 blank), code the type of spread as 99 (no spread used).

- If there is not enough information to give a code for a cooking oil, leave the coding boxes blank. The main nutrient output will be calculated using code 18 (blended vegetable oil) so that the main nutrient output is calculated, but no fatty acid data will be generated for the subject.

- If the subject specifies that they do not use any cooking oil, or only use spray oil, code the type of fat or oil as 99 (no oil used).

Food Frequency Questionnaire (FFQ) Codes	Description
1	Butter
2	Spreadable butter
3	Hard margarine (animal & vegetable fats)
4	Hard margarine (vegetable fats only)
5	Soft margarine, not polyunsaturated
6	Soft margarine, polyunsaturated
7	Blended spread, 70-80% fat
7	Fat spread, 70-80 % fat not polyunsaturated
8	Fat spread, 70% fat polyunsaturated
9	Fat spread, 60% fat, polyunsaturated
10	Fat spread, 60% fat, with olive-oil
11	Blended spread, 40% fat
12	Dairy spread, 40% fat
13	Fat spread, 40% fat, not polyunsaturated
14	Fat spread, 35-40% fat, polyunsaturated
15	Fat spread, 20-25% fat, not polyunsaturated
16	Fat spread, 20-25% fat, polyunsaturated
17	Fat spread, 5% fat
18	Blended vegetable oil
19	Corn oil
20	Olive oil
21	Peanut (groundnut) oil
22	Rapeseed oil
23	Soya oil
24	Sunflower oil
25	Compound cooking fats (solid)
26	Compound cooking fats (polyunsaturated)
27	Lard
28	Suet or beef dripping
29	Palm oil
30	Sesame oil
31	Ghee (butter-based)
32	Ghee (vegetable based)
99	No oil or spread used

Table 1.1 Butters and Margarines – Alphabetical listing by brand name

Code	Name
1	Anchor butter
7	Anchor lighter spreadable (reduced fat)
5	Anchor spreadable
8	Asda Best for Baking
12	Asda butter light
1	Asda English creamy butter
14	Asda light sunflower
10	Asda Olive spread
13	Asda Olive light
13	Asda Pure Gold
1	Asda smart price butter
11	Asda Smart Price reduced fat soft spread
8	Asda Soft spread
8	Asda Sunflower buttery spread
9	Asda Sunflower spread
10	Asda 'You'd butter believe it'
	Be good to yourself (see Sainsbury's)
	Bertolli (see Olivio)
10	Benecol olive spread
14	Benecol light spread
1	Butter (all kinds: Anchor/ Kerrygold/ Tesco value/ West country butter/ Somerfield English, Morrison Betta Buy, slightly salted or unsalted butter etc.)
8	Clover (churned for taste/churned with less salt)
10	Co-op buttery
1	Co-op creamery butter
10	Co-op olive
8	Co-op sunflower spread
1	Country life English butter/ organic butter
2	Country life organic butter
2	Country life spreadable butter
	Filippo Berio (see Philippo Berio)
9	Flora (original/ buttery/ low salt/ no salt)
16	Flora diet
14	Flora light
14	Flora pro-active low fat, lower cholesterol spread (sunflower oil)
14	Flora pro-active low cholesterol spread (olive oil)
16	Gold (St Ivel) lowest
11	Gold (St Ivel) low fat

	Golden Sun (see Lidl)
	Healthy living (see Tesco)
2	Lidl Heusa spreadable
1	Lidl Mibona butter
10	Lidl Golden sun olive gold
8	Lidl Golden sun sunflower spread
14	Lidl Golden sun sunflower 38% fat spread
10	I can't believe it's not butter
2	Kerrygold pure Irish butter spreadable
1	Lurpak salted/slightly salted/unsalted
7	Lurpak lighter
2	Lurpak spreadable (25% vegetable oil)
1	Marks and Spencer 100% entirely natural easy spreading unsalted butter
8	Marks and Spencer dairy free sunflower spread
1	Marks and Spencer freshly churned Scottish salted butter
12	Marks and Spencer half fat freshly churned butter
12	Marks and Spencer low fat butter spread
14	Marks and Spencer low fat dairy free sunflower spread
10	Marks and Spencer reduced fat olive spread
9	Marks and Spencer reduced fat spreadable (slightly salted)
2	Marks and Spencer salted butter naturally spreadable
13	Morrison quality and value low fat olive
13	Morrison quality and value morning gold low fat
10	Morrison quality and value olive spread
14	Morrison quality and value reduced fat sunflower spread
1	Morrison quality and value Scottish butter
8	Morrison quality and value sunflower spread
10	Olivio (see Bertolli)
10	Philippo Berio olive spread
1	President unsalted French butter
9	Pure dairy free soya spread
9	Pure dairy free sunflower spread
9	Pure organic with sunflower
	Quality and value (see Morrison)
1	Reid's dairy Scottish knight slightly salted butter
1	Safeway butter (See Morrison Better buy blended butter)
10	Safeway olive spread (see Morrison Olive spread)
13	Safeway olive light (see Morrison low fat olive)
8	Safeway sunflower spread (see Morrison sunflower spread)
14	Safeway sunflower light (see Morrison reduced fat sunflower spread)
15	Sainsbury's be good to yourself lower fat olive spread
14	Sainsbury's be good to yourself low fat sunflower spread
1	Sainsbury's butter (Taste the difference Normandy butter/ Taste the

	difference salted churned butter/ unsalted Alpine butter/ slightly salted butter)
10	Sainsbury's butterlicious
10	Sainsbury's free from – dairy free
5	Sainsbury's margarine
2	Sainsbury's organic spreadable
10	Sainsbury's organic olive spread
10	Sainsbury's olive spread
9	Sainsbury's sunflower spread
7	Sainsbury's soft spread
2	Sainsbury's spreadable
1	Scottish pride salted butter
10	Somerfield butter gold
10	Somerfield olive
2	Somerfield spreadable
	St Ivel – see Gold/ utterly butterly
9	Stork (perfect for pastry)
10	Stork (perfect for cakes)
9	Stork (wrapped)
5	Tesco baking margarine
10	Tesco butter me up
13	Tesco butter me up light
13	Tesco Healthy living butter me up light
14	Tesco Healthy living enriched sunflower spread
13	Tesco Healthy living olive light spread
17	Tesco healthy living sunflower
10	Tesco olive spread
7	Tesco soft spread
2	Tesco spreadable butter
9	Tesco sunflower spread
17	Tesco sunflower lowest, only 5% fat
7	Utterly butterly original (St Ivel)
9	Vitalite
13	Weight Watchers Olivite
1	Yorkshire butter

You should find most codes on the above list, if not, try table 1.2 and table 1.3

Table 1.2 Butters and Margarines: Alphabetical listing by brand name

Code	Name
13	Anchor half fat butter
7	Anchor butter with olive oil
1	Butter (all kinds: Avonlea/ Co-op country life/ Holly bush/ Wheelbarrow Dutch butter/ Winter sun etc)
13	Calvia (calcium enriched)
10	Carapelli
11	Clover extra light
7	Co-op special blend
13	Delight low fat
15	Delight diet
10	Easily better
3	Echo
13	Gold (St Ivel) light
14	Gold (St Ivel) sunflower
14	Gold unsalted
7	Golden crown
8	Golden churn
13	I can't believe it's not butter light
7	Krona Gold
1	Lockerbie
1	Moonraker (butter)
10	Golden Sun olive gold (Lidl)
14	Outline
8	Pura gold cup sunflower spread
14	Pura slimmers gold light
9	Safeway organic buttery spread
13	Sainsbury's olive light
14	Sainsbury's sunflower light spread
17	Sainsbury's economy reduced fat spread
5	Silver soft
5	Somerfield super soft margarine
5	Stork (tub)
5	Stork SB (special blend)
13	Tesco golden light spread
14	Tesco sunflower light spread
14	Vitalite light

Table 1.3 Butters and Margarines: Alphabetical listing by brand name – DO NOT CODE

Name	
Alsan purely vegetable margarine (palm oil base)	Meadowlea Lea Smooth
Blueband	Marks and Spencer reduced fat spread
Co-op margarine	Morrison baking margarine
Costcutters margarine	Morrison better buy far spread
Dairy crest garlic butter	Morrison better buy soft spread
Lurpark with crushed garlic	Morrison soft spread
Iceland margarine	Nuttelex
Kerry Garlic butter	Safeway margarine
Kerry Low fat spread	Stork low fat
Meadowlea Lea Cholesterol free spread	Tesco soft spread
Meadowlea Lea Canola spread	Tesco value soft spread
Meadowlea Lea Milk free spread	Tesco probiotic sunflower spread
Meadowlea Lea Hi-Omega spread	Tomor dairy free margarine
Meadowlea Lea lite spread	Utterly butterly (St Ivel, Scandinavian style)
Meadowlea Lea Logicol spread	Vegan diary free spread with soya
Meadowlea Lea Logicol, Lite spread	What, not butter?
Meadowlea Lea salt reduced spread	Willow blended spread

Table 2.1 Oils and cooking fats: Alphabetical listing by brand name

Code	Name
22	Again and again (no cholesterol) (includes hydrogenated vegetable oils)
24	Asda Chinese stir fry oil
24	Asda pure grapeseed oil
27	Asda smart price lard
28	Britannia finest beef dripping
22	Canola oil
22	Carotino nature oil (vitamin rich)
25	Cookeen
19	Corn oil (all kinds: Mazola/ Sainsbury's/ Tesco etc)
20	Chalice (lemon infused olive oil)
24	Chalice stir fry oil (blend of sunflower, garlic, ginger)
24	Flax Oil (granovita organic)
26	Flora white
31	Ghee (butter-based)
32	Ghee (vegetable-based)
21	Groundnut oil (all kinds: Chalice/ Sainsbury's/Tesco etc)
19	Lidl Golden Sun frying oil
20	Macadamia nut oil
27	Morrison finest quality lard
20	Olive oil (all kinds)
20	Olive pomace oil
18	Olivio cooking oil (vegetable oil + 15% olive oil)
18	Pura light touch
22	Pura vegetable oil
24	Safeway / Morrison sunflower oil
22	Safeway / Morrison vegetable oil
27	Sainsbury's lard
24	Sainsbury's grapeseed
22	Sainsbury's pure vegetable oil (rape)
24	Sainsbury's sunolive (85% sunflower + 15% olive oil)
30	Sesame oil (toasted), (all kinds: Sainsbury's etc)
22	Somerfield vegetable oil
23	Soyola
99	Spray oil (Tesco sunflower etc.)
24	Sunflower oil (all kinds: Flora/ Sainsbury's/ Somerfield/ Tesco/ Vita d'or)
12	Tesco half fat butter
22	Tesco pure vegetable oil
26	Trex pure vegetable fat
24	Walnut oil (all kinds: Chalice/ Sainsbury's, etc)

