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# Macular Pigment: Practical Implications for Optometric Practice in Preventative Health Care and Visual Performance Enhancement

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# **Macular Pigment: Practical Implications for Optometric Practice in Preventative Health Care and Visual Performance Enhancement**

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Submitted in partial fulfillment of the requirements for the degree of  
Masters of Philosophy.

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Dublin Institute of Technology- School of Physics

Submitted: April 2011

## **ABSTRACT**

### **Background/aims**

The macula is a specialised part of the retina responsible for detailed central and colour vision. The carotenoids, lutein, zeaxanthin and *meso*-zeaxanthin are uniquely concentrated in the inner and central layers of the primate macula, where they are known as macular pigment (MP). It has been shown that MP is entirely of dietary origin and that lutein and zeaxanthin levels in serum, diet and retina correlate. Age-Related Macular Degeneration (AMD) is a disease of the macula and results in loss of central vision. MP, because of its optical filtration and antioxidant properties, may have an important role in the prevention or delay of AMD, and also in the enhancement and preservation of visual performance in healthy individuals.

### **Objectives:**

To assess whether macular pigment optical density (MPOD) is associated with visual performance by attenuating short wavelength light. To modify the McCance and Widdowsons nutritional database to include nutritional data for lutein and zeaxanthin and using this database, assess the accuracy of two self-administered food frequency questionnaires (FFQ's), to estimate dietary lutein and zeaxanthin intake in an Irish population. To compare two heterochromatic flicker photometry (HFP) methods for measuring MPOD and evaluate the suitability of one of such devices, the MPS 9000, for use in clinical practice.

## **Methods**

The spatial profile of macular pigment was measured by customised HFP and values correlated with visual performance psychophysical tests, such as best corrected visual acuity (BCVA), mesopic, photopic contrast sensitivity and glare sensitivity, on 51 healthy subjects. In a separate study, dietary intake of lutein and zeaxanthin was assessed by two different FFQ's and this data was then analysed using two different lutein and zeaxanthin databases, on 22 healthy subjects. The validity of the questionnaires and nutrient databases are determined using biomarkers; serum lutein and zeaxanthin and MPOD. Finally, two flicker photometers, the MPS 9000 and the Densitometer<sup>TM</sup> were compared by measuring MP on 89 healthy subjects. Instrument repeatability was also assessed by taking three MPOD measurements on each instrument for 50 subjects.

## **Results**

We report a positive and statistically significant relationship between BCVA and MPOD at 0.25° and 0.5° retinal eccentricity ( $r = 0.345$ ,  $p = 0.013$ ,  $r = 0.317$ ,  $p = 0.024$ , respectively). When dietary intake of lutein and zeaxanthin was assessed using the Scottish Collaborative Group (SCG) FFQ and modified Italian FFQ, differences of up to 4.88mg/day indicated a poor level of agreement. A strong positive correlation was found between the MPS 9000 and the Densitometer ( $r=0.68$ ,  $p<0.001$ ), however Bland Altman analysis indicated poor agreement between instruments.

## **Conclusion**

Although measures of central visual function, such as visual acuity, are positively associated with MPOD, a longitudinal, placebo-controlled and randomised supplementation

trial would be required to ascertain whether augmentation of MP can influence visual performance. A lack of agreement between FFQ assessment tools and nutrient data bases, highlights the limitations and difficulties inherent in dietary assessment of lutein and zeaxanthin. Finally, underestimation of MP readings by the MPS 9000 may pose some concern for practitioners in clinical practice with regard to advise on preventative health care and visual performance enhancement and/or preservation.

**Keywords:** age-related macular degeneration; heterochromatic flicker photometry; macular pigment; mesopic; photopic; visual acuity.

## **Declaration**

I certify that this thesis which I now submit for examination for the award of Masters of Philosophy, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for post graduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institution or University.

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Signature

Date

Grainne Scanlon

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## **Abbreviations List**

Age Related Maculopathy (ARM)

Age Related Macular Degeneration (AMD)

Age Related Eye Disease Study (AREDS)

Autofluorescence Imaging (AFI)

Basal metabolic rate (BMR)

Best Corrected Visual Acuity (BCVA)

Body Mass Index (BMI)

Butylated hydroxytoluene (BHT)

Cardio Vascular Disease (CVD)

Carotenoids and Co-Antioxidants in Age-Related Maculopathy (CARMA)

Cathode Ray Tube (CRT)

Choroidal Neovascularisation (CNV)

Chromatic Aberration (CA)

Collaborative Optical Macular Pigment Study (COMPASS)

Contrast Sensitivity Function (CSF)

Corrected Distance Visual Acuity (CDVA)

Critical Flicker Frequency (CFF)

Customised Short Wavelength Automated Perimetry (cSWAP)

Cycles per degree (cpd).

Daily Recommended Intake (DRI)

Decibels (dB)

Dublin Institute of Technology (DIT)

Food Frequency Questionnaire (FFQ)



Functional Acuity Contrast Test (FACT)

Eye Disease Case Control Study group (EDCCS)

Geographic Atrophy (GA)

Heterochromatic Flicker Photometry (HFP)

High Density Lipoprotein (HDL)

High Performance Liquid Chromatography (HPLC)

Inter Photoreceptor Matrix (IPM)

Intra Ocular Lens (IOL)

Interquartile Range (IQR)

Light Emitting Diodes (LEDs)

Lutein Antioxidant Supplementation Trial (LAST)

Macular Automated Photostress (MAP)

Macular Pigment (MP)

Macular Pigment Optical Density (MPOD)

Medical Research Council (MRC)

National Health & Nutritional Examination Survey (NHANES)

N-retinylidene-Nretinylethanolamine (A2-E)

Optimal Flicker Frequency (OFF)

Partial Error Scores (PES)

Pearson's correlation coefficient (Pearson's r)

Photostress Recovery Time (PRT)

Polyunsaturated Fatty Acids (PUFA's)

Questionnaire Derived VA Index (VAI)

Recommended Daily Allowance (RDA)

Reactive Oxygen Species (ROS)

Resonance Raman Spectroscopy (RRS)

Retinal Pigment Epithelium (RPE)

Retinitis Pigmentosa (RP)

Revolutions per minute (rpm)

Scottish Collaborative Group Food Frequency Questionnaire (SCGFFQ)

Short Wavelength (SW)

Standard Deviation (SD)

Statistical Software Package (SPSS)

Total Error Scores (TES)

Total Lutein (TL)

Total Zeaxanthin (TZ)

United States Department of Agriculture (USDA)

Visual Function in Normals questionnaire 30 (VFNq 30)

Visual Acuity (VA)

## **Table of Contents**

<b>CHAPTER ONE</b>	<b>Page</b>
<b>Introduction</b>	23
1.1 Background	
1.1.1 Visual performance	24
1.1.2 Age-related macular degeneration (AMD)	24
1.2 Project Aims/Objectives	26
1.3 Expected Results	28
1.4 Research Benefits	28
<b>CHAPTER TWO</b>	
<b>Anatomy and Physiology of the Retina</b>	
2.1 The Retina	30
2.2 Retinal Pigment Epithelium	32
2.2.1 Function of the RPE	32
2.3 Photoreceptor Layer	34
2.3.1 Macular pigment	38
2.4 The Inner Neural Sensory Retina (layers 3 to 10)	39
2.4.1 The outer limiting membrane	39
2.4.2 The outer nuclear layer	39
2.4.3 The outer plexiform layer	39
2.4.4 The inner nuclear layer	39
2.4.5 The inner plexiform layer	40
2.4.6 Ganglion cell layer	40

2.4.7 The nerve fibre layer	41
2.4.8 The inner limiting layer	41
2.5 Blood Supply to the Retina	41
2.6 The Choroid	43
2.6.1 The vessel layer	43
2.6.2 The capillary layer or choriocapillaris	43
2.6.3 Bruch's membrane	43
2.7 The Macula	45
2.7.1 Specialisation of the macula	47
<b>CHAPTER THREE</b>	
<b>Macular Pigment</b>	<b>50</b>
3.1 Carotenoids	51
3.2 Stereochemistry of Macular Pigment	52
3.3 Location of Macular Pigment	54
3.4 Optical Density and Serum Levels of Macular Pigment	55
3.5 Function of Macular Pigment	58
3.6 Oxidative Stress and Reactive Oxygen Species	58
3.6.1 Reactive oxygen species	59
3.6.2 Antioxidant enzymes	60
3.6.3 Generation of reactive oxygen species in the retina	60
3.6.4 Retinal irradiation	61
3.6.5 Polyunsaturated fatty acids	61
3.6.6 Retinal chromophores	62

3.6.7 Lipofuscin	62
3.7 Protection of the Macula by Macular Pigment	64
3.8 The ‘Optical’ Hypothesis of Macular Pigment	66
3.8.1 The ‘acuity’ hypothesis	68
3.8.2 The ‘Visibility’ Hypothesis	73
3.8.3 Photophobia	74
3.8.4 Optical and Anatomic Properties of Macular Pigment	74
3.9 Visual Health Hypothesis	76
3.10 Measurement of Macular Pigment	78
3.10.1 Fundus reflectometry	78
3.10.2 Autofluorescence Imaging	79
3.10.3 Signal based techniques	79
3.10.4 Psychophysical technique	80
3.11 Dietary Assessment of Lutein and Zeaxanthin	81
3.11.1 Evidence that macular pigment can be augmented	82
3.11.2 Dietary factor affecting macular pigment absorption.	85
3.11.3 Recommended daily allowance	86
3.11.4 Dietary sources of lutein, zeaxanthin and <i>meso</i> -zeaxanthin	87

## **CHAPTER FOUR**

<b>Macular Pigment: Its Contribution to Visual Health of the Eye And Visual Performance and Comfort.</b>	92
4.1 Age-Related Macular Degeneration	92
4.1.1 Classification of AMD	93

4.1.2 Early stage AMD	94
4.1.3 Late dry AMD/Advanced form of non-exudative AMD/GA	94
4.1.4 Exudative AMD or wet AMD/CNV	95
4.1.5 Geographic atrophy	96
4.2 Circumstantial Evidence	98
4.2.1 Iris colour	98
4.2.2 Cigarette smoking	99
4.2.3 Female sex	100
4.2.4 Crystalline lens density	100
4.3 Epidemiological Evidence	101
4.4 Experimental Evidence	103
4.5 Clinical Evidence	106
4.6 Macular Pigment and Eye Disease Risk Factors	108
4.6.1 Cardiovascular disease/obesity	108
4.6.2 Sunlight exposure	109
4.6.3 Nutrition and macular pigment	110
4.7 Macular Pigment and its role in Visual Performance and Comfort	111
4.7.1 The Evidence	111
4.7.2 Studies in subjects with retinal pathology	112
4.7.3 Age related macular degeneration	113
4.7.4 Cataract	115
4.7.5 Studies in normal populations	116
4.7.6 Spatial vision	117
4.7.7 Colour Vision	119

4.8 Preservation of Youthful Vision into Old Age	121
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## **CHAPTER FIVE**

### **The Relationship between Macular Pigment and Visual Performance**

5.1 Abstract	125
5.2 Introduction	126
5.3 Materials and Methods	128
5.3.1 Subjects	128
5.3.2 Demographic, medical history, lifestyle and vision case history questionnaires	129
5.3.3 Spectacle refraction, visual acuity, and ocular dominance	129
5.3.4 Glare sensitivity	130
5.3.5 Visual function in normals questionnaire	131
5.3.6 Contrast sensitivity function	132
5.3.7 Photostress recovery	134
5.3.8 Macular pigment optical density	136
5.3.9 Farnsworth-Munsell 100-Hue test (FM100)	138
5.3.10 Customised short-wavelength automated perimetry (cSWAP)	138
5.3.11 Fundus photography	139
5.3.12 Statistical analysis	139
5.4 Results	139
5.4.1 Macular pigment optical density	141
5.4.2 Best corrected visual acuity and MPOD	143

5.4.3 Contrast sensitivity and MPOD	144
5.4.4 Colour vision and MPOD	147
5.4.5 Customised short wavelength automated perimetry	147
5.4.6 Photostress recovery time and MPOD	149
5.4.7 Photostress sensitivity reduction	149
5.4.8 Subjective glare assessment and MPOD	149
5.4.9 Validation of visual function questionnaire in normals (VFNg)	150
5.5 Discussion	152