You should find most codes on the above list, if not, try table 2.2 and table 2.3

Table 2.2 Oils and cooking fats: Alphabetical listing by brand name

Code	Name
22	Asda Pure vegetable oil
22	Chip Shop
27	Crisp 'n dry solid
24	Lidl Golden Sun sunflower oil
21	Safeway groundnut oil
20	Safeway light and mild oil (blend of olive and extra virgin olive oil)
22	Safeway savers oil (see Morrison betta buy vegetable oil)
23	Vita d'Or vegetable oil (soya)
19	Vita d'Or corn oil

Table 3.2 Oils and cooking fats: Alphabetical listing by brand name – DO NOT CODE

Chalice chilli infused sunolive oil	Nisa vegetable oil
Chalice chilli infused oil made with fresh chillis	Pura vegetable lard
Chalice (garlic infused oil/basil infused oil)	Pura vegetable oil (solid)
Heart content oil	Pure additive free vegetable oil
Lidl Vita d'Or pure vegetable oil	
Loscoe chilled foods Ltd pork dripping with jelly	

Food codes

14	Benecol Light spread
02	Connacht Gold
02	Dairygold
14	Dairygold Light
14	Flora Proactive
14	Flora Light
10	Golden Olive
09	Golden Pasture
01	Kerrygold
02	Kerrymaid
14	Light sunflower spread
14	Low Low
08	Move Over Butter
09	Sunflower spread
07	Utterly Butterly

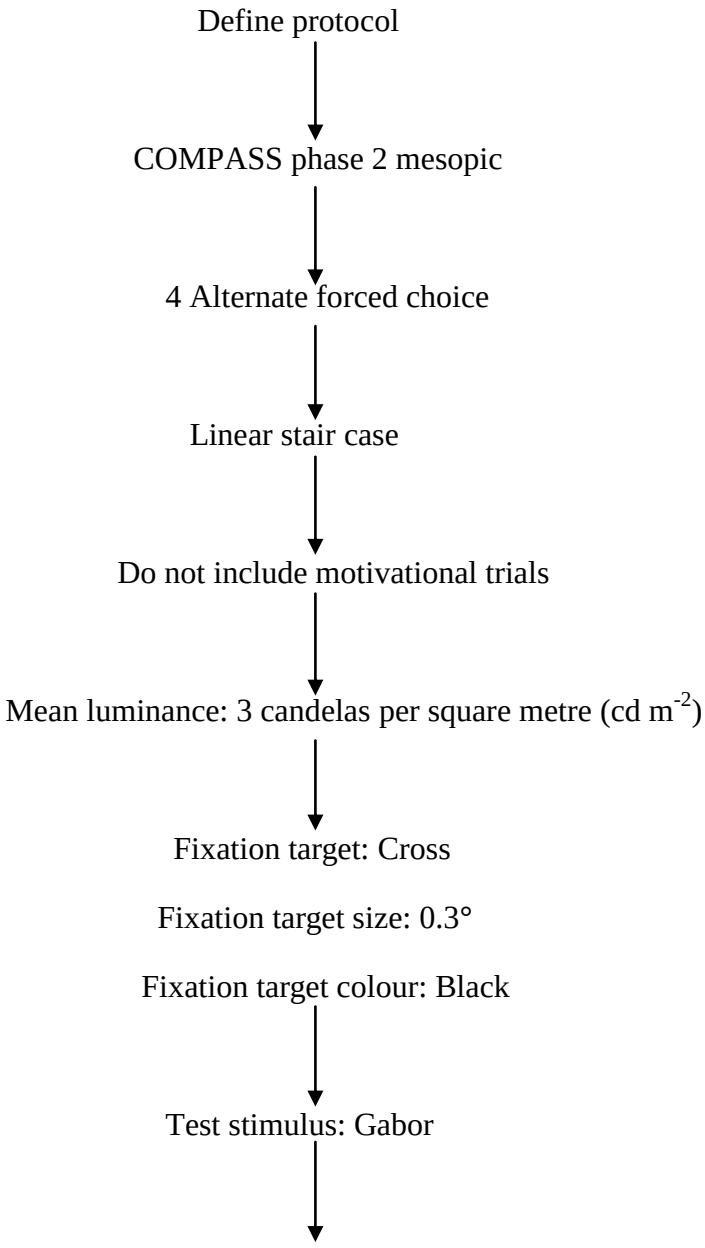
APPENDIX 7.10: STANDARD OPERATING PROCEDURE FOR MEASURING GLARE DISABILITY USING FUNCTIONAL VISION ANALYZER™

1. Plug in Functional Vision Analyzer™ ensuring cable from device to the control pad is attached.
2. Turn on switch at back of instrument.
3. Press button on control pad to select right or left eye (only one Light Emitting Diode (LED) should be illuminated).
4. Press forward on control pad so that position 5 on the dial at the right side of the instrument is in line with the vertical hole adjacent to the word 'far'.
5. Press the far/near button on the control pad so that 'far' LED is illuminated.
6. Press the day/night button so that the 'night' LED is illuminated.
7. Press the glare button so that the LED is off.
8. Ensure the subject wears their best optical correction.
9. Show the subject the demo card and give precise instructions such as 'some people have difficulty with night driving for example when facing oncoming headlights, this test will tell us how sensitive you are to glare….. I would like you to tell me whether the lines are oriented right, left or straight up as seen in the card. If you do not know what the orientation is please do not guess- just say you do not know' and answer all boxes.
10. Enter the subject's details in the Functional Vision Analyzer™ software.
11. Tear off forehead tissue strip.
12. Place the occluder on the eye not being tested.
13. Instruct the subject to lean their head forward to press against the headrest – the test chart should appear in their field of view.

14. If the subject makes a correct response to the first patch, instruct them to answer for the second patch and so on until they make an incorrect response or say they do not know the orientation of a particular patch.
15. Enter the responses in the Functional Vision Analyzer^{TM} software.
16. Repeat the test for dial numbers 6, 7, 8 and 9.
17. 'Night testing without glare' is now finished.
18. Press the 'back' button on the control pad 4 times to get back to position 5.
19. Press the glare button so that the medium glare LED is now on.
20. The subject should see the same chart but surrounded by radial medium glare lights.
21. Repeat the same testing procedure as above.
22. 'Night testing with medium glare lights' is now complete.
23. Press the 'back' button on the control pad 4 times to get back to position 5.
24. Press the glare button so that the high glare LED is now on.
25. The subject should see the same chart but surrounded by radial high glare lights.
26. Repeat the same testing procedure as above.
27. 'Night testing with high glare lights' is now complete.
28. Once complete, three contrast sensitivity function (CSF) plots should be complete and the test is over.

APPENDIX 7.11: CONTRAST SENSITIVITY FUNCTION TEST SETTINGS ON METROPSIS

Mesopic Contrast Sensitivity Function (CSF):



Spatial offset of the stimulus from the fixation target

Location: 2°

↓

Stimulus presentation time: 5000 ms

Stimulus temporal profile/envelop: Square

↓

Spatial frequency	Initial contrast%
1.0 cycle per degree (cpd)	11.5%
4.1 cpd	10%
7.5 cpd	12%
11.8 cpd	30%
20.7 cpd	70%

Photopic CSF:

Define protocol

↓

COMPASS phase 2 photopic

↓

4 Alternate forced choice

↓

Linear stair case

↓

Do not include motivational trials

↓

Mean luminance: 100 cd m^{-2}

↓

Fixation target: Cross,

Fixation target size: 0.3°,

Fixation target colour: Black

↓

Test stimulus: Gabor

↓

Spatial offset of the stimulus from the fixation target

Location: 2°

↓

Stimulus presentation time: 5000 ms

Stimulus temporal profile/envelop: Square

↓

Spatial frequency	Initial contrast%
1.0 cpd	11%
4.1 cpd	10%
7.5 cpd	10%
11.8 cpd	18%
20.7 cpd	60%

Metropsis Monitor configuration:

Current settings:

Device: DVP14 – 256 MB (VSG 81.02.0196)

Monitor: SONY GDM – F520

Frame rate: 85.01

Scan rate: 85.65

Width: 1280

Height: 960

Select Auto

↓

Monitor type: SONY GDM − F520

Required parameters:- Required frame rate − 85 Hz

Required resolution – 1280 X 960 pixels

Select manual

↓

Frame rate – 85 Hz

Max page width − Auto

Line scan rate – 85.65 KHz

Clock rate – 148.00 MHz

Frame sync width – 35 µs	Line sync width – 973 ns
Frame front porch – 12 µs	Line front porch – 541 ns
Frame back porch – 514 µs	Line back porch – 1514 ns
Frame sync polarity − +	Line sync polarity − −

Select calibration

↓

Test pattern – Pixel size calibration

Size of square: 100 pixels

Height of square: 31.60 mm

Default viewing distance: 1500 mm

Gamma correction set up:

Select options

↓

Gamma fit type: Linear interpolation

Input device: ColorCAL on USB

Output patch: centre

Selection: H−bias

Readings: 32

Select Advanced

↓

Do not enable curve validation

Scale factors

Red: 1.00

Green: 1.00

Blue: 1.00

Active configuration: Config.vsg

Phosphor coordinates	CIE		Luminance (cd m^{-2})	
	x	y	min	max
Red	0.604	0.327	0.00	34.13
Green	0.274	0.586	0.00	115.70
Blue	0.154	0.071	0.00	17.55
White			–	–

APPENDIX 7.12: STANDARD OPERATING PROCEDURE FOR MEASURING CONTRAST SENSITIVITY FUNCTION USING METROPSIS

1. Turn on Metropsis, Computer, CRT and flat screen monitors.
2. Select Gamma correction icon.
   Select calibrate.

   Press ColCAL against a flat table to setup in total light absence.

   Press ColCAL against the centre of the CRT screen whilst calibration is taking place.

3. Ensure vision testing unit is also plugged in.
4. Switch off room lights.
5. Select Metropsis icon.
6. Select Contrast Sensitivity icon.
   Select Define protocol. Give the protocol a title ‘COMPASS phase 2 mesopic’. Press OK.

7. Click ►at top of screen to run test. The targets will be presented in random sequence diagonally in each quadrant.
8. Save the test results.

9. Select Contrast Sensitivity icon.
   Select Define protocol. Give the protocol a title ‘COMPASS phase 2 photopic’. Press OK.

10. Click ►at top of screen to run test. The targets will be presented in random sequence diagonally in each quadrant.
11. Save the test results.

Subject Instructions

- The subject should be instructed to look at the central fixation cross.
- An audio beep will signal the target presentation.
- The subject should be instructed to search the area surrounding the fixation cross for the presence of the target once the subject hears this beep.
- The subject should respond using the handheld responder (use the responder with 6 buttons).
- The subject should indicate the location of the target in relation to the fixation cross using the appropriate button e.g. press the top right button if the target is above and to the right of the cross.
- There are 4 possible positions – top right, top left, bottom right, bottom left.
- The subject should be instructed that on certain occasions they may be unable to detect the target. It is important to remind the subject that this is normal.
- The subject should be advised that if no target is visible within 2 seconds that they should guess the location of the target and press the appropriate button. It does not matter whether the response is correct or incorrect.
- Each response the subject makes will be indicated by another audio tone. If the subject presses a button and no tone is heard, then the subject should press the button again until a tone is sounded.
- After making a response the subject should direct their fixation back towards the central fixation cross.

APPENDIX 7.13: STANDARD OPERATING PROCEDURE FOR MEASURING COLOUR VISION USING FARNSWORTH-MUNSELL 100 HUE TEST

PRELIMINARY:

1. Load software onto PC.
2. Ensure that PC graphics mode is set to: 1024 x 768 resolution, 32-bit full colour.
3. Place shortcut on desktop.

COLOUR VISION TESTING:

1. Double click on shortcut.
2. Double click on 'FM100' icon.
3. Place test under colour-corrected lighting, ensuring that:
    a. Illumination is even.
    b. No specular reflections (high-spots) exist.
    c. Background is neutral (white or grey) & of reduced illuminance.
4. Capsize colour caps in each tray onto the lid so that the numbers are invisible.
5. Scramble colour caps in each tray so that numbers underneath the caps are in a random order.
6. Check that no consecutively numbered caps are adjacent.
7. Give instructions to the subject:
    a. Please arrange the caps in order of colour between the fixed colours at each end of the box.
    b. Please do not touch the colours!
    c. Accuracy is more important than speed.
8. Give each tray one after the other.
9. Score each tray (while subject is arranging colours on the next tray) using the software:
    a. Drag & drop samples to replicate the same order of colours used by the subject.

b. Click ‘Show scores’ (LHS, below colour samples).
c. Enter patient’s details (name, DOB).

10. Save.
11. Manually record at your convenience the following (by clicking on ‘Database’ page, then selecting saved record):
    a. Total Error Score (TES) (from ‘Analysis’ page).
    b. Calculate error scores for each quadrant (with correction for minimum possible score).
    c. Colour type.
    d. Colour discrimination rank.
    e. Calculate Partial Error Score (PES) for caps from 50 to 68.
    f. Calculate Partial Error Score (PES) for caps from 36 to 54.
12. Calculate percentage of PES (%PES) for caps from 50 to 68 using the formula as shown below and record.

    % PES 50-68 caps = PES 50-68 caps/TES

13. Calculate percentage of PES (%PES) for caps from 36 to 54, using the formula as shown below and record.

    % PES 36-54 caps = PES 36-54 caps/TES

APPENDIX 7.14: STANDARD OPERATING PROCEDURE FOR MEASURING COLOUR VISION USING HEIDELBERG MULTI COLOUR ANOMALOSCOPE

1. Check that power cables for Oculus 'Anomaloskop' & for PC are connected & cable secured between 'Anomaloskop' & PC.

2. Switch on Anomaloscope & PC. Boot the PC.

3. Load Oculus software by double-clicking on Oculus icon.

4. Icon will now fill the screen – click again.

5. Dim overhead lights (background to Anomaloscope: 25-50 candelas per square metre (cd m^{-2})).

6. Click 'Start'.

7. Click 'Screen'.

8. Enter patient's surname, 1st name, date of birth (dd/mm/yyyy), ID # (if required) (Or for subject re-test, select existing patient).

9. Click 'File', 'File new patient', & 'Anomaloskop'.

10. From menu bar at top of screen, Click 'Program' & select 'Manual'.

11. Click 'Color Test' & select 'Moreland'.

12. Click 'Eye' & select 'Right or Left'.

13. Click 'Matching Range' & select 'Without neutral adaptation'.

14. Check that the following are now displayed:

Program:	Manual
Color test:	Moreland
Eye:	Right
Width of Accept:	NeutOff

15. Click 'Start' and 'Yes' (on 'Start the examination?').

16. Ask subject to look at a neutral (white) background for about 10 seconds.

17. Ask subject to adjust eyepiece focus until circumference of the field & dividing line between upper & lower fields are sharp (with any spectacles or contact lenses that are being worn).

18. Give subject instructions:

'We would like you to make the top part of the display seen through the eyepiece match the bottom part in colour. Please adjust the UPPER display to give the best possible match using upper control only, using a bracketing technique.' (Give demonstration now.)

19. Set 'Mixing Light' to **70** & 'Reference Light' to **50** using the mouse to drag the cross, then click.

20. Check on the monitor throughout test procedure that subject is following instructions (watch the black & white cross).

21. Ask subject to adjust UPPER display only match using upper control only, using the bracketing technique, to make a **colour** match.

22. Ask subject 'Do you wish to re-adjust the top display or are you satisfied that the 2 halves of the display match exactly?' If subject NOT satisfied, allow re-adjustment of upper display.

23. Ask subject to press '=' button when finished. Computer display will now show a red dot.

24. Set 'Mixing Light' to **30** & 'Reference Light' to **50 -** repeat steps 21 to 24.

25. Set 'Mixing Light' to **70** & 'Reference Light' to **50 -** repeat steps 21 to 24.

26. Set 'Mixing Light' to **30** & 'Reference Light' to **50 -** repeat steps 21 to 24.

27. Check that there are now **four** red dots on screen.

28. Click 'End' & (on 'Save Examination?) click 'Yes'.

29. Click 'X' above 'Help'.

30. Double click on ‘Previous examination’ to review data for that subject: minimum & maximum values will now be displayed for: Anomaloquotient, Mixing Light, and Reference Light.

31. Backup all data at the end of the day.

APPENDIX 7.15: STANDARD OPERATING PROCEDURE FOR PHOTOSTRESS RECOVERY TEST

1. Turn on Humphrey® Field Analyzer, model 745*i*. (Please note that this machine takes approximately ten minutes to warm up).
2. Explain the subject about the testing procedure using a demo chart.
3. Select the appropriate test which is; 'Central 24-2'.
4. Select the eye to be tested and occlude the subject's other eye with an occluder which is provided.
5. Enter the subject's details. Enter the subject's spectacle Rx. You can choose an option to calculate the appropriate trial lens or you can manually in put the spectacle Rx.
6. Take note of the appropriate trial lens that should be used and place it in the holder in front of the subject's eye that is being tested.
7. Ask the subject to place their chin on the chin rest and to ensure that their forehead is pressed against the headrest (if the study eye is right, use the left chinrest and if the study eye is left, use the right chinrest).
8. Adjust the room lighting so that only a dimmer light remains on.
9. Now press 'Proceed'. Test will appear on the screen. Select 'Change Parameters': Set 'Foveal Threshold' from 'OFF' to 'ON' and press 'Selection Complete'.

   Note: There is a fixation window on the screen where you can check if the subject's eye is in the correct position or not. If the subject's pupil is off centre, buttons on the screen can be adjusted to manoeuver the chinrest so that the central fixation cross is at the level of the lower edge of the pupil.
10. Present the subject with a clicker.
11. Press 'Start' on the test screen. Instruct the subject to look at the centre of the four fixation lights and ask them to press the clicker whenever they see a flashing white light stimulus in the centre of the four lights

during the test. Now press ‘Start’. This test will calculate the foveal threshold.

12. Test lasts about twenty to thirty seconds, until a beep sound comes and an instruction appears on the screen, which asks the subject ‘Look at the central fixation target’. Press ‘Start’.
13. Instrument asks to ‘Initialize gaze monitor’. Press ‘Start’.
14. There will be a value appears on the centre of the screen which you note down. This is the subject’s foveal threshold under normal viewing conditions.
15. Next, an instruction appears on the screen saying ‘Initializing gaze failed, give additional instructions’. When you press ‘Cancel’, test screen appears.
16. Repeat the same test two more times and calculate the average. This is the subject’s baseline foveal threshold.
17. Now start the clock and expose the subject’s eye to a very bright light (300 W, 230 V tungsten lamp) for five seconds from a metre distance. Make sure the lamp is aligned at the subject’s eye level and the subject is looking at the centre of the lamp. Once the macula has been bleached, repeat the test immediately.
18. Take note of the new threshold reading and time. We would expect the reading to be lower because the macula has been bleached and it will take some time to recover.
19. Repeat the test and take note of the threshold and time readings consecutively until ten minutes. If the eye does not recover to the baseline foveal threshold, even after ten minutes, stop the clock and test.
20. Ask the subject to sit back and relax.
21. Record percentage of sensitivity reduction following post-bleaching.
22. Subtract five seconds from each time reading (which is a photostress time).
23. Calculate the time to taken to return to the 95% of the baseline foveal threshold.