## **CHAPTER SIX**

### **Validation of Two Food Frequency Questionnaires to Assess Dietary**

#### **Lutein and Zeaxanthin in Irish Adults**

6.1 Abstract	158
6.2 Introduction	160
6.3 Materials and Methods	163
6.3.1 Subjects	163
6.3.2 Study design	164
6.3.3 Demographic details questionnaire	166
6.3.4 Food frequency questionnaire	166
6.3.5 Scottish collaborative group (SCG) FFQ	166
6.3.6 Modified Italian FFQ	167
6.3.7 Macular pigment optical density measurement	168
6.3.8 Analysis of serum samples	168



6.3.9 Data input and coding	169
6.3.10 Modification of WISP	170
6.3.11 Data cleaning of the SCG FFQ	171
6.3.12 Statistical analysis	172
6.4 Results	173
6.4.1 Demographic data	173
6.4.2 Comparability of the two FFQ's	175
6.4.3 Correlation between both FFQs	175
6.4.4 Assessment of agreement	
between FFQs: Bland-Altman Plot	176
6.4.5 Comparability of the two nutrient databases	177
6.4.6 Correlation between the nutrient databases	178
6.4.7 Assessment of agreement	
between databases: Bland Altman Plot	178
6.4.8 Classification into quintiles of consumption	180
6.4.9 The relationship between	
dietary lutein and zeaxanthin and serum	180
6.4.10 The relationship between	
dietary lutein and zeaxanthin and MPOD	181
6.4.11 The relationship between	
serum lutein and zeaxanthin and MPOD	182
6.4.12 Analysis of sources of	
lutein and zeaxanthin in the Irish population	182
6.5 Discussion	183

## **CHAPTER SEVEN**

### **An Evaluation of a Novel Instrument for Measuring**

#### **Macular Pigment Optical Density: The MPS 9000**

7.1 Abstract	197
7.2 Introduction	198
7.3 Materials and Methods	201
7.3.1 MPS 9000	202
7.3.2 Densitometer	203
7.3.3 Statistical analysis	204
7.4 Results	205
7.4.1 Instrument Concordance	205
7.4.2 Test - Retest Repeatability	207
7.5 Discussion	209

## **CHAPTER EIGHT**

8.1 Discussion	219
8.2 Macular Pigment and its Role in Clinical Practice	219
8.2.1 AMD	219
8.2.2 Visual performance and comfort	222
8.3 Dietary Intake of Macular Pigment Carotenoids	224
8.3.1 Special precautions	224
8.4 Lutein, Zeaxanthin and meso-Zeaxanthin Supplementation	225
8.5 Measuring Macular Pigment Density in Practice	227

8.6 Macular Pigment Protocol for Clinical Practice	228
8.7 Future Research	230
8.8 Conclusion	231
<b>Bibliography</b>	233
<b>List of publications</b>	271
<b>Appendix</b>	272
A. Visual Health Supplement Formulations, Dosages, and Comparison with Recommended Intake Levels	
B. Estimated dietary requirement range based on optimal serum lutein and zeaxanthin concentration.	
C. Food sources of lutein and zeaxanthin	
D. SCG FFQ	
E. Modified Italian FFQ	
F. Consent form for Diet study	
G. Consent form HFP study	
H. CRF COMPASS	

## **Table of figures**

### **Chapter Two**

Figure. 2.1 Schematic drawing of the human eye (Tunnacliffe, 1993).

Figure. 2.2 a) Schematic drawing of human retina (Heath and Young 2000) and b) histological section of human retina (Freeman and Hull 2003).

Figure 2.3 Diagram showing the relationship between the photoreceptor cells and the RPE.

Figure 2.4 The structure of a rod cell

Figure 2.5 Absorption spectrum of rods and cones

Figure 2.6 Graph showing number of rods and cones per mm

Figure 2.7 Showing blood supply to the retina

Figure 2.8 Basement membrane, comprising the basal lamina and reticular lamina, in a diagram of a section through epithelial tissue.

Figure 2.9 (a) Schematic drawing of human foveola (Kanski and Milewski 2002).

(b) Histological section of human foveola (Heath and Young 2000).

Figure 2.10 An anatomical view of the macula region as viewed in cross section (adapted from Thibos *et al* 2000).

Figure 2.11 The central human fovea shown as graphics (Modified from Trieschmann et al. 2008).

### **Chapter Three**

Figure 3.1 The structure of the major components of Macular Pigment (Bone et al. 1993).

Figure 3.2 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). (Modified from Trieschmann et al. 2008).

Figure 3.3 Longitudinal chromatic aberration-focal length for incident white light varies across wavelength (Loughman et al. 2007).

Figure 3.4: Diagrammatic representation of the amount of blur induced by longitudinal CA when an eye is focused optimally for 555 nm. Note that the blue end is significantly more blurred than the red (Loughman et al. 2010 ).

## **Chapter Four**

Figure 4.1 Comparison of the gross appearance of the two major drusen phenotypes observed at the fundus level. “Hard” drusen (A) tend to be smaller with relatively distinct margins, whereas “soft” drusen (B) are larger and typically have less distinct borders (Modified from Hageman et al. 2001).

Figure 4.2: Disease severity scale with ARM 1, 2, 3 representing combinations of drusen and pigmentary irregularities, and 4 when CNV or GA is seen (Modified from Hogg & Chakravarthy, 2010).

Figure 4.3. The estimated 5-y probability of progression to advanced AMD was reduced (AREDS)

Figure 4.4. Morphological progression in study eyes (Chakravarthy et al. 2009).

Figure 4.5. The mean (95% CI) differential change in BCVA in study eyes between Treatment and Placebo Group. X axis is number of letters read. (Change is from baseline to each visit ) (Chakravarthy et al. 2009)

## **Chapter Five**

Figure. 5.1. Spectral Irradiance at 1 m fixation distance from Arri 300 photostress lamp.

Figure. 5.2 Densitometer; showing the foveal test field on the left and the parafoveal test field on the right (for illustration only, not drawn exactly to scale) (Modified from Loane et al. 2007).

Figure 5.3 A second-order decreasing exponential function fit the averaged subjects' profile data well.

Figure 5.4 The relationship between MPOD at 0.25° and BCVA

Figure 5.5 The relationship between MPOD at 0.25° and log contrast sensitivity at 5.7 cpd for mesopic conditions.

Figure 5.6 The relationship between MPOD at 0.25° and log contrast sensitivity at 5.7 cpd for photopic conditions

Figure 5.7 Glare subjective satisfaction score and MPOD at 1 degree of retinal eccentricity

Figure. 5.8 Relationship between BCVA and visual acuity assessed by questionnaire

## **Chapter Six**

Figure 6.1 Study Design

Figure.6.2(a) Correlation between lutein and zeaxanthin intakes assessed by the SCG FFQ and the modified Italian FFQ.

Figure.6.2(b) Differences between lutein and zeaxanthin intakes assessed by the SCG FFQ and the modified Italian FFQ plotted against the average of the two measurements (Bland Altman Plot)

Figure. 6.3(a) Correlation between lutein and zeaxanthin intake analysed using the WISP database and the MRC database

Figure. 6.3(b) Differences between lutein and zeaxanthin intakes analysed using the WISP and the MRC database plotted against the average of the two measurements (Bland-Altman Plot)

## **Chapter Seven**

Figure 7.1. Relationship between MPOD readings at 0.5° retinal eccentricity obtained with each instrument, with the line  $y = x$  superimposed.

Figure 7.2. Bland-Altman plot for MPOD values at 0.5° retinal eccentricity, showing 95% limits of agreement between the MPS 9000 and Densitometer

Figure 7.3. Bland Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the MPS 9000.

Figure 7.4. Bland Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the Densitometer.

## **Chapter Eight**

Figure 8.1 Life expectancy of men and women between 1950 and 2050.

Figure 8.2: Genetic and environmental influence on AMD development

## **List of tables**

Table 3.1 Lutein and zeaxanthin 6 mg/day exchanges.

Table 5.1: Parameters assessed and their sequence for a typical study visit

Table 5.2: Demographic, medical, lifestyle, anthropometric, and ocular related data for the entire study group.

Table 5.3: Mean and SD's of MPOD at all retinal eccentricities:

Table 5.4: The Pearson's correlation coefficient (Pearson's r) for MPOD at each eccentricity measured and log mesopic and photopic contrast thresholds at different spatial frequencies.

Table 5.5: cSWAP and MPOD

Table 6.1: Demographic characteristics of sample population (n=22)

Table 6.2. Correlation Matrix showing relationships between the study parameters for the entire study group

Table 7.1. Inter - sessional MPOD (mean  $\pm$  SD) and coefficient of repeatability for the MPS 9000 and Densitometer

Table 8.1 Visual Health Supplements



## CHAPTER ONE

### Introduction

#### 1.1 Background

The Yellow colouration of the macula lutea is attributable to the presence of MP in the centre of the retina (Snodderly et al. 1984). MP consists of the xanthophylls, lutein and zeaxanthin and the retinal metabolite of lutein: *meso*-zeaxanthin (Handleman et al. 1988; Bone et al. 1997; Johnson et al. 2005). Although the definitive role of MP remains uncertain, several functions have been hypothesised and these include: (1) reduction of the effects of light scatter and chromatic aberration (CA) on visual performance (Nussbaum et al. 1981; Reading & Weale, 1974); (2) non-optical enhancement of visual performance and comfort due to ocular, retinal and cortical health benefits; (Craft et al. 2004; Carboni et al. 2010) (3) limitation of the damaging photo-oxidative effects of short wave (SW) light through selective absorption (Bone & Landrum, 1984); (4) protection against the adverse effects of photochemical reactions because of the antioxidant properties of these carotenoids (Snodderly, 1995).

The selective accumulation of only three specific dietary carotenoids at the macula, suggests an exquisite biological selectivity for lutein, zeaxanthin and *meso*-zeaxanthin at the site of best vision in the human retina, and indicates a role for these carotenoids which is uniquely suited to this anatomic location. Lutein, zeaxanthin and *meso*-zeaxanthin are located at the macula to the exclusion of all other carotenoids in nature. Given that Darwinian natural selection confers advantage before and until the period of procreation, it follows that the biological selectivity of MP's accumulation in the retina primarily

represents an advantage in youth and middle age. The anatomic location, optical and antioxidant properties of MP have thus prompted two distinct lines of research, which seek to explore the definitive role of MP in vision and visual health.

### **1.1.1 Visual performance**

The ‘optical’ hypothesis posits that MP could improve visual resolution by absorbing SW light, which is easily scattered and poorly focussed. The yellow colouration of MP is such that it selectively absorbs blue green incident light with maximum absorption circa 460 nm, and with little or no absorption above 530 nm. Importantly given that peak retinal sensitivity is at 555 nm and the proportion of blue SW sensitive cones in the central macula is far lower than that of red and green (long and medium sensitive) cones, it is logical to suggest that the ‘optical’ properties of MP are such that it attenuates the component of light that is least beneficial and potentially destructive (given that the effects of both light scatter and longitudinal chromatic aberration (CA) are non-linear and SW dominated to visual performance and experience (Bone et al. 1992).

### **1.1.2 Age-related macular degeneration**

Although it is likely that the primary role of MP involves its contribution to visual performance, an alternative to this hypothesis is that MP protects the retina against light damage and as a powerful antioxidant protects the eye against age-related maculopathy (ARM) and AMD. AMD has received considerable attention from MP researchers, as it is the leading cause of visual loss in people over the age of 65 years in the Western world (Leibowitz et al. 1980). Although the aetiopathogenesis of AMD remains a matter of debate, there is a growing body of evidence to indicate that oxidative damage (Winkler et

al. 1999; Beatty et al. 2000), and associated inflammation (Hollyfield et al. 2010) play a role. Consequently, the possibility that the absorption characteristics and antioxidant properties of MP confer protection against AMD has been postulated (Snodderly, 1995; Landrum et al. 1997).

The fact that MP is modifiable means that ongoing research into its role in prevention or progression of AMD, and optimisation of visual health is important. As we can see the function of MP may be to improve visual performance during life, as it acts as a SW light filter, and is a powerful antioxidant. Whether improved (and preserved into old age) visual performance is primarily due to its filtration effects or its antioxidant ability is still unknown. The long-term benefit of these effects however is that MP may improve the visual health of the eye, and may therefore retard the development of AMD, which is a late onset disorder. The ‘protective’ and ‘visual performance’ hypotheses of MP are, therefore, not mutually exclusive.

## **1.2 Project Aims/Objectives**

The aim of this thesis is to provide an overview of the relevance of MP to eyecare practitioners, including its role in protecting the eye against age-related eye conditions such as AMD, and its role in visual performance. Given the inherent variability in the optical density of MP in population studies, and given the potential importance of this pigment for preventative eyecare systems, the current study sought to further explore the variety of methods available for measuring MP, including serum levels of lutein and zeaxanthin, dietary intake using FFQ’s and clinical devices for the measurement of the optical density

of MP in the eye. Three separate studies were carried out, and will be explored in this thesis;

- a) The first section of this study was designed to identify and investigate relationships, if any, between MPOD and visual performance using a battery of techniques. A number of recent studies have reported positive and statistically significant associations between MP and several parameters of visual performance including visual comfort (Stringham et al. 2003), photophobia (Wenzel et al. 2006), veiling glare (Stringham & Hammond, 2007) and photostress recovery (Stringham & Hammond, 2007; Stringham & Hammond, 2008). The ‘optical’ hypothesis of MP was originally discussed by Reading & Weale (1974) and later by Nussbaum et al. (1981) and includes MP’s putative ability to enhance visual performance and/or comfort by attenuation of the effects of CA and light scatter via its light filtering properties (Walls & Judd, 1933). In this study we investigated the relationship between MPOD at various degrees of retinal eccentricity and clinically important parameters of central visual performance including BCVA, contrast sensitivity, glare sensitivity, subjective (questionnaire derived) visual function, hue discrimination, customised short-wavelength automated perimetry (cSWAP) and photostress recovery.
- b) Although MP’s absorptive and transport characteristics have yet to be fully elucidated it has been shown that MPOD can be augmented through dietary modification. Another aim of this study was to assess dietary intake of lutein and zeaxanthin in a small sample population, using two different FFQs, and to analyse this data using two different nutrient databases. The validity of the dietary assessment tools and nutrient databases was subsequently assessed using nutrient

biomarkers; blood serum and MPOD. Valid measurements of retrospective lutein and zeaxanthin intake, as well as ability to track lutein and zeaxanthin intake longitudinally, are crucial components for elucidating the role of lutein and zeaxanthin in health and disease.

- c) Of the antioxidants found in the human retina, only the macular carotenoids can be quantified non-invasively. MPOD determinations, as opposed to blood serum may be more precise and indicative of long term ocular nutrient status (Snodderly et al. 2004). In the absence of effective treatment strategies for non-neovascular AMD, interest has focused on prevention and/or retardation of progression, therefore, the ability to measure MP in-vivo could prove valuable in determining, the possible risk of developing AMD and in monitoring treatment. A reliable clinical method to measure and quantify MPOD, and changes in MPOD in response to dietary fortification and/or supplementation, would afford optometrists the capacity to detect low MP, and therefore monitor response to treatment protocol, in order to reduce the risk of AMD and/or improve visual performance. The MPS 9000 is a new commercial instrument for measuring MP, and it also employs the HFP technique. It is a portable device, designed to be minimally demanding for both patient and practitioner, enabling clinicians to quickly measure MP. While based on the same optical principles of HFP, significant design and methodological differences do exist. The current study, therefore, aims to assess the accuracy and repeatability of the MPS 9000 in relation to the gold standard, the Densitometer.

### **1.3 Expected Results**

It is important that optometrists and primary eyecare practitioners are aware of the functional role of MP. There is evidence to support the view that MP plays a role in preventing or retarding the progression of AMD (Bone et al. 1997; Age Related Eye Disease Study (AREDS), report no 8, 2001; Johnson et al. 2005). AMD is a late onset disorder and changes may be occurring in the eye decades before any visual signs or symptoms of the disease. MP is believed to be associated with reduced risk of development and progression of AMD and can be augmented, not only by eating food rich in these carotenoids, such as spinach, but also by dietary fortification with one of the many commercially available food supplements (Bone et al. 2003; Murray & Carden, 2008). A large part of this thesis will focus on the relevance of MP to primary eyecare practice, including the prevention of debilitating eye conditions such as AMD, and the optimisation and preservation of visual performance at youthful levels in an era of increasing life expectancy and increasing aged population. The current series of investigations aim to elucidate the nature of any expected visual performance benefits that might be associated with higher MPOD, the value (if any) of current means of assessment of dietary intake of MP carotenoids, and the validity of a novel MPOD measurement device currently marketed to primary eyecare practitioners.

### **1.4 Research Benefits**

Optometrists and primary eyecare practitioners remain surprisingly ignorant about MP's potential role, both in relation to visual symptoms such as glare and photophobia, and in long term preventative eyecare practice. Increased awareness, coupled with coherent and structured implementation of clinical practice policies and guidelines may confer long-term

visual health benefits, which may translate as improved visual comfort during life, and also as a reduced risk of developing AMD in later years. At a community level, screening for lutein, zeaxanthin and MP levels would allow the identification and establishment of appropriate dietary requirements, both in the lower (risk factor) and the upper levels (preventive factor), and as a result it would be possible to determine the adequacy and efficacy of dietary intake, or nutritional interventions, with clinical impact on disease prevention and visual improvement.

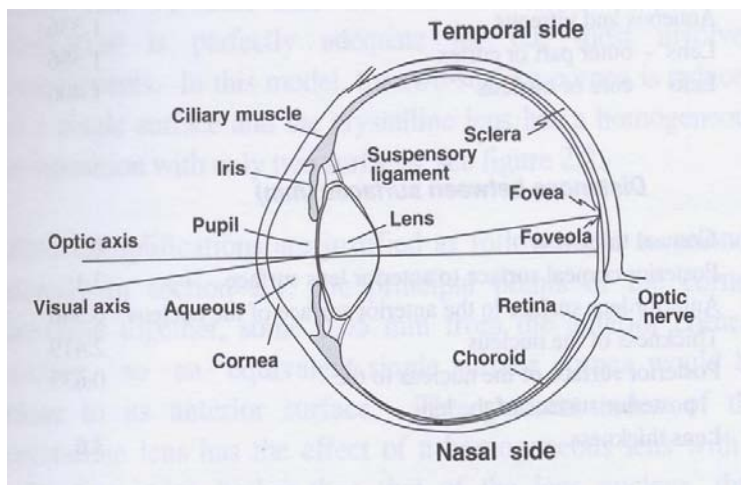
Using the information gathered from these studies, it may be possible to develop a clinical guide for optometrists, explaining the practical implications of MP and its constituent carotenoids, for optometric practice in preventative health care and visual performance enhancement, and outlining the most appropriate means of quantifying and assessing MP and MP carotenoid consumption in a clinical population.

## CHAPTER TWO

### Anatomy and Physiology of the Retina

#### 2.1 The Retina

The retina is the light sensitive layer which extends over the inner surface of the back of the eyeball, lying in contact with the vitreous internally and with the vascular layer, the choroid, externally.

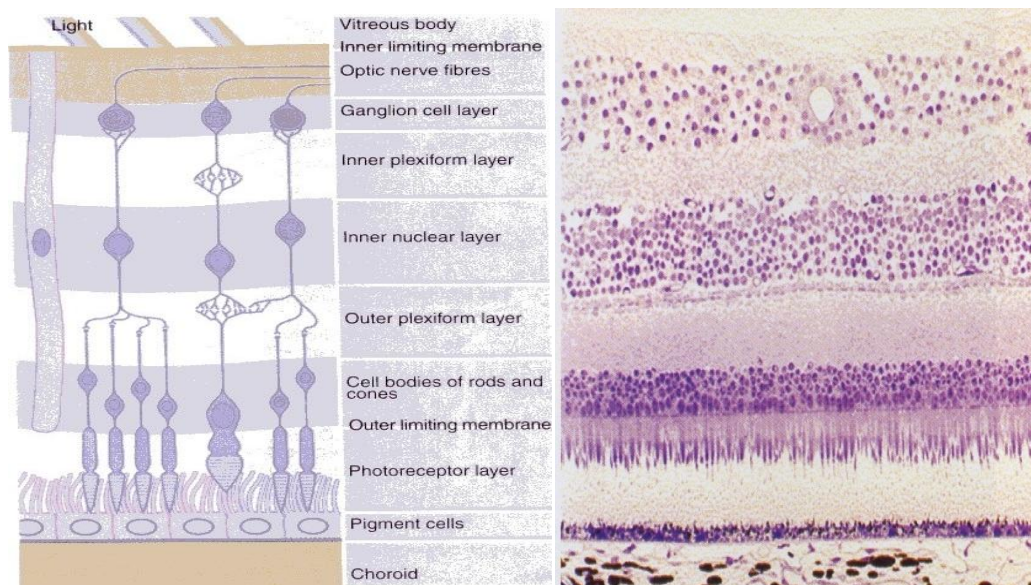


**Figure. 2.1 Schematic drawing of the human eye (Tunnacliffe, 1993).**

The gross anatomy of the retina is divided into the outer pigmented epithelium layer and the inner neural sensory layers. These layers form a functional unit essential for vision. The neural components of the eye are an extension of the forebrain, and thus form part of the central nervous system. The retina is approximately 310 microns with significant geographical and inter-subject variation (Yang & Du, 1999). Retinal thickness varies from 0.56 mm near the optic disc to 0.1 mm at the ora serrata. It is thinnest at the centre of the fovea. The structure of the retina consists of ten layers (Figure. 2.2) which include:



1. The Retinal Pigment Epithelium (RPE)
2. Photoreceptors-rods and cones
3. Outer limiting layer
4. Outer nuclear layer-rod and cone nuclei
5. Outer plexiform or synaptic layer
6. Inner nuclear layer-bipolar, horizontal and amacrine nuclei.
7. Inner plexiform or synaptic layer
8. Ganglion cell layer
9. Optic nerve fibres-ganglion cell axons
10. Inner limiting membrane



**Figure. 2.2 a) Schematic drawing of human retina (Heath and Young 2000) and b) histological section of human retina (Freeman and Hull 2003).**

## **2.2 Retinal Pigment Epithelium**

Morphologically RPE cells form a highly polarised epithelial sheet that separates the choroid from the retina. It is a single layer of cells that act as a major barrier between the leaky blood vessels of the underlying choroid and the neural retina. The epithelial cells are bound together by junctional complexes with tight junctions that separate the cells into an apical half that faces the retina and a basal half that faces the choroid. The basal end of each cell is much infolded and rests on the basement membrane which forms part of Bruch's membrane. The apical end of the cells have multiple microvilli and these project between and surround the outer segments of the rods and cones. Some cells in the body are capable of ongoing replication, while others such as those of the RPE have very limited ability to divide before reaching cellular senescence (Gao & Hollyfield, 1992; Marshall, 1987; Boulton, 1991). The RPE cells are for the most part non-mitotic cells and the epithelium is currently believed to consist of a stable, non-dividing pool of cells (Tso & Friedman, 1967).

### **2.2.1 Function of the Retinal Pigment Epithelium**

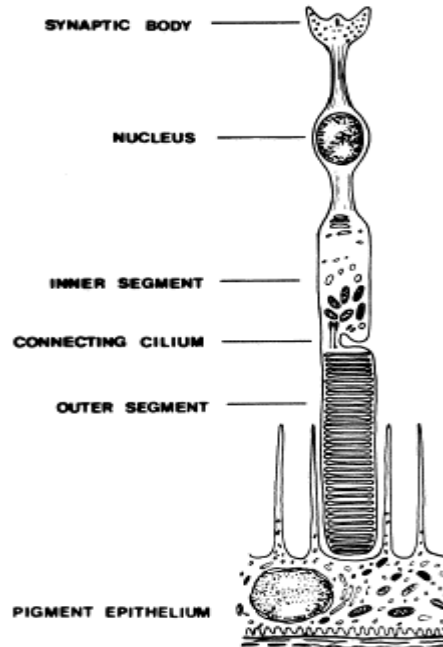
The RPE performs highly specialised unique functions essential for homeostasis of the neural retina. The RPE cells provide the principal mechanism for transfer of nutrients between the choriocapillaris and the photoreceptors, which is essential for maintaining normal photoreceptor cell function and survival. The RPE cells are arranged in a regular hexagonal pattern and form a barrier that limits the flow of ions and prevents diffusion of large toxic molecules from the choroid capillaries to the photoreceptors. The RPE acts in the absorption of light passing through the retina and phagocytosis of spent outer segment discs (Young & Bok, 1969). RPE cells are also the principal storage depot for vitamin A in

the eye and these cells supply the essential visual cycle intermediate, 11-cis retinal, to the photoreceptors for the regeneration of rhodopsin (Berman, 1994).