24. Calculate the time taken to return to the 100% (maximum) of the baseline foveal threshold.

APPENDIX 7.16: STANDARD OPERATING PROCEDURE FOR SHORT WAVELENGTH AUTOMATED PERIMETRY TEST

1. Turn on Humphrey® Field Analyzer, model 745*i* perimeter. (Please note that this machine takes approximately ten minutes to warm up).
2. Explain the subject about the testing procedure using a demo chart.
3. Select the appropriate test which is; COMPASS 5-2 SWAP 56. This is a threshold test to test the short wavelength sensitive cone sensitivity.
4. Select the eye to be tested and occlude the subject's other eye with an occluder which is provided.
5. Enter the subject's details. Enter the subject's spectacle Rx. You can choose an option to calculate the appropriate trial lens or you can manually in put the spectacle Rx.
6. Take note of the appropriate trial lens that should be used and place it in the holder in front of the subject's eye that is being tested.
7. Ask the subject to place their chin on the chin rest and to ensure that their forehead is pressed against the headrest (if the study eye is right, use the left chinrest and if the study eye is left, use the right chinrest).
   Ask the subject to fixate on the central yellow light target and advise them that they should remain fixated on this target during the test.
8. Adjust the room lighting so that only a dimmer light remains on.
9. Now press 'Proceed'. Test will appear on the screen. Press 'Start' and extend the visor before blue-yellow test and then press 'OK'. Now it prepares the instrument for testing. At the end of the instrument's preparation, a bright yellow background light appears to adapt the medium and long wavelength cones there by reducing their sensitivity to blue stimulus. Ask the subject to look at the lower fixation lights and expose the subject's eye to this adapting light for 3 minutes.
   Note: There is a fixation window on the screen where you can check if the subject's eye is in the correct position or not. If the subject's pupil is

off centre, buttons on the screen can be adjusted to manoeuver the chinrest so that the central fixation cross is at the level of the lower edge of the pupil.

10. Present the subject with a clicker.
11. Instruct the subject to look at the centre of the four fixation lights and ask them to press the clicker whenever they see a flashing blue light stimulus in the centre of the four lights during the test. Now press 'Start'. This test will calculate the foveal threshold. A 440nm Goldmann size blue light stimulus is used to determine the sensitivity of the short wavelength cone system, both in the central and peripheral retina.
12. At the end of this test, ask the subject to fixate at the central yellow fixation target and advise them that they should remain fixated on this target during the rest of the testing period. Instruct the subject to press the clicker whenever they see a flashing blue light stimulus peripherally, while fixating at the central yellow target.

    Note: There is a fixation window on the screen where you can check if the subject's eye is in the correct position or not. If the subject's pupil is off centre, buttons on the screen can be adjusted to manoeuver the chinrest so that the central fixation cross is lined up in the centre of the subject's pupil.
13. Now press 'Start'.
14. Test will begin after foveal and/or gaze monitor initialization.
15. This test takes approximately 5 minutes to complete.
16. Save the test and print a copy. Attach the copy to the case report form.

APPENDIX 7.17: STANDARD OPERATING PROCEDURE FOR DAILY CALIBRATION OF THE MACULAR DENSITOMETER™

MACULAR METRICS DENSITOMETER™

DAILY CALIBRATION - Waterford

Macular Pigment Research Group (MPRG) Department of Chemical & Life Science Waterford Institute of Technology	
SOP-MPRG-007 Issue 1	Issued by: Dr. Edward Loane Reviewed by: Dr. John Nolan Issue Date: 4/01/2007

1. Perform a complete visual inspection of the instrument, ensuring all switches and cables are in the correct position

2. Perform checklist to ensure all switches are in the correct position:
   a. Switch on control unit pointed towards YOKED
   b. Switch on control unit for FIX LED set to off position (down)
   c. Switch on control unit pointed towards blue
   d. Switch at back of subject control box set to linear ('Lin')

3. Calibration:
   a. Turn on all power switches and turn switch on at back of control unit
   b. Ensure master knob on control unit is set to 'Run'
   c. Set Stimulus to position 5 (red manual advance button on series controller; visually check to ensure its large 2^0 disc)
   d. Ensure port at front of black box is closed (sliding knob to right and underneath eyepiece)
   e. Turn/set switch at left of main unit to low (i.e. switch at left is down. N.B. This is a three-way switch, so make sure it is all the way down)
   f. Turn master knob on control unit is set to 'Off'
   g. Dim/switch off the room lights
   h. Adjust dark current ('Photometer' reading) to zero using twisty knob (blue in colour) at left of main unit
   i. Turn master knob to 'Background' (reading on photometer should be 1153 ± 15)
   j. If within ± 15 of 1153 on photometer turn master knob to 'Off'

MPRG Vision Laboratory

k. Turn/set gain switch at left of main unit to middle position (i.e. switch at left is in mid position; remember that this is a three-way switch)

l. Adjust dark current ('Photometer' reading) to zero using twisty knob (blue in colour) at left of main unit

m. Turn master knob to 'Blue' and adjust radiance using subject control knob to ~ 1823

n. Reading on photometer should be 400 ± 5

o. Now turn master knob to "Green"; reading on photometer will be between 26 and 28 and radiance 1826 ± 5

p. The Macular Metrics Densitometer™ is now calibrated and ready to measure macular pigment

APPENDIX 7.18: YOKED PROTOCOL FOR MEASURING MACULAR PIGMENT OPTICAL DENSITY USING THE MACULAR DENSITOMETER™

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
Standard Operating Procedure (SOP)-MPRG-006 **Issue 2**	**Issued by: Dr. John M Nolan** **Reviewed by: Dr. Billy Wooten** **Date: 10/01/2008**

Prior to testing each day, the instrument must be calibrated, using a separate calibration protocol (see Appendix 7.17).

I. VIDEO

Have the subject view the video that describes the flicker appearance and the overall task (5 minutes).

II. INSTRUMENT PREPARATION

1. Ensure Densitometer has been previously calibrated (see Densitometer calibration SOP for instructions).
2. Set the Filter Wheel Controller Box to Target 2 (1^{o} stimulus).
3. Set the silver toggle switch on the main unit to up/yoked position (for location, see point 7 in manual).
4. Set the silver toggle switch on the main control box to off/CFF position (for location, see point 5 in manual).

5. Open the ocular opening on the front of the enclosure by (sliding knob to left underneath eyepiece).
6. Turn the dial on the main control box to the 'RUN' position (for location see point 1 in manual).
7. Turn the red light emitting diode (LED) toggle switch to the down/FIX LED position (red LED is now off when you look into the ocular unit).
8. Set the low frequency flicker to approximately 10.00 (dial on left side of main control box, for location see point 10 in the manual).

III. SUBJECT PREPARATION

1. Have the subject sit in the chair in front of the instrument (subject can wear their corrective lens if required).
2. Have the subject place his/her eye being tested on the eyepiece unit and ensure the subject can do so comfortably (e.g. the Densitometer is at correct height for the subject etc.).
3. Dim the lights in the room.

Examiner to Subject: *You should see a round blue field with a smaller flickering disc in the centre.*

IV. DETERMINING CRITICAL FLICKER FUSION FREQUENCY (CFF)

1. Adjust the *subject* black tuning knob so that the radiance is 1000.
2. Turn off the room lights.

Examiner to Subject: *We will first be doing a series of measurements to help us determine the speed of flickering light that is best for you to complete the macular pigment testing. As in the video, you will be asked to look at a light*

target and I will turn a dial until you feel that the light has just stopped flickering. Now look at the flickering disc in the centre of the larger blue field and confirm that the disc is in fact flickering. You should also see a small dot in the centre of the flickering disc. For these measurements, I want you to look at the flickering disc in the centre of the larger blue field. Please focus on the small dot in the centre of the disc and tell me when you feel that the disc has stopped flickering (the examiner slowly adjusts the flicker frequency knob in a clockwise motion). *Good. I will record this measurement (the examiner resets the flicker frequency dial to approximately 10.00). Please blink and we will do the measurement again* (remind the subject that after blinking the flicker will appear again*). We will do **three measurements** in total for this part of the test. Let's begin.*

3. Record each of the three frequency values in the appropriate place on the data entry sheet and calculate the average CFF (see Collaborative Optical Macular Pigment ASsessment Study (COMPASS) case report form).

4. This value will be used to calculate the subject's Optimal Flicker Fusion Frequency (OFF) for each target during testing (refer to **'CFF conversion to OFF'** on data entry sheet (see below).

Location	Predicted OFF	Target used
0.25^{o}	CFF-8	1
0.5^{o}	CFF-7	2
1^{o}	CFF-7	3
1.75^{o}	CFF-7	4
3^{o}	CFF-9	2
7^{o}	CFF-14	5

5. Record the subject's OFFs on the data entry sheet (see COMPASS case report form).

V. MACULAR PIGMENT OPTICAL DENSITY TESTING

1. Set the Filter Wheel Controller Box to Target 1 using the Filter Wheel control box (see target on Densitometer card display for explanation to the subject).
2. Ensure the silver toggle switch on the main control box is set to down/BLUE position (for location, see point 5 in manual).
3. Set the low frequency flicker to the value obtained for the **OFF** for Target 1 (refer to COMPASS data entry sheet for actual value).
4. Set the Subject Knob radiance to a value between 25 and 100.

Examiner to Subject: *We are now ready to measure your macular pigment. You are doing very well. As for the above measurements, you will be turning the knob clockwise and counter clockwise until you are in the middle of the zone in which there is no flicker* (use radio analogy if required). *When you have reached that point, let the dial go and let me know and I will record the reading. We will do* ***five measurements*** *at each one of the light targets. Let's begin.*

5. Instruct subject as before, noting that he/she should focus on the small central dot, turning the dial until the flickering stops.
6. Remind the subject to blink normally.
7. Do 5 measurements at Target 1.
8. During the testing, alternate the starting radiance so that the subject is not always turning the knob in the same direction (for example: one measurement beginning at 25-100, the next measurement beginning at 1800-2000).
9. Record the radiance values in the 'COMPASS data entry sheet' at which the subject noted that the disc had stopped flickering.
10. After completing Target 1, tell the subject that he/she may rest (lean

back from the Densitometer) while you prepare for the next part of the test.