The RPE is an integral part of the visual cycle. The photoreceptor outer segments must undergo continuous turnover in order for normal photoreceptor cell function and survival to be maintained (Herron et al. 1969). Rhodopsin, phospholipids and other components of the disc membranes are transported to the base of the outer segments for assembly into new discs. These components are then displaced distally along the length of the rod outer segment, and finally small packets of terminal discs are shed. The outer segment tips shed in this process are rapidly phagocytosed by the pigment epithelium. Degradation of these ingested outer segments is achieved through a highly developed phagolysosomal system first described in human RPE by Feeney (Feeney, 1973).

The continuous phagocytic load imposed upon RPE cells through life leads to a striking age-related accumulation, from about aged 40, of auto fluorescent lipofuscin granules (Feeney, 1973; Marshall, 1987). RPE lipofuscin is derived almost exclusively from phagocytosed outer segments (Boulton et al. 1989; Kennedy et al. 1995). With advancing age these particles may in some cases fill almost the entire RPE cell (Wing et al. 1978). Most of the discs appear to be degraded quickly in lysosomes of young healthy individuals, however, over time incompletely degraded membrane material builds up in the form of lipofuscin within secondary lysosomes or residual bodies (Boulton et al. 1989). In older individuals up to 25% of the volume of RPE cells may be occupied by lipofuscin, therefore room for normal cellular machinery is consequently limited. As a result, lysosomes and mitochondria of RPE suffer age-related alterations which, eventually, may lead to apoptotic

cell death. There is an age-related loss of RPE cells, particularly in the fovea and mid-periphery, forcing compromised lipofuscin-engorged cells to provide metabolic maintenance for the retina.

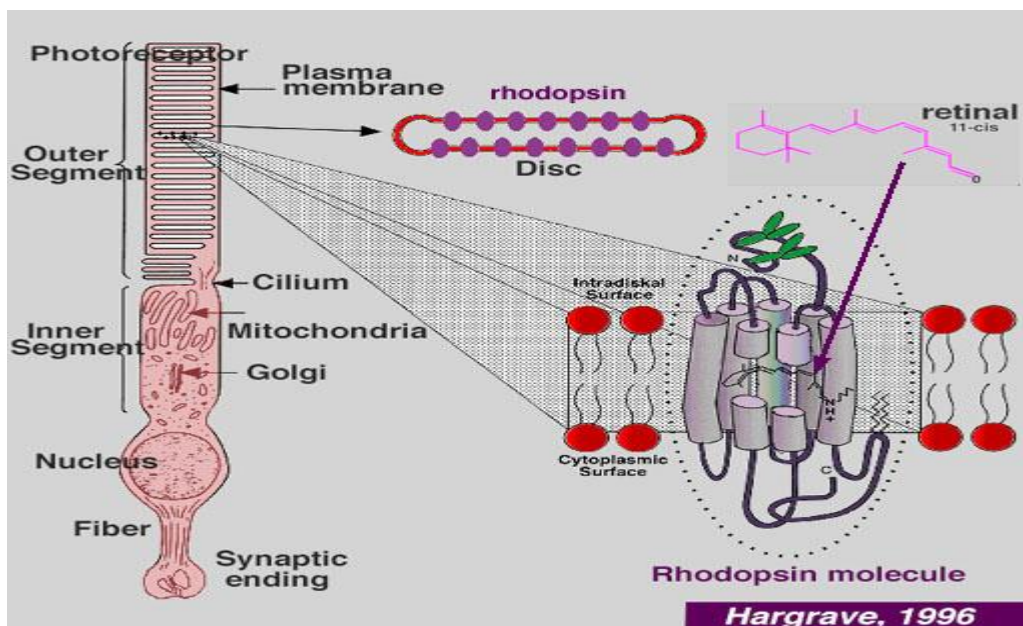


**Figure 2.3 Diagram showing the relationship between the photoreceptor cells and the RPE. The outer segments undergo a continual process of renewal. Periodically, the tips of the outer segments are shed from the photoreceptors and are then phagocytosed by the RPE (Modified from Katz & Robison, 2002).**

### **2.3 Photoreceptor Layer**

Photoreceptors are highly specialised cells that are sensitive to light and responsible for converting light into a neural impulse. There are two main types of photoreceptors: rods and cones. Rods are responsible for peripheral and scotopic vision, whereas cones operate best in photopic conditions and are responsible for central vision and colour perception. Cones are capable of high spatial and temporal resolution. The outer segments of both rods and cones contain discs with a double membrane and the visual pigments which capture

photons are mainly built into these discs and consist of aldehyde of vitamin A and various proteins (Steinberg et al. 1980; Adler and Martin, 1982; Papermaster et al. 1985). The photopigment in rods is rhodopsin and the photopigment in cones is iodopsin. The inner synaptic end of the photoreceptors transmits the effect of light to the bipolar and horizontal cells (Daw et al. 1990). Typically there are approximately 110 to 125 million rods and 6.5 million cones in an eye. Overall rods are longer and thinner than cones (Heath & Young, 2000).

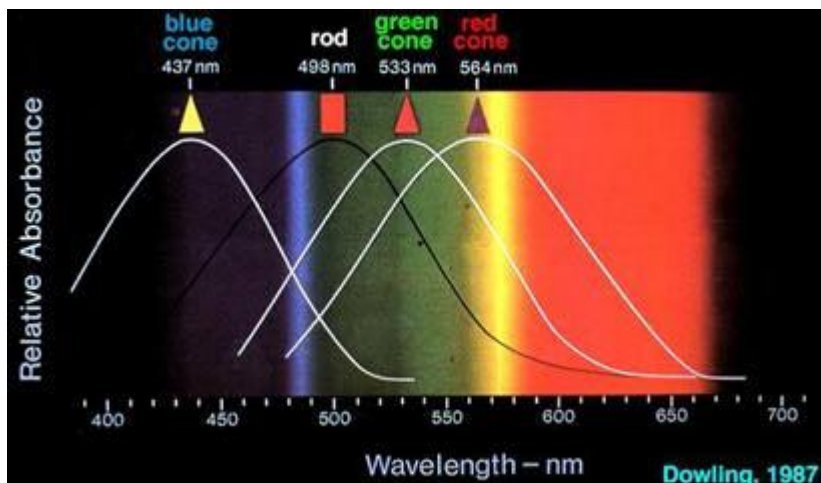


**Figure 2.4 The structure of a rod cell (Accessed from <http://www.phys.utl.edu/~avery/course/3400/gallery/gallery-vision.html>).**

The photoreceptor outer segments protrude into a unique compartment bounded by the RPE and Müller cells. The molecular composition of this compartment, the interphotoreceptor matrix (IPM), contains components secreted by rod and cone photoreceptors, Müller cells and RPE cells. In addition to transporting molecules among the cells bordering the IPM, various proteins and glycosaminoglycans provide a scaffold for the photoreceptor outer

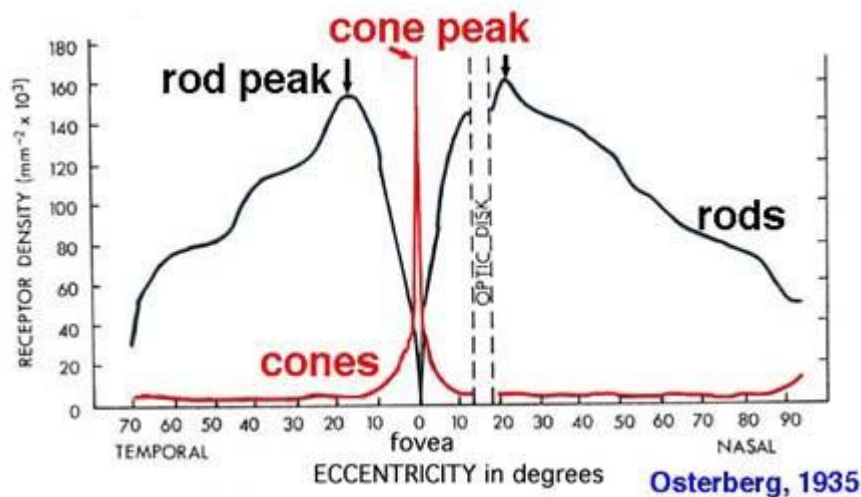
segments and regulate the water and ion content of their environment (Hollyfield, 1999). RPE cells and Müller glial cells form the major component of the blood retinal barrier for the choroidal and retinal circulation, respectively. They regulate nutrient flow to photoreceptors and control the composition of the extra cellular environment surrounding rods and cones.

There are three types of cones; the differing absorption spectra between their pigments give rise to colour perception. The SW class is the least populated cone type and is absent at the very centre of the foveola but has a peak intensity at approximately one degree. This in theory would have implications for reducing sensitivity to CA in the eye. The two remaining cone classes (medium and long wavelength cones) combine with the SW class to provide a typical range of sensitivity to wavelengths between 390 nm & 760 nm (Tunacliffe, 1993). Red-green cones seem to be more resistant than blue cones to aging and may also increase in size in AMD (Sarks et al. 1988; Curcio et al. 1996).



**Figure 2.5 Absorption spectrum of rods and cones.** (Accessed from <http://www.phys.utl.edu/~avery/course/3400/gallery/gallery-vision.html>).

The small cone dominated fovea of only 0.8 mm ( $2.75^\circ$ ) in diameter is surrounded by a rod dominated parafovea (Curcio et al. 1990). In young adults, rods outnumber cones in the macula by 9:1. In the entire eye, rods outnumber cones 20:1. So the macula can be considered cone enriched but not cone dominated (Curcio et al. 2000). The retinal region with the highest acuity, the macula, has the highest cone density, rising to about 146,000 cones per  $\text{mm}^2$  (Tunacliffe, 1987).



**Figure 2.6. Graph showing number of rods and cones per mm (Accessed from <http://www.phys.utl.edu/~avery/course/3400/gallery/gallery-vision.html>).**

In the maculas of older adults lacking grossly visible drusen and pigmentary change (i.e. they do not have AMD), the number of cones in the cone dominated part of the macula is stable at approximately 32,000 through to the ninth decade (Curcio et al. 1993). In contrast, the number of rods in the macula of the same eyes decreases by 30%. The greatest loss occurs in the parafovea (1-3 mm from the fovea at  $3.5^\circ$ - $10^\circ$  from fixation). Psychophysical studies of photopic and scotopic sensitivity have identified that rods are at risk for

degeneration in aging and ARM and that scotopic impairment is greater than photopic impairment in older adults (Curcio et al. 2000).

With respect to photoreceptor topography at different stages of ARM, the foveal cone mosaic of eyes with large drusen and thick basal deposits is surprisingly similar to that of aged matched controls (Curcio et al. 1996), and the total number of foveal cones remain normal. Furthermore, in eyes with late ARM, virtually all surviving photoreceptors in the macula are cones, a reverse of the normal predominance of rods. While rods gradually disappear with age, even without evidence of overt RPE disease, cones only begin to degenerate by advanced stages in non-exudative AMD (Curcio et al. 1993; 1996), and may finally result in disappearance of all photoreceptors in the presence of geographic atrophy (GA) or disciform degeneration (Sarks et al. 1988; Curcio et al. 1996). Although death of cone photoreceptors and consequent loss of vision are end points of AMD, it is far from clear whether the disease begins in these cells or elsewhere.

### **2.3.1 Macular pigment**

Although MP is found throughout the tissue of the eye, it is concentrated in the macula lutea region of the retina, including the central retinal depression called the fovea, where cone photoreceptors reach their maximal concentration. The localisation of MP within the retina is in the fibres of Henle in the fovea and parafoveally MP is located in the inner and outer plexiform layers (Trieschmann et al. 2008). MP will be discussed in detail in chapter three.