11. If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), progress to step 12. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find the frequency at which the subject can achieve an absolute null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).
12. Set the Filter Wheel Controller Box to Target 2 using the Filter Wheel control box (see target on Densitometer card display for explanation to the subject).
13. Set the low frequency flicker to the value obtained for the **OFF** for Target 2 (refer to data entry sheet for actual value).
14. Set the Subject Knob radiance to a value between 25 and 100.

Examiner to subject: *We are now ready to do our next target. This target will be very similar to the first; however, the small flickering disc will be a bit bigger. Again, while focusing on the small dot in the centre of the disc I would like you to turn the knob clockwise and counter clockwise until you feel that you are in the middle of the zone in which there is no flicker. Good. Now I will have you repeat the measurement, just like before.*

15. Record the five radiance values in the 'COMPASS data entry sheet' at which the subject noted that the disc had stopped flickering.
16. After completing Target 2, tell the subject that he/she may rest (lean back from the Densitometer) while you prepare for the next part of the test.
17. If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), progress to step 18. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find frequency at which the subject can achieve an absolute

null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).

18. Set the low frequency flicker to the value obtained for the **OFF** for location 3^{o} eccentricity (refer to COMPASS case report form data entry sheet for actual value).
19. Set the Subject Knob radiance to a value between 25 and 100.

Examiner to subject: *Now I want you to look off to the left at the edge of the blue background and you will see a small black dot* (use target on Densitometer card display to explain to the subject). *In order to do the measurements for this target, you will need to focus or fixate on the black dot to the left of the blue field. Your eye may have a tendency to drift over to look at the target. It is important to keep your focus on the small black dot at all times. As you did above, you will be turning the knob clockwise and counter clockwise until you are in the middle of the zone in which there is no flicker of the blue flickering target. When you have reached that point, let the dial go and let me know and I will record the reading.*

20. Record the five radiance values in the 'COMPASS data entry sheet' at which the subject noted that the disc had stopped flickering.
21. After completing this Target, tell the subject that he/she may rest (lean back from the Densitometer) while you review the radiance values.
22. If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), progress to step 21. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find frequency at which the subject can achieve an absolute null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).
23. Set the Filter Wheel Controller Box to Target 3 using the Filter Wheel control box (see target on Densitometer card display for explanation to the subject).

24. Set the low frequency flicker to the value obtained for the **OFF** for Target 3 (refer to COMPASS case report form data entry sheet for actual value).
25. Set the Subject Knob radiance to a value between 25 and 100.

Examiner to subject: *This next target differs slightly. Here you will see a blue ring and a small central dot. You will need to focus on the small central dot and turn the knob to eliminate the flickering of the outside ring. If you look at the outside ring directly it may never stop flickering. It is important to keep your focus on the small central dot as you do this measurement.* Repeat measurement five times as outlined above.

26. Record the five radiance values in the 'COMPASS data entry sheet' at which the subject noted that the disc had stopped flickering.
27. After completing Target 3, tell the subject that he/she may rest (lean back from the chin rest) while you prepare for the next part of the test.
28. If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), progress to step 29. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find frequency at which the subject can achieve an absolute null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).
29. Set the Filter Wheel Controller Box to Target 4 using the Filter Wheel control box (see target on Densitometer card display for explanation to the subject).
30. Set the low frequency flicker to the value obtained for the **OFF** for Target 4 (refer to COMPASS case report form data entry sheet for actual value).
31. Set the Subject Knob radiance to a value between 25 and 100.

Examiner to subject: *This next target is very similar to the previous one. The outside ring may appear to be larger. Just remember to keep your focus on the small central dot as you turn the knob to eliminate the flickering of the outside ring.* Repeat measurement **five** times as outlined above.

32. Record the radiance values in the in the 'COMPASS data entry sheet' at which the subject noted that the disc had stopped flickering.
33. After completing Target 4, tell the subject that he/she may rest (lean back from the chin rest) while you prepare for the next part of the test.
34. If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), progress to step 35. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find frequency at which the subject can achieve an absolute null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).
35. Set the Filter Wheel Controller Box to Target 5 using the Filter Wheel control box (see target on Densitometer card display for explanation to the subject).
36. Set the low frequency flicker to the value obtained for the **OFF** for Target 5 (CFF-14, refer to data entry sheet for actual value).
37. Set the red fixation LED toggle switch to the up/on position (red LED turned on when looking into ocular unit, for location see point 6 in manual).

Examiner to subject: *Now I have changed the light to the one with the red light off to the left. As you may recall from the video, this target consists of a large blue field and a smaller red light off to the left. In order to do the measurements for this target, you will need to focus or fixate on the red light to the left of the blue field. Your eye may have a tendency to drift over to look at the blue field. It is important to keep your focus on the small red light at all times. As you did for the other tests, you will be turning the knob clockwise and counter*

clockwise until you are in the middle of the zone in which there is no flicker of the blue field. When you have reached that point, let the dial go and let me know and I will record the reading. Now we'll take the first measurement. Remember to focus on the red light and turn the knob until you feel you are in the middle of the zone in which there is no flicker of the large blue field in your peripheral vision. Good. Now blink and we'll do the measurement again.

38. Remind the subject to blink normally.
39. Do five measurements at Target 5.
40. During the testing, alternate the starting radiance so that the subject is not always turning the knob in the same direction (for example: one measurement beginning at 25-100, the next measurement beginning at 1800-2000).
41. After completing Target 5, tell the subject that he/she may rest (lean back from the Densitometer) while you prepare for the next part of the test.

If the radiance values obtained are within an acceptable range (i.e. 10-15% of each other), you are finished. If not, the examiner may need to adjust the low frequency flicker (by 1 Hz in either direction) for this Target, to find frequency at which the subject can achieve an absolute null (no flicker whatsoever) point (see below, 'points to note number 2' for instructions).

VI. POINTS TO NOTE

1. Throughout the testing, reassure the subject that they are doing the test correctly and repeating instructions as needed. Do not rush the subject. Continue to describe each target and remind the subject to focus on the central dot (or red fixation light) while doing the test. Always watch what the subject is doing and watch the radiance values to ensure they are passing back and forth through the no flicker zone and not just approaching it from one side each time.

2. The predicted OFF formulas are based on trials run at the Medical College of Georgia. The values are intended to customise an individual's OFF in order to perform the task without difficulty. However, if a subject reports that he/she is unable to find a zero flicker point, or if the range of zero flicker is too large, the following rules should be applied:

   - If the subject cannot get the flicker to stop, increase the frequency by 1 Hz in a stepwise fashion until the subject is satisfied that he/she can find a zero flicker point.
   - If the subject reports that the range of no flicker is very large, decrease the flicker frequency by 1 Hz in a stepwise fashion until the subject is satisfied that the range is narrow, and he/she can find a zero flicker point.

3. Remind the subject to blink normally (about once every 3-4 seconds) throughout the test.

APPENDIX 7.19: SETTINGS AND STANDARD OPERATING PROCEDURE FOR FUNDUS PHOTOGRAPHY USING NON-MYDRIATIC AUTO FUNDUS CAMERA

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
MPRG-Standard Operating Procedure(SOP)-005 **Issue 2**	**Issued by: Dr. Edward Loane** **Reviewed by: Dr. John M Nolan** **Issue Date: 07/08/2007**

1. INSTRUMENT PREPARATION

A. Start-up sequence for NIDEK fundus camera:
   i. Turn on computer.
   ii. Turn on Canon EOS 5D camera (middle position of three-way switch on back of camera).
   iii. Turn on NIDEK AFC 210 base unit.
   iv. Open Navis Lite program (password is 'nidek', lower case).

B. Camera settings for Canon EOS 5D camera (these should not be changed):
   i. ISO 400
   ii. White Balance: Flash
   iii. WB Shift/BKT: B2, G2/±0
   iv. Shutter speed: 1/60th of a second
   v. Image size: Medium step
   vi. Colour Temperature: 5200 K
   vii. Picture Style: User defined settings:

Sharpness: +1 (5)

Contrast: 0

Saturation: 0

Colour Tone: 0

C. NIDEK fundus camera settings (this automatically starts on fundus mode):
    i. Fundus image capture:

        Flash +10

    ii. Anterior segment (Iris) image capture:

        Flash +5 for dark irides; +3 for light irides (adjust with *Flash Intensity Buttons*)

        Focus setting central (adjust with *Focusing Knob*)

D. Enter subject details
    i. Click on the 'New' button at the top left in the Navis Lite window.
    ii. Enter patient ID, name, sex, and date of birth.
    iii. Click on 'Capture'.

2. SUBJECT PREPARATION

A. Inform the subject that you are going to take several photographs of the front and back of their eye(s) and that there will be a bright flash each time.
B. Adjust the table height, as required, with the up/down button, which is located just below the centre of the instrument table on the operator's side.
C. Ask the subject to place their chin on the *Chinrest.*
D. Adjust the *Chinrest* with the *Chinrest Up/Down Buttons* so that the *Eye Level Marker* is in line with the outer canthus (outer corner) of the subject's eye. * This does not have to be too precise.
E. Ensure that the subject has their forehead pressed against the *Forehead Rest* and that they are comfortable in this position.
F. Turn off the room lights.

3. FUNDUS IMAGE CAPTURE

A. Using the *Joystick*, move the *Main Unit* left or right to align the *Objective Lens* with the eye under examination.
B. Tell the subject that they will see a green flashing light and ask them to fixate on this.
C. While watching the *Liquid Crystal Display (LCD) Monitor*, align the *Target Mark* (the two centre-most concentric rings) with the subject's pupil by moving the *Main Unit* up or down by turning the head of the *Joystick* clockwise or counter clockwise.
D. Push the *Main Unit* towards the subject using the *Joystick*, until the *Movement Distance Indicator* goes from green to yellow.
    i. The camera will then auto-focus on the fundus.
    ii. If the *Movement Distance Indicator* goes pink, you have advanced the *Main Unit* too close to the subject and need to pull it back towards yourself.
E. Fundal details should now be faintly observable on the *LCD Monitor*.
    i. Fundal illumination can be adjusted using the *Observation Illumination Intensity Knob.*
F. Using the *Focusing Knob*, adjust the *Focus Bars* so that they are in line with each other.
G. Using the *Joystick*, adjust the *Optical Working Dots* so that they are in line with the *Optical Working Dot Charts* and are in sharp focus on the *LCD Monitor*. This will result in greater image detail than if the *Optical Working Dots* are not sharply focused.
H. Press the *Release Button* on the top of the *Joystick* to capture the fundus image.
I. Repeat for the fellow eye, as required.
J. If the subject's pupil is very small, it may be necessary to press the *Small Pupil Photography Mode Switch* and proceed as above from Step D.