## **2.4 The Inner Neural Sensory Retina (layers 3 to 10)**

### **2.4.1 The outer limiting membrane**

In the outer retina, the junctional complexes between Müller glial cells and photoreceptor inner segments, form the outer limiting membrane. It is not a true membrane structure but rather an artifact created by extensive interconnections between adjacent photoreceptors.

### **2.4.2 The outer nuclear layer**

The outer nuclear layer contains the nuclei of the photoreceptors and their surrounding cytoplasm.

### **2.4.3 The outer plexiform layer**

Photoreceptor axons and synaptic processes extend up to the outer plexiform layer (a communication layer) where they synapse with the neural layers.

### **2.4.4 The inner nuclear layer**

The inner nuclear layer contains the nuclei of

- a) Bipolar cells, which communicate between one or more ganglion cells and one or more photoreceptors. They also may synapse with horizontal or amacrine cells.
- b) Horizontal cells which may have short processes and one long process. They form lateral connections between photoreceptors, and between photoreceptors and bipolar cells.
- c) Amacrine cells which communicate with bipolar cells, fellow amacrine cells, ganglion cells and occasionally provide a feedback loop to photoreceptors.
- d) Interplexiform cells which communicate from ganglion cells to photoreceptors and

e) Müller cells which are non-neural support cells (Heath and Young, 2000).

There are many sub-types of bipolar cells which are important for summation and retinal processing. Horizontal cells are used in coding colour vision and they can act to dampen rod signal if the cone signal is strong and visa versa. Amacrine cells are large cells predominantly located in the retinal periphery.

#### **2.4.5 The inner plexiform layer**

The inner plexiform is thicker than the outer plexiform layer, except at the fovea where it is absent. It is a communication layer where impulses are relayed between bipolar, amacrine and ganglion cells.

#### **2.4.6 Ganglion cell layer**

Ganglion cells are the only cell type in the ganglion cell layer and there are 1.2 million ganglion cells in the retina. Their cell size increases and the layer thickness decreases with retinal eccentricity. A 1:1 relationship between cones and ganglion cells exist at the fovea, compared to a 100:1 rod to ganglion cell relationship at the periphery. The difference in coupling ratios exemplifies the greater resolution at the fovea, which is important for the eye to extract the maximum image it can from the retinal image provided by the optics of the eye. The reduced resolution of the peripheral retina is economically matched in terms of biological demands to the reduced image quality provided by the eye's optics in the peripheral visual field.

#### **2.4.7 The nerve fibre layer**

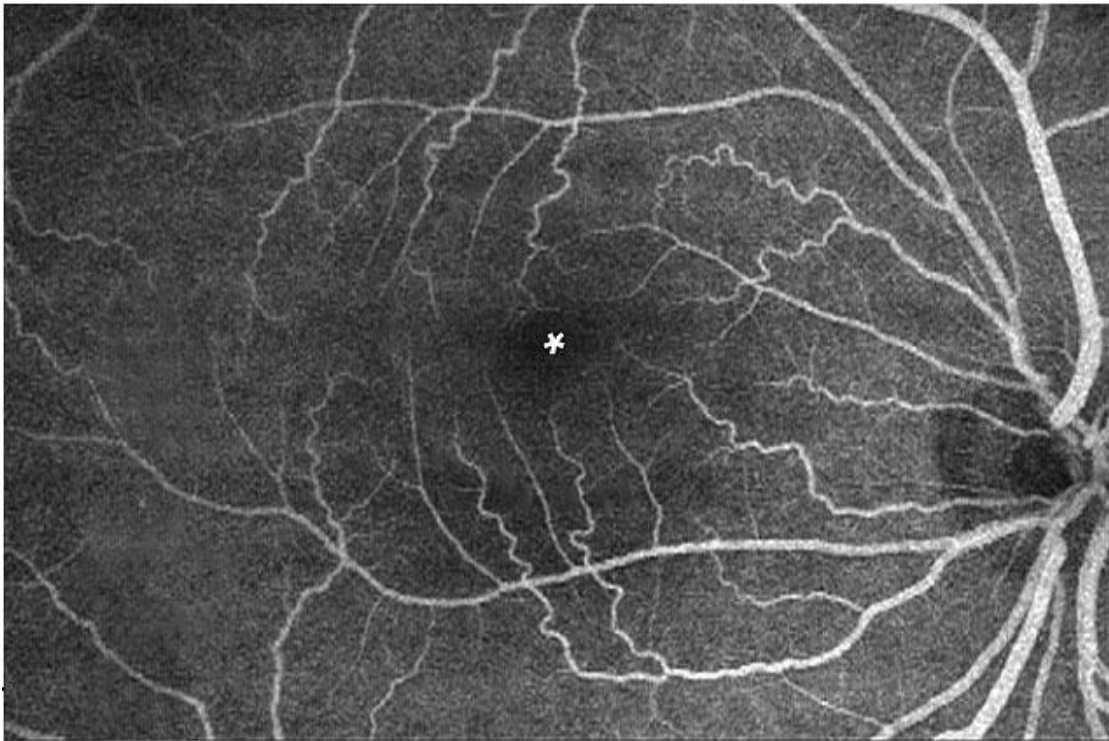
The nerve fibre layer contains the axons from ganglion cells. It takes a radial pathway across the nasal retina and an arcuate pathway across the temporal retina to the optic nerve head. The only exception is the papillomacular bundle where some axons in the central macular region take a radial path to the optic nerve head. The nerve fibre paths are strictly demarked between the superior and inferior retina by the horizontal raphe. Although the nerve fibres are unmyelinated, they are insulated by glial cells.

#### **2.4.8 The inner limiting layer**

The inner limiting membrane marks the boundary between the vitreous and the retina. A true basement is present and it is associated with condensing Müller cell membranes and a thickening of the vitreous basal lamina. The inner limiting membrane has variable adherence to both the sensory retina and the vitreous.

### **2.5 Blood Supply to the Retina**

The retina receives vascular supply from both the choriocapillaris and the retinal vasculature system. The retinal vessels are absent centrally at the fovea. The central retinal artery enters the optic nerve some 10 to 15 mm behind the globe. On entering the eye the vessels branch into inferior and superior divisions which continue to subdivide and proceed to all parts of the retina, where, as capillaries, they supply the inner two-thirds of the retina. The outer third is supplied by transport across the pigment epithelium from the choroid. The retinal venous system exits the eye via the central retinal vein. The corresponding retinal venous branches have much the same distribution as arteries.



*Fig. 17. Fundus photograph showing fluorescein imaging of the major arteries and veins in a normal human right eye retina. The vessels emerge from the optic nerve head and run in a radial fashion curving towards and around the fovea (asterisk in photograph). (Image courtesy of Isabel Pinilla, Spain).*

**Figure 2.7 Showing blood supply to the retina (Accessed from Simple Anatomy of the Retina – webvision**<http://webvision.med.utah.edu/book/part-i-foundations/simple-anatomy-of-the-retina/>**)**

The choroid is supplied by the short posterior ciliary arteries, and these arteries are branches of the Ophthalmic artery. They form a rich anastomotic network that quickly empties large quantities of blood into the choriocapillaris (sinusoidal network). The choriocapillaris supplies the RPE and outer retinal layers. Four or five vorticoses veins drain the choroid and pierce the sclera to join the Ophthalmic veins.

## **2.6 The Choroid**

The choroid is a thin layer lining the inner surface of the sclera. It is extremely vascular and its principal function is to nourish. The choroid extends from the optic nerve posteriorly to the ciliary body anteriorly. Its inner surface is smooth and firm and is attached to the pigmented layer of the retina. Its outer surface is roughened and is firmly attached to the sclera in the region of the optic nerve and where the posterior ciliary arteries and ciliary nerves enter the eye.

The choroid may be divided into three layers.

(i) The vessel layer (ii) the capillary layer and (iii) Bruch's membrane.

### **2.6.1 The vessel layer**

The external layer consists of loose connective tissue containing melanocytes in which are embedded numerous large and medium sized blood vessels.

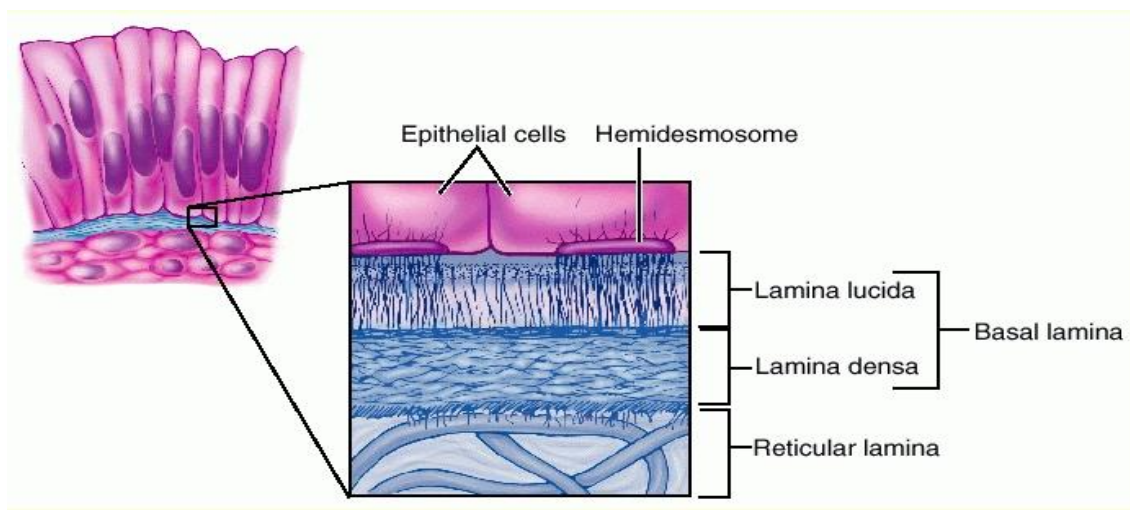
### **2.6.2 The capillary layer or choriocapillaris**

This intermediate layer consists of a network of wide bore capillaries with sac like dilatations. They are fed by arteries from the vessel layer and drained by veins into the vessel layer.

### **2.6.3 Bruch's membrane**

Bruch's membrane has five anatomic layers with known structure and function. The innermost layer (i.e. closest to the RPE) of Bruch's membrane is the RPE basal lamina, which serves as the anchoring surface for the RPE. Proceeding externally is the inner collagen layer, which is a dense collagen matrix that interconnects the basal lamina and

elastin layers of Bruch's membrane. Most of the dysfunction within AMD starts in the inner collagen layer. Drusen like material can accumulate either on the inner or outer aspect of the basal lamina layer. Next is the outer collagen layer, which is similar to the inner collagen layer at the ultrastructural level. Structural changes occur within the outer collagen layer as a function of advancing patient age, including collagen cross-linking. Lastly, the basal lamina layer separates the outer collagen layer from the choriocapillaris.



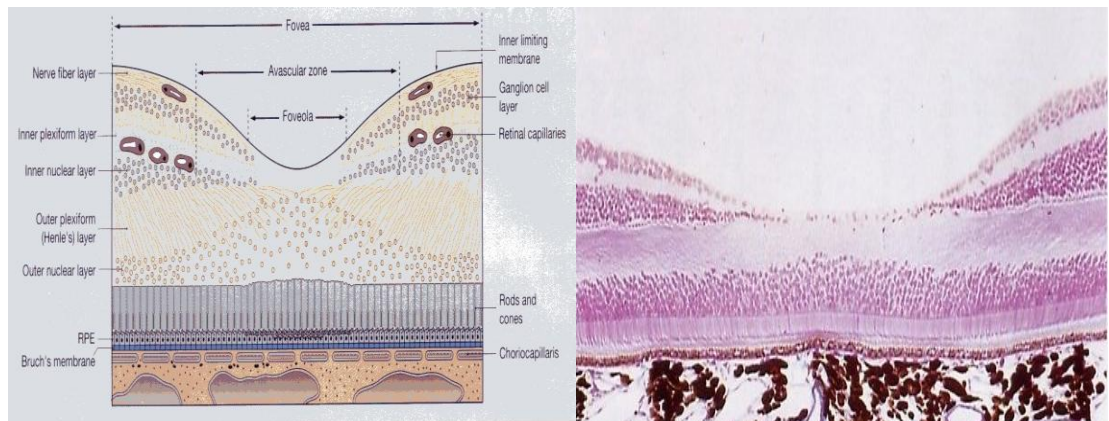
**Figure 2.8 Basement membrane, comprising the basal lamina and reticular lamina, in a diagram of a section through epithelial tissue.** (Accessed from <http://medical-dictionary.thefreedictionary.com/Bruch's+membrane>)

Deposition of waste material occurs between the RPE and Bruch's membrane in the form of basal deposits. Basal laminar deposits accumulate between the RPE cell plasma membrane, and its basement membrane (Green & Enger, 1993; Green, 1999), whereas basal linear deposits accumulate external to the basement membrane of the RPE (e.g in the inner collagenous zone of Bruch's membrane). Basal laminar and basal linear deposits contribute to thickening of Bruch's membrane with age (Green & Enger, 1993).

## 2.7 The Macula

The macula is recognised as the specialised region of the retina capable of high-resolution visual acuity (VA). Francisco Buzzi was the first to anatomically define the macula at the end of the 18<sup>th</sup> century (Buzzi,1782) (Cited by Loughman et al. 2010). He described it as the yellow portion of the posterior retina, lateral to the optic nerve, with a depression in its centre. Wald (1945) documented the first xanthophylls extracted from the human retina and found they were concentrated in the macula. He suggested that the yellow pigment absorbed wavelengths between 430 and 490 nm, with maximum absorption at 465 nm. It has been established that the xanthophylls, lutein and zeaxanthin are responsible for the yellow colour and the antioxidant capabilities of these xanthophylls combined with their ability to trap SW light may serve to protect the outer retina, RPE and choriocapillaris from oxidative damage (Weale, 1951; Brown & Wald, 1963; Ruddock, 1963; Bone et al. 1985). Bone et al. (1985), identified lutein and its structural isomer, zeaxanthin as the specific xanthophylls in the retina, and Snodderly et al. (1984), located the xanthophyll pigment in the Henle fibre layer of primates.

Anatomically the macula (macula lutea or central retina) is defined as that portion of the posterior retina that contains xanthophylls and two or more ganglion cells. This region is about 5.85 mm in diameter and is centered approximately 4 mm temporal and 0.8 mm inferior to the centre of the optic disc (Hogan et al. 1971). On the basis of microscopic anatomy, the macular area can be subdivided into several zones: the macula lutea (~5.85 mm diameter), fovea centralis (~1.85 mm diameter) and the foveola (~0.35 mm diameter) (Kanski, 2003).

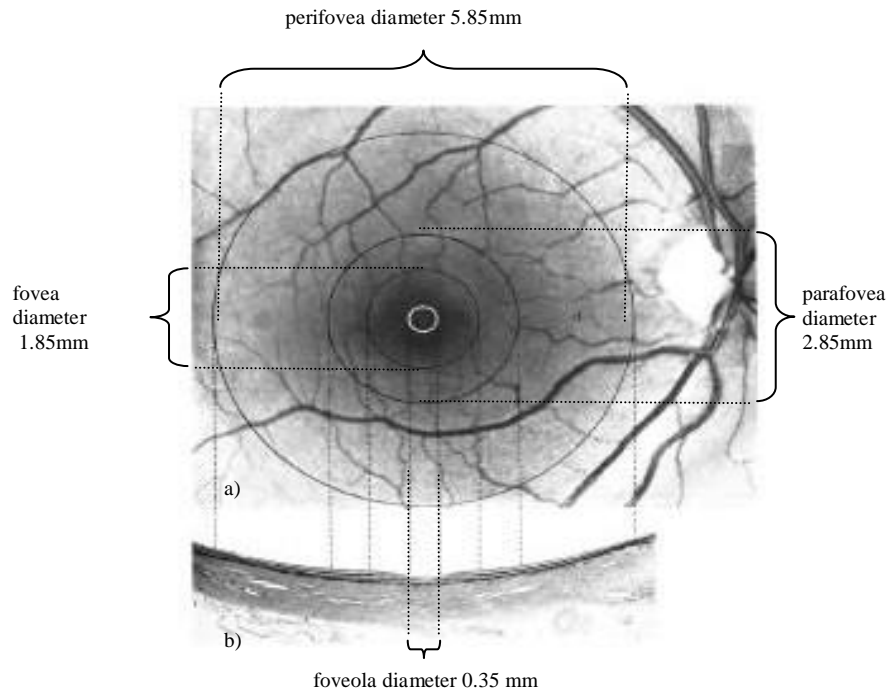


**Figure 2.9 (a) Schematic drawing of human foveola (Kanski and Milewski 2002). (b) Histological section of human foveola (Heath and Young 2000).**

The fovea (fovea centralis) is a depression in the inner retinal surface in the centre of the macula and it is more heavily pigmented than the surrounding retinal tissue. The central floor of the fovea is called the foveola. It lies within the capillary free zone. A small depression in the centre of the foveola is called the umbo where the retina is only 0.13 mm thick. The anatomic subdivisions of the macula are ill-defined ophthalmoscopically. The foveal reflex is present in most normal eyes and it lies just in front of the centre of the foveola. The macular area can also be described and divided in terms of different areas:



(i) Fovea containing foveola (ii) Parafovea (iii) Perifovea (Figure. 2.11).

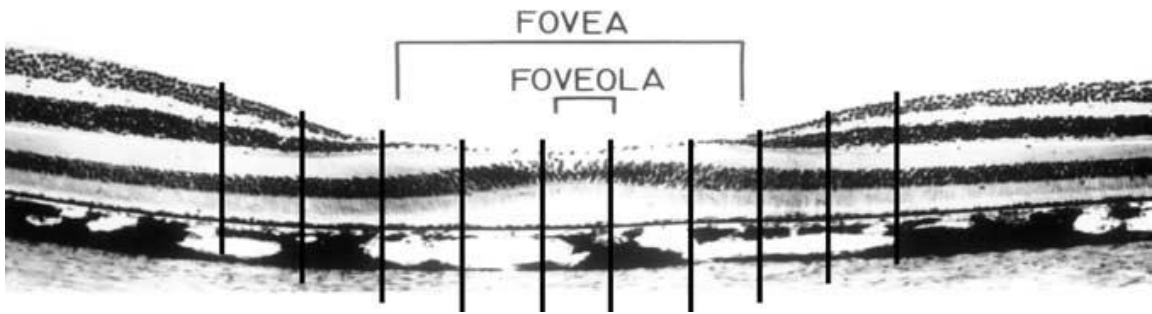


**Figure 2.10 An anatomical view of the macula region as viewed in cross section (adapted from Thibos *et al* 2000).**

The foveola is surrounded by a wide ring zone, where the ganglion cell layer, inner nuclear layer and outer plexiform layer of Henle are thickest, and this is called the parafoveal area. This zone is in turn surrounded by the perifoveal area.

### **2.7.1 Specialisation of the macula**

Most layers of the sensory retina are displaced sideways at the foveola to create the foveal pit. Thus, photoreceptors have unimpeded stimulation from light forming the retinal image. The small cone size and tight cell packing at the fovea, increases the spatial resolution of the visual system for on axis imaging (Heath and Young, 2000).



**Figure 2.11 The central human fovea shown as graphics (Modified from Trieschmann et al. 2008).**

The fovea accounts for almost all of our useful photopic vision, even though it comprises less than 4% of the total retinal area. The retinal region with the highest acuity, the fovea, has the highest cone density and the central 100  $\mu\text{m}$  of the foveola contain only red and green cones. Blue cone density is highest in a zone between 100 and 300  $\mu\text{m}$  from the centre of the fovea. The foveola is entirely rod free. Rods, ganglion cells and all inner nuclear layer neurons are absent from the foveola so that light is directly incident on photoreceptors.

The retina is principally organised on a vertical basis. Receptors transmit activity to the bipolar and ganglion cells and then to the higher brain centres. A given receptor may activate several bipolar cells, which may in turn activate more than one ganglion cell which causes a horizontal spread of the effect of the light stimulus. Horizontal and amacrine cells further increase the probability of horizontal interaction. There are three types of bipolar cells. Midget bipolars make contact with one cone only, whilst the flat bipolar connects with 6 or 7 cones. The third type, the rod bipolar, may make synapses with up to 50 rods (Tunnacliffe, 1987). There are two types of ganglion cells: the midget ganglion cell which connects with a midget bipolar cell and therefore has a very close relationship with a single

cone, and a diffuse type connecting with several bipolar cells. The single cone/midget bipolar/midget ganglion cell route is partly the basis of high VA achievable at the fovea. Midget ganglion cells probably only respond to cone stimulation, whereas diffuse ganglion cells respond to both rod and cone stimulation.

The variable density and distribution of photoreceptors and ganglion cells across the retina, the differential light sensitivity of photoreceptors, and the convergence of information from the extra-foveal retina means that a hierarchy exists in the architecture of retinal processing and foveal information is given higher priority. This hierarchy continues back to the striate cortex, where a high percentage of cortical cells are dedicated to foveal information. The fovea is particularly important for functional vision (e.g. acuity); blindness results when this area is lost to disease.

## **CHAPTER THREE**

### **Macular Pigment**

The centre of the retina at the back of the eye contains a yellow pigment which gives this region its name, macula lutea (yellow spot). The yellow colouration of the macula is attributable to the presence of MP (Snodderly et al. 1984). Although all humans appear to have some quantity of MP within their retina, foveal concentrations tend to vary quite dramatically (Pease et al. 1987; Hammond et al. 1995). Lutein and zeaxanthin are introduced to the human body through dietary means alone, and although not considered to be essential micronutrients, they have powerful antioxidant and photoreceptive properties (Snodderly, 1995; Khachik et al. 1997; Landrum et al. 1997). Findings are currently emerging that higher levels of dietary and serum levels of lutein and zeaxanthin are associated with a lower risk of AMD (Seddon et al. 1994; Chakravarthy et al. 2009). The fact that MP is modifiable means that ongoing research into its role in prevention or progression of AMD is important. The functional role of macular carotenoids, have not been completely defined, however two major non-exclusive hypotheses have been proposed. The protection hypothesis has received the most attention and is based on the possibility that MP could reduce the cumulative effects of damage due to light and oxidation, and thereby retard the development of age-related eye disease. The optical hypothesis suggests that MP could influence visual resolution by absorbing SW light, which is easily scattered and poorly focused. The optical hypothesis is expanded further to include acuity, visibility and visual health hypotheses. These hypotheses are discussed in detail in section 3.8; 3.9.

### 3.1 Carotenoids

De novo synthesis of carotenoids does not occur in animals and the MP of primates can be traced to its dietary origins. Lutein and zeaxanthin are two of the 600 plant pigments in the carotenoid class, and they belong to the xanthophyll family of carotenoids. There are between 40 and 50 carotenoids present in a typical western diet (Khachick et al. 1992; 1997), but only 14 have been detected in human blood (Khachick 1992; 1997). The most prominent plasma carotenoids include lycopene,  $\alpha$ -carotene,  $\beta$ -carotene, lutein and zeaxanthin (Khachick et al. 1997). *Meso*-zeaxanthin has been found in trace amounts in blood serum (Connolly et al. 2010); however until recently it had not, in the absence of supplementation, been detected or reported on in human serum. Lutein, zeaxanthin and *meso*-zeaxanthin are found in the retina (Bone et al. 1985; Khachik et al. 1997) to the exclusion of all other carotenoids in nature and are termed MP. Lutein and zeaxanthin are also the only carotenoids found in the lens of the human eye (Landrum & Bone, 2001).

Lutein and zeaxanthin are found in a typical Western diet, of fruit and vegetables, although zeaxanthin is found in much smaller quantities than lutein (Sommerburg et al. 1998). In the diet lutein is found in highest concentrations in dark green leafy vegetables (spinach, kale, collard greens and others) (Sommerburg et al. 1998). Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges and tangerines. The highest molar percentage of lutein and zeaxanthin are found in egg yolk and maize (Sommerburg et al. 1998). In serum, the ratio of zeaxanthin to lutein is 1:4 (Bone et al. 1997). Although *meso*-zeaxanthin has been identified in some less commonly consumed foods including fish (e.g. salmon and trout), shrimp, and turtle (Maoka et al. 1986), it is generally believed to be generated at the macula following a biochemical transformation of lutein (Bone et al. 1997; Johnson et al.