4. ANTERIOR SEGMENT (IRIS) IMAGE CAPTURE

A. Switch on the room lights to induce pupillary constriction, so that more of the iris detail is available for imaging.
B. Press the *Anterior Eye Image Capturing Switch.*
C. Inform the subject that there is no focus point for this step and that they should just look straight ahead.
D. Adjust the flash intensity to +5 (or +3; see Section 1. Instrument Preparation) using the *Flash Intensity Buttons.*
E. Adjust the focus setting to its central position using the *Focusing Knob.*
F. Use the *Joystick* to align the anterior segment (iris) centrally on the *LCD Monitor* so that it occupies approximately three quarters of the screen.
    i. The iris will not appear to be in sharp focus on the screen at this point.
G. Press the *Release Button* on the top of the *Joystick* to capture the anterior segment (iris) image.
H. Repeat for the fellow eye, as required.

5. STEREO FUNDUS IMAGE CAPTURE

A. Prepare the subject for image capture, as outlined in Section 2, above.
B. Change the image capturing mode to '1', using the *Stereo Photography Mode Switch*, located to the bottom right of the *LCD monitor.*
C. Push the *Main Unit* towards the subject using the *Joystick*, until the *Movement Distance Indicator* goes from green to yellow.
    i. The camera will then auto-focus on the fundus.
    ii. If the *Movement Distance Indicator* goes pink, you have advanced the *Main Unit* too close to the subject and need to pull it back towards yourself.
D. Fundal details should now be faintly observable on the *LCD Monitor.*

i. Fundal illumination can be adjusted using the *Observation Illumination Intensity Knob*.

E. Using the *Focusing Knob*, adjust the *Focus Bars* so that they are in line with each other.

F. Using the *Joystick*, adjust the *Optical Working Dots* so that they are in line with the *Optical Working Dot Charts* and are in sharp focus on the *LCD Monitor*. This will result in greater image detail than if the *Optical Working Dots* are not sharply focused.

G. For the right eye, move the *Image Capturing unit* of the instrument to the temporal side of the right eye, to shift the *Optical Working Dot* on the left side of the screen about three scales of the *Stereo Gauge* to the right.

H. Press the *Release Button* on the top of the *Joystick* to capture the fundus image.

I. Change the image capturing mode to '2', using the *Stereo Photography Mode Switch*, located to the bottom right of the *LCD monitor*.

J. Repeat Steps C-H, above.

K. Repeat for the left eye, as required, noting that the *Optical Working Dot* must be moved in the opposite direction to that for the right eye, as described above.

6. ADVANCED OPERATION

A. Refer to the 'NIDEK NON-MYDRIATIC AUTO FUNDUS CAMERA Model AFC-210 OPERATOR'S MANUAL' for further details on advanced operation, description of apparatus components, and trouble-shooting.

APPENDIX 7.20: STANDARD OPERATING PROCEDURE FOR HUMAN PHLEBOTOMY

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
MPRG- Standard Operating Procedure(SOP)-009 **Issue 1**	**Issued by: Dr. Edward Loane** **Reviewed by: Dr. John M Nolan** **Issue Date: 08/01/2008**

1. REQUIREMENTS

A. Spirigel® (or similar) alcohol hand gel (Ecolab Ltd., Garforth, England)
B. Alcohol swabs
C. Sharps disposal container
D. Tourniquet
E. Vacutainer® needle system (Becton Dickinson Vacutainer™ Systems)
    i. Vacutainer® (BD, Plymouth, U.K.)
    ii. Sterile needles (preferably BD Vacutainer® Flashback Blood Collection Needles, 21G; Ref. 301746; BD, Plymouth, U.K.)
F. Blood tubes (Greiner Bio-One GmbH, Kremsmünster, Austria)
G. Cotton wool
H. Tape

2. PREPARATION

A. Select appropriate blood tubes and quantities of each.
B. Invite your volunteer to sit comfortably in one of the armchairs and enquire if they have a 'good arm' for taking blood from. This is often the case.

C. Appear confident. Being well organised and mentally visualising the steps involved will promote your own confidence! Idle chit-chat may also help to relax your volunteer.
D. Prepare a small ball of cotton wool and a length of tape, approximately 10cm long.

3. PROCEDURE

A. Apply the tourniquet above the elbow of the selected arm and adjust so that it is tight, but not uncomfortable.
B. Clean your hands thoroughly with alcohol gel.
C. Feel for an appropriate vein in the volunteer's ante-cubital fossa. An appropriate vein will feel firm but compressible compared to surrounding tissues. Remember that the vein you will select to take blood from will generally NOT be one of the visible superficial veins; i.e. 'go for the vein you can feel, not the vein you can see'. This rule will occasionally be broken, and only experience will guide this decision.
D. Clean the skin thoroughly overlying the selected vein with an alcohol swab.
E. Feel again for the selected vein and make note of the direction in which it is passing.
F. Close the port on the end of the vacutainer by pressing the white tab.
G. Take a sterile needle and twist off the end to reveal a narrow grey rubber tube that covers one end of the needle. Attach this end of the sterile needle to the vacutainer by screwing it on. Be very careful not to touch the grey tubing.
H. Ask your volunteer to look away and tell them that you will let them know when there will be a sharp pinch. Remind them that they should hold their arm very steady and not jump.
I. Remove the other end of the sterile needle to reveal a bare needle. Make sure not to touch the needle.

J. Ensure that the bevel (or ‘opening’) of the needle is facing away from the volunteer’s arm before inserting the needle.
K. Stabilize the distal end (the side closer to your volunteer’s hand) of the vein with the thumb of your left hand (for right-handed phlebotomists), by gently drawing the skin back towards your volunteer’s hand.
L. Holding the plastic vacutainer with your right hand, smoothly and firmly insert the needle through the skin overlying the selected vein at an angle of approximately 30° to the skin surface. Don’t forget to remind your volunteer that they will feel a sharp pinch!
M. Watch the clear plastic part at the base of the needle for the ‘flash-back’ (blood entering this part of the needle). Once this happens, stop advancing the needle and hold it precisely in this position with your left hand (having switched hands at this point). One may also appreciate a slight ‘give’ once the vein has been correctly entered.
N. Click-on each blood tube into the plastic vacutainer, by pushing the coloured end of each tube onto the needle that is covered by the grey tubing within the vacutainer.
O. Each blood tube should fill automatically. Allow this to happen to the required amount.
P. Once the required number of tubes have been filled, RELEASE THE TOURNIQUET.
Q. Gently rest a ball of cotton wool over the end of the needle where it enters your volunteer’s skin, and then quickly and smoothly remove the needle and immediately apply firm pressure with your thumb over the ball of cotton wool.
R. Ask your volunteer to take over from you in applying pressure over the cotton wool.
S. Immediately dispose of the needle into the sharps disposal container by pressing the green tab on the vacutainer, releasing the needle.

T. Tape down the ball of cotton wool onto your volunteer's arm and encourage them to continue applying pressure for at least one minute. This will minimise any potential bruising.
U. Ensure that your volunteer feels OK.

APPENDIX 7.21: AGILENT 1200 SERIES METHOD FOR LUTEIN AND ZEAXANTHIN STANDARDS

Macular Pigment Research Group (MPRG) Department of Chemical & Life Sciences Waterford Institute of Technology	
MPRG-Standard Operating Procedure(SOP)-001 Issue 1	**Issued by: Dr. John M Nolan Reviewed by: Dr. Brian Murphy Issue Date: 25/07/2007**

INSTRUMENT

HP 1200 Series High Performance Liquid Chromatography (HPLC) system, PC, Software (Agilent Chem Station for LC System)

1. METHOD SETUP

1.1 Power on computer connected to HPLC

1.2 Click OK (no password is required)

1.3 Click Instrument 1 online

2. SETTING METHOD PARAMETERS

2.1 Click **Method** on top toolbar

2.1.1 Select **Edit Entire Method**

2.1.2 **Edit Method** Dialog box appears

2.1.3 All boxes in this dialog box should be automatically selected, if not select, press OK

2.1.4 **Method Information:** Dialog box appears

2.1.5 Comments Box, enter relevant comments

2.1.6 Click OK

2.2 New Dialog box appears **Set Up Pump.** Adjust parameters to appropriate values for your chromatography

2.2.1	Flow-rate	:	1ml/min
2.2.2	Stop-time	:	6 mins
2.2.3	Solvents	:	Methanol 97%:
			Tetrahydrofuran (THF) 3%
2.2.4	Set Pressure limits	:	max = 400 bar, min = 0 bar

2.2.5 Click OK

2.3 New Dialog box appears **Set Up Injector**

2.3.1 Select Standard Injection

2.3.2 Select Injection Volume: 100 μL

2.3.3 Click OK

2.4 New Dialog box appears **DAD Signals**

2.4.1 In store A set λ to 450 nm for carotenoids

2.4.2 Press OK

2.4.3 Store: Select All

2.4.4 Set your nm range from min to max from 400 nm to 500 nm

2.4.5 Select step 2.0 nm

2.4.6 Set Threshold to 1.0 mAu

2.4.7 Select UV and Vis lamps

2.4.8 Set Peak Width: 0.1 min (2 s)

2.4.9 Ensure Autobalance is set for prerun and postrun

2.4.10 Set Slit at 4 nm

2.4.11 Time and Timetable doesn't need to be set

2.4.12 Click OK

2.5. New Dialog box appears **Column Thermostat Method**

2.5.1 Set at 25º C

2.5.2 Click OK

2.6 New Dialog box appears **Signal Details**

2.6.1 Ensure all signals of interest are selected and added to method (e.g.450nm)

2.6.2 Click OK

2.7 New Dialog box appears **Edit Integration Events**:

Integration Events	**Value**
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical

Time	**Integration Events**	**Value**
Initial	Slope Sensitivity	1
Initial	Peak Width	0.04
Initial	Area Reject	1
Initial	Height Reject	1.7
Initial	Shoulders	OFF