2005). Xanthophylls are a subclass of carotenoids, a large group of plant pigments responsible for the colour of bright fruit and vegetables. They act as energy sinks in plants and provide the essential first step toward photosynthesis in association with chlorophyll. Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one at each side of the molecule. Zeaxanthin is a stereoisomer of lutein differing only in the location of a double bond in one of the hydroxyl groups. The hydroxyl groups appear to control the biological function of these two xanthophylls (Johnson et al. 2005). Carotenoids are linear hydrocarbons and xanthophylls are the oxygenated form (Goodwin, 1980). *Meso*-zeaxanthin, which represents the product of chemical processes involving lutein in the retina, will be discussed in section 3.2.

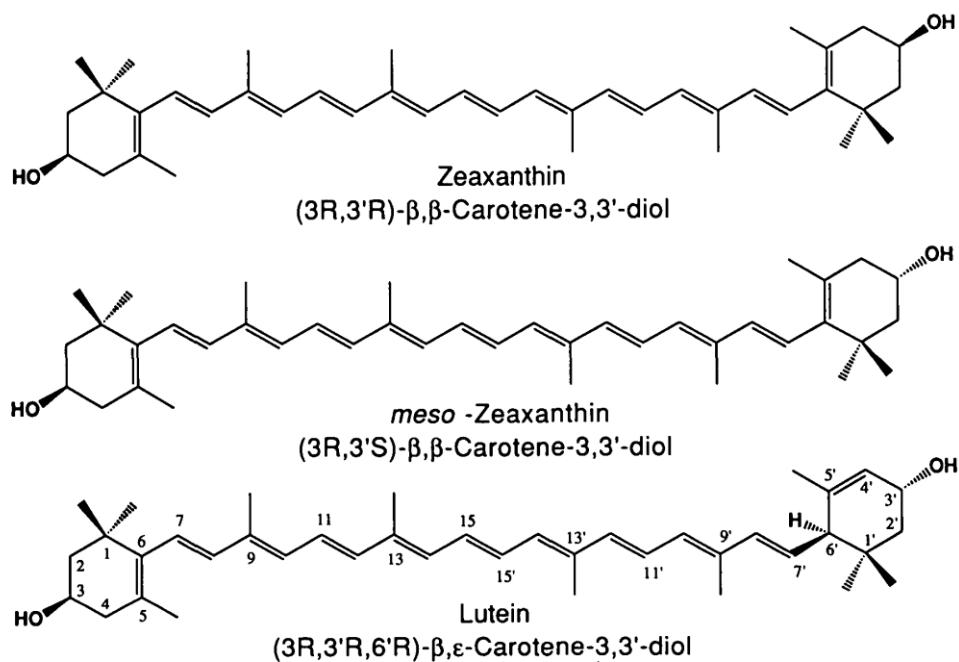
### **3.2 Stereochemistry of Macular Pigment**

The yellow MP was shown in 1985, by Bone et al. and later by Handleman et al. (1988), using high-performance liquid chromatography (HPLC), to be composed of two chromatographically separable components, namely lutein and zeaxanthin. Subsequently MP was shown to be characterised by the presence of specific stereoisomers of these two carotenoids (Bone et al. 1993).

While lutein is present as a single stereoisomer, [(3R,3'R,6'R)- $\beta,\epsilon$ -carotene-3,3'-diol], zeaxanthin occurs primarily as a mixture of three isomers; [(3R,3'R)- $\beta,\beta$ -carotene-3,3'-diol], [(3R,3'S)- $\beta,\beta$ -carotene-3,3'-diol], with a much smaller amount of [(3S,3'S)- $\beta,\beta$ -carotene-3,3'-diol] (Bone et al. 2007). The first two predominant zeaxanthin isomers are referred to as zeaxanthin and *meso*-zeaxanthin respectively.

Lutein is the dominant carotenoid in the blood (Bone et al. 1993), whereas zeaxanthin and *meso*-zeaxanthin in particular, are less commonly consumed in a typical Western diet. Despite a lutein-zeaxanthin ratio of approximately 4 to 1 in human plasma (Bone et al. 1997), and only trace amounts of *meso*-zeaxanthin have been detected in human serum (Connolly et al 2010), *meso*-zeaxanthin accounts for about one third of total MP at the macula, which is consistent with the finding that retinal *meso*-zeaxanthin is produced primarily by isomerisation of retinal lutein in the eye (Bone et al. 1993, Johnson et al. 2005). This hypothesis is supported by the observation that lutein can be isomerised to *meso*-zeaxanthin by a base-catalysed reaction (Bone et al. 1997).

### Stereochemistry of Human Macular Carotenoids

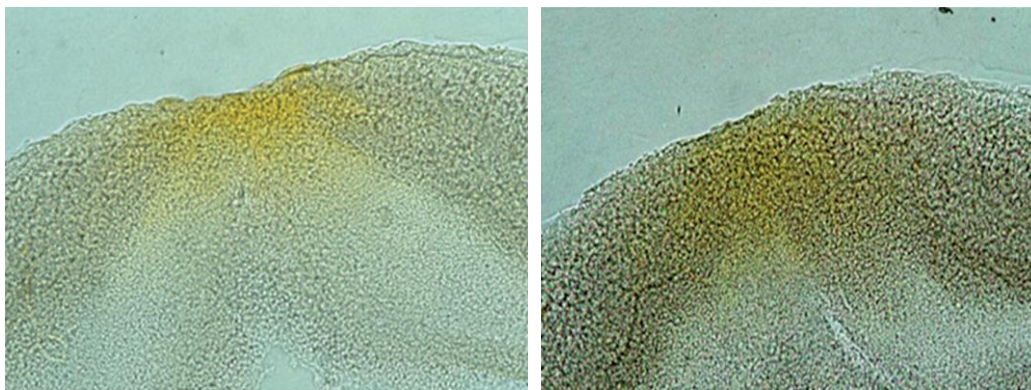


**Figure 3.1** The structure of the major components of Macular Pigment (Bone et al. 1993).

MP therefore refers to the accumulation at the macula of a single isomer of lutein and 3 stereoisomers of zeaxanthin (RRZ, *meso*-zeaxanthin, and SSZ), to the exclusion of all other carotenoids which are found in the blood (Snodderly et al. 1984; Bone et al. 1993).

### 3.3 Location of Macular Pigment

The spectral distribution of lutein and zeaxanthin, were studied in the retinas of macaque monkeys and squirrel monkeys by Snodderly et al. (1991). Lutein and zeaxanthin were found to reach their highest concentration at the centre of the fovea, in the Henle fibre layer, with a sharp drop-off with increasing eccentricity from the macula (Snodderly et al. 1991). Trieschmann et al. (2008) evaluated MP in the human retina of donor eyes and found comparable results with those obtained with primates (Snodderly et al. 1984; 1984). Within the central fovea, the carotenoids are most concentrated within the photoreceptor axons of the Henle nerve fibre layer (Snodderly et al. 1984). In the perifoveal region lutein and zeaxanthin are present in the outer segments of rod photoreceptors (Trieschmann et al. 2008). *Meso*-zeaxanthin is observed to reach its maximum in the central macula (Bone et al. 1993; 1997).



**Figure 3.2 Histology of human macular pigment 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). (Modified from Trieschmann et al. 2008).**

MP density and distribution however varies considerably between individuals (Trieschmann et al, 2003; 2008), and it is not yet known if either or both the density and/or the distribution is important in protection of the retina.

Although MP is found throughout the whole retina (Handleman et al. 1988; Bone et al. 1988), these carotenoids reach their maximum concentrations at the foveola. Bone et al. (1997) reported that the proportions of *meso*-zeaxanthin:zeaxanthin in the central 3 mm of the macula was 0.83, which decreased with increasing distance from the fovea. The ratio of lutein to zeaxanthin and *meso*-zeaxanthin varies linearly with the ratio of rods to cones with increasing eccentricity up to approximately 6 degrees from the fovea (Bone et al. 1988). The hypothesis that zeaxanthin is found only in the rods is refuted by the fact that the fovea contains predominantly cones, as well as by the fact that squirrel monkey and macaque retina have their highest concentration of lutein and zeaxanthin in the central fovea (Snodderly et al. 1991). It is believed that at 7 degrees eccentricity retinal carotenoids become optically undetectable (Bone et al. 1988).

### **3.4 Optical Density and Serum Levels of Macular Pigment**

There is a consensus among investigators that MP density varies between individuals (Hammond et al. 1997; Trieschmann et al. 2008), but there is good individual interocular agreement of MPOD, with mean differences of only 5% for zeaxanthin and 11% for lutein, between fellow eyes (Hammond et al. 1992; Handleman et al. 1991). Hammond et al.

(1996) conducted a prospective study to investigate the relationship between plasma, dietary and macular carotenoids. The investigators were able to draw some reasonable conclusions:

(i) There are individual differences in the response to dietary modification with carotenoid supplements and there is dramatic variability of MPOD between individuals (Pease et al. 1987; Hammond et al. 1992; 1997). It has been suggested that people who are less responsive to lutein supplementation may be so because of genetic differences, which results in reduced or less efficient lutein binding proteins in some people. Lutein-binding proteins have a high affinity to lutein and have been discovered in the retina of human eyes (Landrum & Bone, 2004). Hammond et al. (1997) found no retinal response in three people out of 11, despite dietary modification with spinach and corn for 14 weeks. Two retinal ‘non-responders’ showed substantial increases in serum lutein but not in MPOD. One serum and retinal ‘non-responder’ showed no changes in serum lutein, zeaxanthin or  $\beta$ -carotene and no change in MPOD.

(ii) Serum levels of lutein and zeaxanthin reflect recent nutritional intake. MPOD on the other hand is said to have a slower biological turnover, as it reflects the local balance between pro-oxidant stresses and antioxidant defences in the retina (Nolan et al. 2007). A sudden change in diet will be reflected in much more rapid changes of serum concentrations of lutein and zeaxanthin, whereas it is unlikely to affect MPOD in the retina for several weeks. However, a recent study reported a significant increase in MPOD after only two weeks of supplementation (Connolly et al. 2010) and a study by Loughman et al (2011), observed a decline of 31% and 43% of central (0.25 degrees) and average MPOD respectively, after a period of only 21 days of dietary exclusion of the MP carotenoids.

Macular concentrations of the carotenoids in the retina may not appear to be as consistent over long periods of time, in individuals on a relatively constant diet, as was originally thought (Bone et al. 1988), highlighting even more the importance of measuring MP in clinical practice.

(iii) There are discrepancies between tissue and serum responses to lutein and zeaxanthin supplements, which suggest that blood levels of carotenoids in isolation, provide insufficient information when investigating the possible protective effect of carotenoids against retinal degenerative disorders. Although a positive and significant relationship exists between the density of MP and serum concentrations of lutein and zeaxanthin (Landrum et al. 1997), lutein/zeaxanthin ratios of blood and macula do not correlate and this is partly attributable to the stereochemistry of macular carotenoids (Bone et al. 1993), processes which influence digestion, absorption and transport of compounds in question, and accumulation and stabilisation of the carotenoids in the tissues. Because xanthophylls are fat soluble nutrients, bioavailability to tissues is dependent on a number of factors including nutrient source (whole food or supplement), state of food (raw, cooked or processed), extent of disruption of the cellular matrix via mastication and digestive enzymes, and absorption by the enterocytes of the intestinal mucosa (primarily the duodenum). Cooking of lutein/zeaxanthin foods may increase bioavailability by disrupting the cellular matrix and the carotenoid protein complexes (Castenmiller, 1999). Non dietary factors affecting absorption and bioavailability of lutein and zeaxanthin include age, body composition, gender, malabsorption of fats, alcohol consumption, smoking and liver and kidney disease.

Lutein and zeaxanthin are also known to accumulate in liver, spleen and adipose tissue (Thomson, 2002). There is evidence of an inverse relationship between body fat and MPOD in humans (Johnson et al. 2000; Hammond et al. 2002), and a similar relationship with retinal lutein (not zeaxanthin) in female quail (Thomson, 2002), suggesting that fat and retina compete for lutein.