2.7.1 Click OK

2.8 New Dialog box appears Specify Report:

2.8.1 Under Destination**,** Select Printer and File

2.8.2 Click OK

2.9 New Dialog box appears **Instrument Curves:**

2.9.1 Click OK (No instrument curves should be selected)

2.10 New Dialog box appears **Run Time Checklist:**

The following should be automatically selected:

2.10.1 Data Acquisition

2.10.2 Standard Data Analysis

2.10.3 Click OK

2.11 Click **Method** on top toolbar

2.11.1 Click Save Method

2.11.2 Add comment and click OK

APPENDIX 7.22: AGILENT 1200 SERIES METHOD FOR RETINOLS, TOCOPHEROLS AND CAROTENOIDS IN SERUM SET-UP

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
MPRG-Standard Operating Procedure (SOP)-002 Issue 1	**Issued by: Dr. John M Nolan** **Reviewed by: Dr. Brian Murphy** **Issue Date: 25/07/2007**

INSTRUMENT

HP 1200 Series High Performance Liquid Chromatography (HPLC) system, PC, software (Agilent Chem Station for LC System)

1. METHOD SETUP

1.1 Power on computer connected to HPLC

1.2 Click Ok (no password is required)

1.3 Click Instrument 1 online

2. SETTING METHOD PARAMETERS

2.1 Click Method on top toolbar

2.1.1 Select Edit Entire Method

2.1.2 **Edit Method Instrument 1** Dialog box appears

2.1.3 All boxes in this dialog box should be automatically selected, if not select, press OK

2.1.4 **Method Information:** Dialog box appears

2.1.5 Comments Box, enter relevant comments

2.1.6 Click OK

2.2 New Dialog box appears **Set Up Pump**. Adjust parameters to appropriate values for your chromatography

2.2.1 Flow-rate : 1 ml/min

2.2.2 Stop-time : 15 mins

2.2.3 Solvents : Methanol 97%:
Tetrahydrofuran (THF) 3%

2.2.4 Set Pressure limits : max = 400 bar, min = 0 bar

2.2.5 Click OK

2.3 New Dialog box appears **Set Up Injector**

2.3.1 Select Standard Injection

2.3.2 Select Injection Volume: 100 μL

2.3.3 Click OK

2.4 New Dialog box appears **DAD Signals**

2.4.1 In store A set λ to 450 nm for carotenoids

2.4.2 In store B set λ to 292 nm tocopherols

2.4.3 In store C set λ to 325 nm retinols

2.4.4 Press OK

2.4.5 Store: Select All

2.4.6 Set your nm range from min to max from 200 nm to 500 nm

2.4.7 Select step 2.0 nm

2.4.8 Set Threshold to 1.0 mAu

2.4.9 Select UV and Vis lamps

2.4.10 Set Peak Width: 0.1 min (2 s)

2.4.11 Ensure Autobalance is set for prerun and postrun

2.4.12 Set Slit at 4 nm

2.4.13 Time and Timetable doesn't need to be set

2.4.14 Click OK

2.5. New Dialog box appears **Column Thermostat Method**

2.5.1 Set at 25º C

2.5.2 Click OK

2.6 New Dialog box appears **Signal Details**

2.6.1 Ensure all signals of interest are selected and added to method (e.g.450nm, 325 nm, and 292 nm)

2.6.2 Click OK

2.7 New Dialog box appears **Edit Integration Events:**

Integration Events	**Value**
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical

Time	Integration Events	Value
Initial	Slope Sensitivity	1
Initial	Peak Width	0.04
Initial	Area Reject	1
Initial	Height Reject	1.7
Initial	Shoulders	OFF

2.7.1 Click OK

2.8 New Dialog box appears **Specify Report:**

2.8.1 Under Destination, Select Printer and File

2.8.2 Click OK

2.9 New Dialog box appears **Instrument Curves:**

2.9.1 Click OK (No instrument curves should be selected)

2.10 New Dialog box appears **Run Time Checklist:**

The following should be automatically selected:

2.10.1 Data Acquisition

2.10.2 Standard Data Analysis

2.10.3 Click OK

2.11 Click **Method** on top toolbar

2.11.1 Click Save Method

2.11.2 Add comment and click OK

APPENDIX 7.23: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PERFORMANCE CHECK

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
MPRG-Standard Operating Procedure(SOP)-003 **Issue 1**	**Issued by: Dr. John M Nolan** **Reviewed by: Dr. Brian Murphy** **Issue Date: 13/07/2007**

INSTRUMENT

HP Agilent Technologies 1200 High Performance Liquid Chromatography (HPLC)

MATERIALS

Mobile Phase: 97% methanol: 3% tetrahydrofuran (THF)

Column: 5 micron analytical/preparative, 4.6 x 250 mm 201TP Speciality Reverse Phase Column (Vydac), with in-line Guard Column

Standard Solutions: Lutein solutions – 0.1, 0.2, 0.3, 0.4, 0.5, & 0.6 µg/ml

Zeaxanthin solutions - 0.05, 0.1, 0.15, 0.2, 0.25, & 0.3 µg/ml

Combined standard solution - 0.3 µg/ml lutein & 0.15 µg/ml zeaxanthin

Blank standard solution – methanol

Standard Reference Material: National Institute of Standards and Technology (NIST) Standard Reference Material 968c for Fat-Soluble Vitamins, Carotenoids and Cholesterol in Human Serum

Miscellaneous: 5 ml graduated cylinder, stop watch, various glassware

A. INSTRUMENT OPERATIONAL CHECK

Frequency: Quarterly

Objective: To examine the operational performance of the HP 1200 HPLC system

Task: Perform the following instrument checks and record the results in the 'Operational Performance Check' Logbook

1. Pump Performance

I. Manual Flow Rate Check

- Manually check the following flow rates using a 5 ml graduated cylinder and stop watch

  0.5 ml/min x 2

  1 ml/min x 2

  1.5 ml/min x 2

  2 ml/min x 2

II. Pressure Stability Check

- Set the flow rate to 2 ml/min
- Record instrument pressure every 10 seconds for 2 minutes

2. Injector - Injection Volume Check

- Standard solution - 0.3 µg/ml lutein

50 µL	x 2
100 µL	x 2
150 µL	x 2
200 µL	x 2
250 µL	x 2

- Check the linearity of Peak Area
- Please note that this is NOT an Accuracy Check

3. Quick Auto-Sampler Check

- Place filled vials (methanol) in locations 1 – 20
- Make injections from 5 random vials
- Check that septa on correct vials are pierced

4. Detector - Wavelength Check

- Standard Solution - 0.3 µg/ml lutein
- 3 injections
- Examine UV scan of lutein peak using Agilent software – Record and compare λ_{max}

B. METHOD VALIDATION CHECK

Frequency: Quarterly

Objective: To check the validity of the instrument method developed to separate and quantify serum lutein and zeaxanthin using the HP 1200 HPLC System

Validation: Validation is the procedure which proves that a method yields results with reliability, precession, linearity, sensitivity, selectivity and accuracy

1. Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose 'true value' is known) is analysed and the measured value should match the true value.

- Using the NIST Standard Reference Material 968c for Fat-Soluble Vitamins, Carotenoids and Cholesterol in Human Serum, extract lutein and zeaxanthin from above NIST standard using the assay designed for this method (MPRG-SOP-002)
- Specification: The allowed ranges for NIST serum lutein and zeaxanthin are as follows:

Lutein:	0.049 - 0.065 µg/ml
Zeaxanthin:	0.017 - 0.035 µg/ml

2. Linearity Plot (Standard Curve)

- Plot a graph of Peak Area *versus* Analyte Concentration for lutein at six different concentrations: 0.1, 0.2, 0.3, 0.4, 0.5, & 0.6 µg/ml (2 injections for each solution)

- Plot a graph of Peak Area *versus* Analyte Concentration for zeaxanthin at six different concentrations: 0.05, 0.1, 0.15, 0.2, 0.25, & 0.30 µg/ml (2 injections for each solution)
- Specification: $R^2 = 1 \pm 0.002$

3. Selectivity

Selectivity is the ability to find and quantify the compound of interest in the presence of other compounds. For chromatographic methods, this means that the analyte of interest can be separated with sufficient resolution from all other peaks.

- Run a combined standard solution (0.3 µg/ml lutein & 0.15 µg/ml zeaxanthin) on the HP 1200 Liquid Chromatography system 5 times
- Specification: The average resolution between these two analytes (calculated using the ChemStation Software) shall be ≥ 2

4. Precision

Precision is defined as the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The relative standard deviation (RSD) is a measure of precision, calculated by dividing the standard deviation (SD) for a series of measurements by the average measurement:

$$RSD\ (\%) = 100\ SD/Average$$

- Inject lutein standard solution (0.3 µg/ml) five times and calculate %RSD
- Inject zeaxanthin standard solution (0.15 µg/ml) five times and calculate %RSD
- Specification: %RSD $\leq$ 2% for both analytes

5. Sensitivity

Sensitivity is the ability to analyse samples with low content. The *Limit of Detection* (LOD) can be defined as the smallest level of analyte that gives a measurable response. The *Limit of Quantitation* (LOQ) can be defined as the smallest concentration of analyte which gives a response that can be accurately quantified.

- LOD: 3 times the baseline noise level
- LOQ: 10 times the baseline noise level
- Run a blank solution (i.e. methanol) and calculate the baseline noise level (height)
- Based on the peak height for standard solutions (lutein = 0.3 µg/ml; zeaxanthin = 0.15 µg/ml) calculate LOD and LOQ for both lutein and zeaxanthin

APPENDIX 7.24: MEASURMENT OF RETINOLS, TOCOPHEROLS AND CAROTENOIDS IN SERUM AND STANDARD(S) PREPARATION FOR ROUTINE ANALYSIS PROTOCOL

Macular Pigment Research Group (MPRG) **Department of Chemical & Life Sciences** **Waterford Institute of Technology**	
MPRG-Standard Operating Procedure(SOP)-004 **Issue 2**	**Issued by: Dr. John M Nolan** **Reviewed by: Dr. Brian Murphy** **Issue Date: 04/02/2008**

INSTRUMENT

HP 1200 Series High Performance Liquid Chromatography (HPLC) system, PC, Software (Agilent Chem Station for LC System)

MATERIALS

Mobile Phase: 97% methanol: 3% tetrahydrofuran (THF)

Column: 5 micron analytical/preparative, 4.6 x 250 mm 201TP Speciality Reverse Phase Column (Vydac), with in-line Guard Column

Standard Solutions: Lutein – 0.1, 0.2, 0.3, 0.4, 0.5, & 0.6 µg/ml

Zeaxanthin - 0.05, 0.1, 0.15, 0.2, 0.25, & 0.3 µg/ml

α-tocopherol – 5, 10, 15, 20, & 25 µg/ml

Blank Standard Solution – methanol

Chemicals: Ethanol, methanol, heptane, α-tocopherol acetate, butylated hydroxytoluene (BHT)

Sample: 1 ml of serum sample(s) to be tested

Miscellaneous: Analytical balance, 50 ml and 1000 ml graduated cylinders, 10 ml, 50 ml & 100 ml volumetric flasks, various other glassware, micro pipettes (0 to 200 µL & 0 to 1000 µL)

Note – HPLC grade solvents are used throughout analysis

A. MOBILE PHASE PREPARATION [97% METHANOL: 3% THF

1. 30 ml of THF is added to 970 ml of methanol and mixed thoroughly
2. The mobile phase is automatically degassed by the HP 1200 Series system

B. SAMPLE PREPARATION

- Every sample is analysed **in duplicate**

1. A 400 µL aliquot of serum is pipetted into a labelled clear microcentrifuge tube [W] (1.5 ml total capacity)
2. 200 µL of internal standard (α-tocopherol acetate in ethanol @ 25 µg/ml) is added to each tube [W]
3. Ethanol (300 µL) containing 25 µg/ml BHT is added to each tube
4. Heptane (500 µL) is added to each tube [W]
5. Samples are vortexed vigorously for 1 min and then centrifuged at 2000 rpm for 5 min (MSC Micro Centaur, Davison & Hardy Ltd., Belfast, UK)
6. The resulting upper heptane layer is retained (400 µL with automated pipette) and transferred to a labelled amber (light sensitive) microcentrifuge tube [X]
7. Steps 4 – 6 are repeated (i.e. a second heptane extraction is performed; 400 µL of the upper heptane layer is retained)

8. The combined heptane layers are evaporated to dryness under a stream of nitrogen
9. These dried samples may either be assayed immediately, or can be stored for short periods of time (up to 6 months) at minus 70º C
10. When ready to run samples for HPLC analysis, the dried sample(s) are reconstituted in 200 µL of methanol and BHT and gently vortexed for 1 min
11. Tube X is allowed to stand for 1 min following vortexing
12. The contents of tube X are transferred into a designated HPLC vial insert [Y] and placed into a light sensitive HPLC vial [Z]

C. LUTEIN, ZEAXANTHIN AND α-TOCOPHEROL ACETATE STANDARD PREPARATION

- **Lutein standard preparation in methanol containing BHT (25 µg/ml)**

1. Lutein stock solution **SS** is prepared as follows (and stored at minus 70º C): 1 mg of lutein powder is accurately weighed (using appropriate glassware and analytical balance), transferred to a light sensitive 100 ml volumetric flask and made up to just below the mark with methanol containing BHT. The solution is mixed and sonicated and then made up to the mark with methanol containing BHT. Oxygen is displaced from the storage bottle using a stream of nitrogen. The lutein powder is immediately placed back in the freezer after weighing out.

*Lutein **SS** = 10 µg/ml*

2. Lutein working standard **WS** solution is prepared as follows: 1 ml of SS is made up to 10 ml with methanol and BHT using appropriate glassware and pipette and transferred to a light sensitive storage bottle. Oxygen is displaced using a stream of nitrogen.

*Lutein **WS** = 1 µg/ml*

3. Serial dilutions are performed on the **WS** to provide the desired concentration range (0.1, 0.2, 0.3, 0.4, 0.5, & 0.6 µg/ml) as follows: The dilutions are carried out in amber (light sensitive) microcentrifuge tubes before being transferred to light sensitive HPLC vials.

    - STD 1 – 0.6 µg/ml = 600 µL WS + 400 µL methanol and BHT
    - STD 2 – 0.5 µg/ml = 500 µL WS + 500 µL methanol and BHT
    - STD 3 – 0.4 µg/ml = 400 µL WS + 600 µL methanol and BHT
    - STD 4 – 0.3 µg/ml = 300 µL WS + 700 µL methanol and BHT
    - STD 5 – 0.2 µg/ml = 200 µL WS + 800 µL methanol and BHT
    - STD 6 – 0.1 µg/ml = 100 µL WS + 900 µL methanol and BHT

Note: Before sealing vials oxygen is displaced using nitrogen

- **Zeaxanthin standard preparation in methanol**

1. Zeaxanthin **SS** is prepared as follows (and stored at minus 70º C): 1 mg of zeaxanthin powder is accurately weighed (using appropriate glassware and analytical balance), transferred to a light sensitive 100 ml volumetric flask and made up to just below the mark with methanol and BHT, 10 drops of THF (~ 5 mls) is added before sonication. After sonication the solution is then made up to the mark. Solution is then placed under a stream of nitrogen before storing in the freezer in light sensitive glassware. Zeaxanthin powder is immediately placed back in the freezer.

    *Zeaxanthin **SS** = 10 µg/ml*

2. Zeaxanthin **WS** solution is prepared as follows: 1 ml of SS is made up to 10ml with methanol (using appropriate glassware and pipette).

    *Zeaxanthin **WS** = 1 µg/ml*

3. Serial dilutions are performed on the **WS** to provide the desired concentration range (0.05, 0.1, 0.15, 0.2, 0.25, and 0.30 µg/ml) as follows:

    - STD 1 – 0.30 µg/ml = 300 µL WS + 700 µL methanol and BHT
    - STD 2 – 0.25 µg/ml = 250 µL WS + 750 µL methanol and BHT
    - STD 3 – 0.20 µg/ml = 200 µL WS + 800 µL methanol and BHT
    - STD 4 – 0.15 µg/ml = 150 µL WS + 850 µL methanol and BHT
    - STD 5 – 0.10 µg/ml = 100 µL WS + 900 µL methanol and BHT
    - STD 6 – 0.05 µg/ml = 50 µL WS + 950 µL methanol and BHT

- **α-tocopherol acetate standard preparation in methanol**

1. α-tocopherol acetate **SS** is prepared as follows (and stored at 4º C): approximately 25 mg of α-tocopherol acetate is weighed out and is made up to 100 ml with methanol (using appropriate glassware). Please note that α-tocopherol acetate is a very viscous material.

*α-tocopherol acetate **SS** = 250 µg/ml*

2. α-tocopherol **WS** solution is prepared as follows: 10 ml of **SS** is made up to 100 ml with methanol (using appropriate light sensitive glassware and pipette).

*α-tocopherol acetate **WS** = 25 µg/ml*

Note: α-tocopherol acetate is made up in methanol for injection onto the HPLC in system suitability, and made up in ethanol for use in samples.

- **α-tocopherol standard preparation in methanol**

1. α-tocopherol SS is prepared as follows (and stored at 4º C): 100 mg of α-tocopherol is weighed out accurately and made up to 100 ml with methanol (using appropriate glassware).

*α-tocopherol **SS** = 100 µg/ml*

2. α-tocopherol WS is prepared as follows: 1 ml of SS is made up to 10 ml with methanol (using appropriate glassware and pipette). Solution is then placed in a light sensitive storage bottle.

*α-tocopherol **WS** = 10 µg/ml*

3. Serial dilutions are performed on the **WS** to provide the desired concentration range (5, 10, 15, 20, and 25 µg/ml) as follows:

- STD 1 – 5 µg/ml = 50 µL WS + 950 µL methanol
- STD 2 – 10 µg/ml = 100 µL WS + 900 µL methanol
- STD 3 – 15 µg/ml = 150 µL WS + 850 µL methanol
- STD 4 – 20 µg/ml = 200 µL WS + 800 µL methanol
- STD 5 – 25 µg/ml = 250 µL WS + 750 µL methanol

SYSTEM SUITABILITY

- System suitability checks will be performed for every HPLC run.

1. As part of every HPLC run lutein, zeaxanthin, α- tocopherol, α- tocopherol acetate standards, and a control sample are assayed for system suitability testing as follows:

- Before run – lutein 0.3 µg/ml x 3 injections, zeaxanthin 0.15 µg/ml x 1 injection, α- tocopherol 15 µg/ml x 1 injection, α- tocopherol acetate 25 µg/ml x 1 injection, and control sample x 1 injection
- After run – lutein 0.3 µg/ml x 3 injections, zeaxanthin 0.15 µg/ml x 1 injection, α- tocopherol 15 µg/ml x 1 injection, and α- tocopherol acetate 25 µg/ml x 1 injection (same samples as before the run)

2. System suitability calculations:

Peak Area

- Before run – lutein 0.3 µg/ml x 3 injections % RSD ≤ 2%
- After run – lutein 0.3 µg/ml x 3 injections % RSD ≤ 2%
- Before and after run for above (i.e. 6 injections for each standard) % RSD ≤ 2%

Tailing Factor

- Average of 3 injections before run (for each standard)
- Average of 3 injections after run (for each standard)
- Changes over time are monitored

Column Efficiency (number of theoretical plates)

- Average of 3 injections before run (for each standard)
- Average of 3 injections after run (for each standard)
- For expected values see column certificate

HPLC RUN

- System Suitability – Start (lutein 0.3 µg/ml x 3 injections)
- Zeaxanthin 0.15 µg/ml x 1 injection
- α-tocopherol 15 µg/ml x 1 injection

- Internal standard (α-tocopherol acetate) 25 µg/ml x 1 injection
- Control sample
- Samples
- System suitability (lutein 0.3 µg/ml x 3 injections)
- Zeaxanthin 0.15 µg/ml x 1 injection
- α-tocopherol 15 µg/ml x 1 injection
- Internal standard (α-tocopherol acetate) 25 µg/ml x 1 injection
- Control sample
- Shutdown