### **3.5 Function of Macular Pigment**

Although the function of MP remains uncertain, several possibilities have been suggested. Blue light filtration effects, including glare reduction, minimisation of CA, contrast enhancement, improved fine detail distinction, and free radical scavenger by neutralising reactive oxygen species (ROS), are some of the major proposed functions of these ocular carotenoids (Whitehead et al. 2006). Two major but non-exclusive hypotheses regarding the function of MP exist and these include the 'Protection' hypothesis and the 'Optical' hypothesis.

### **3.6 Oxidative Stress and Reactive Oxygen Species**

The 'protection' hypothesis of MP is based on the possibility that MP could reduce the cumulative effects of damage due to light and oxygen and retard the development of age-related eye diseases such as AMD.

Oxidative stress is caused by an imbalance between the production of ROS and a biological systems ability to readily detoxify or easily repair the resulting damage. In aerobic conditions, ROS are generated at a very high rate. In the retina, the generation of ROS can occur as the by-products of cellular metabolism (Kukreja & Hess, 1992) or as a result of

photochemical reactions (Dargal, 1992). These molecules are highly reactive and will readily react with lipid, protein and nucleic acids, thereby resulting in impaired cell function or cell death (Halliwell, 1997). Most of the ROS are byproducts of normal physiological processes and are eliminated immediately by antioxidant systems. ROS form a natural by product of normal metabolism of oxygen and have an important role in cell signalling, however during times of environmental stress (UV and SW light, smoking or heat exposure), ROS levels can increase dramatically, which can result in significant damage to cell structures. This cumulates into a situation known as oxidative stress.

### **3.6.1 Reactive oxygen species**

ROS is an umbrella term used to describe free radicals, hydrogen peroxide and singlet oxygen. Free radicals are molecules that contain one or more unpaired electron in their outer orbits, and examples include the superoxide anion ( $O_2^{\cdot -}$ ), the hydroxyl free radical ( $OH\cdot$ ), the hydroperoxyl radicals ( $HO_2\cdot$ ) and the lipid peroxy radicals (Halliwell, 1991). Hydrogen peroxide ( $H_2O_2$ ) and singlet oxygen ( $^1O_2$ ) contain their full complement of electrons, but in an unstable or reactive state. Environmental factors such as cigarette smoking intense light exposure, irradiation, aging and inflammation are known to increase the production of ROS (Borish et al. 1987; Machlin & Bendich, 1987). In order to achieve a stable state, free radicals extract electrons from other molecules, which are themselves rendered unstable by this interaction, and a cytotoxic oxidative chain reaction results. Hydrogen peroxide, although containing no unpaired electrons, can generate free radicals through the Fenton reaction and singlet oxygen can damage molecules as it converts back to normal oxygen (Beatty et al. 2000).

Carbohydrates, membrane lipids, proteins and nucleic acids are all vulnerable to damage caused by ROS, and this damage is believed to contribute to the pathogenesis of many diseases, including ischemia, atheroma, diabetes, aging and Parkinson's disease (Davis, 1991; Halliwell, 1991).

### **3.6.2 Antioxidant enzymes**

The antioxidant system in cells and tissues include enzymes (catalase, glutathione peroxidase and superoxide dismutase) and smaller antioxidant molecules, such as water soluble vitamin C and glutathione, and lipid soluble antioxidants, such as xanthophylls, retinoids and vitamin E, which back up the enzymatic system as direct scavengers (Beatty et al. 2000; Cai et al. 2000).

### **3.6.3 Generation of reactive oxygen species in the retina**

The retina is an ideal environment for the generation of ROS for a number of reasons. The outer retina, especially membranes of the outer segments of the photoreceptors, has a high concentration of polyunsaturated fatty acids (PUFAs) that are susceptible to photo-oxidation (De La Paz & Anderson, 1992). Oxygen consumption by the retina is much greater than by any other tissue and the outer retina has a high oxygen tension (70 mmHg), almost that of arterial blood. The retina is also subject to high levels of cumulative irradiation and the process of phagocytosis by the RPE itself creates oxidative stress, which results in the generation of ROS (Tate, 1995). The turnover of photoreceptors is high and the shed membranes have the highest concentration of PUFA's of any human tissue and are promptly phagocytosed by RPE. Peroxidation of these lipids can induce damage in the RPE.

A rich oxygen supply, combined with high-energy SW light stimulation, and a vulnerable substrate creates ideal conditions for oxidative damage. Another potential anatomical site for damage mediated by a photochemical response is the choriocapillaris.

#### **3.6.4 Retinal irradiation**

It has been shown that photochemical injury at the level of the RPE is related to wavelength, the threshold for damage being lowest for SW light region of the visible spectrum. Photochemical retinal injury was first described by Ham et al. (1978). The authors reported on the histopathologic findings of rhesus monkey retinas that had been exposed to blue light (441 nm) for 1000 seconds. It was noted that SW light resulted in damage to the photoreceptor outer segments and the generation of AMD like lesions in monkey retinas following exposure to light of varying wavelengths required 70-1000 times less power when using blue light compared to infrared wavelengths (Ham et al. 1978).

#### **3.6.5 Polyunsaturated fatty acids**

The photoreceptor membranes of both rods and cones contain a lipid bilayer. PUFAs, account for about 50% of the lipid bilayer of rod outer segment membranes and proteins make up the remaining 50%. PUFAs are particularly susceptible to free radical damage because their conjugated double bonds are convenient sources of hydrogen atoms, which contain one electron. The lipid radical combines with oxygen to form lipid peroxy radicals and lipid peroxides, which can achieve a steady state only by stealing electrons from other PUFAs, thus creating a cytotoxic cascade of reactions that consume valuable PUFAs and produce damaged molecules (Beatty et al. 2000).

Docosahexaenoic acid (DHA) is the most oxidisable fatty acid in the body, and it makes up ~ 50% of the vertebrate rod photoreceptor phospholipid (Anderson et al. 1976). DHA contains six double bonds so the retina is inherently susceptible to lipid peroxidation. High levels of oxygen, delivered to the photoreceptor outer segments and the RPE from the choriocapillaris, coupled with the intense focus of environmental light on the macula, provides a highly permissive environment for the generation of reactive oxygen that can damage DHA (Hollyfield, 2010). Lipid peroxidation of membrane PUFAs results in loss of membrane function and structural integrity (Anderson & Krinsky, 1973).

### **3.6.6 Retinal chromophores**

Chromophores or photosensitisers are molecules that absorb light to produce a chemical reaction that would not occur in their absence. Photochemical damage may be defined as injury arising from absorption of UV and visible light by a chromophore, resulting in the generation of ROS. The retinal chromophores include rhodopsin, melanin and lipofuscin.

### **3.6.7 Lipofuscin**

Lipofuscin is a lipid-protein aggregate which accumulates within the lysosomes of a variety of metabolically active post-mitotic cells, especially the RPE (Eldred & Lasky, 1993). The RPE is unusual in the amount of retinoids and PUFA's that each of its cells must process through life. Waste by-products, which are not susceptible to the process of phagocytosis, contribute to the formation of lipofuscin. Lipofuscins have three defining characteristics. They consist of intracellular secondary lysosomes, they have a yellow auto fluorescent emission when excited by UV or blue light, and they accumulate during normal senescence (Katz & Robison, 2002).



The accumulation of lipofuscin with age within the RPE has been well documented (Wing et al. 1978; Feeney-Burns et al. 1984). Regional differences have also been noted with the highest concentrations in the macular region (including a dip at the fovea where MP is highest), and the lowest in the peripheral regions (Wing et al. 1978). Lipofuscin is likely composed of by-products of vitamin A metabolism, as well as products of lipid peroxidation (Feeney-Burns et al. 1984; Boulton, 1991). There is a growing body of evidence indicating that lipofuscin compromises RPE cellular function, and histopathological studies have demonstrated an association between high levels of lipofuscin and degeneration of RPE cells and the adjacent photoreceptors (Dorey et al. 1989). Possible mechanisms exist whereby lipofuscin may disrupt RPE cellular activities include; metabolic processes may fail simply because of the reduction in functional cytoplasmic space which results from the presence of intracellular lipofuscin (Young, 1987; 1988) and lipofuscin may actually induce oxidative damage of surrounding tissues, as it acts as a photosensitiser for generation of ROS (Boulton et al. 1993; Suter et al. 2000).

Blue light induced generation of ROS by lipofuscin has been demonstrated *in vitro* by Rozanowska et al. (1995). Blue light damage to the RPE cells is proportional to the amount of light received and the amount of lipofuscin within the RPE cells (Sparrow et al. 2003). Lipofuscin has been established as the major chromophore of the RPE and that aerobic photo activation of lipofuscin forms several potentially cytotoxic ROS. N-retinylidene-N-retinylethanolamine (A2-E) is a component of lipofuscin and there is strong experimental evidence that it can damage the RPE, is toxic to mitochondria and when exposed to blue light, induces apoptosis of cultured RPE cells (Suter et al. 2000; Sparrow & Cai, 2001).

Further studies have revealed that the lipofuscin mediated generation of ROS in response to irradiation is wavelength dependant, being greatest for the blue region of the visible spectrum (Rozanowska et al. 1995).

### **3.7 Protection of the Macula by Macular Pigment**

Oxidative stress has been proposed as a major pathogenic factor in AMD (Beatty et al. 1999). Exposure to light and oxidative stress induce photoreceptor death *in vitro* (Wiegand, 1983). The process is accompanied by an increase in ROS and is diminished by use of antioxidants (Conn, 1991). It is likely that MP acts to protect the retina from photochemical damage both directly, by acting as a free radical scavenger (Snodderly et al 1984; Snodderly et al 1984; Khachik et al. 1997), and indirectly, by filtering out the potentially damaging SW blue light (Bone et al. 1992).

The possibility that the absorption characteristics and antioxidant properties of MP confer protection against AMD has been postulated (Seddon et al. 1994; Snodderly, 1995; Landrum et al. 1997; Chakravarthy et al. 2009). Lutein, zeaxanthin and *meso*-zeaxanthin are oxygenated carotenoids (xanthophylls), and are present as the major diet-based compounds of MP (Landrum & Bone, 2001). Several studies examining the association between dietary intake of these carotenoids and AMD have yielded positive protective relationships. The risk of developing advanced AMD is lower among those reporting the highest lutein/zeaxanthin intake (Seddon et al. 1994; Mares-Perlman, 2001). Eyes with AMD have consistently been found to demonstrate low levels of MP (Eye Disease Case Control Study group (EDCCS), 1993; Beatty et al. 2001; Bone et al. 2001; Bernstein et al. 2002). It is unclear however whether this is the cause or a consequence of the condition.

The Age Related Eye Disease Study (AREDS) demonstrated the effectiveness of supplementation with vitamins and minerals with antioxidant properties in preventing progression to advanced AMD among people with large drusen, GA or neovascular AMD in the other eye (AREDS, report no 8, 2001).

The carotenoids as a family, have clear antioxidant properties, have been shown to react with singlet oxygen, free radicals and also to prevent lipid peroxidation (Khachik et al. 1997). Oxidation products of lutein and zeaxanthin have also been identified in the retina (Khachik et al. 1997), suggesting that lutein and zeaxanthin may act as antioxidants to protect the macula against SW visible light. Chucair et al. (2007) demonstrated that lutein and zeaxanthin efficiently protect photoreceptors in culture from apoptosis induced by oxidative stress and also promoted photoreceptor differentiation. Interestingly, the average levels of MP have been reported as 32% lower in eyes with AMD than in normal age-matched control eyes, among subjects not consuming high-dose lutein supplements (Bernstein et al. 2002).

*In-vitro* experiments indicate that zeaxanthin is a more potent antioxidant, quenching singlet oxygen more efficiently than lutein by a factor of ~ 2 (Cantrell et al. 2003). The reason presumably due to the extended conjugation of zeaxanthin compared to lutein. The perpendicular orientation of zeaxanthin close to the oxidisable PUFA's, may also mean that zeaxanthin offers more protection than lutein (Bone et al. 1997).

Almost 50% of total zeaxanthin in the human retina is in the form *meso*-zeaxanthin, which is not found in a typical Western diet. It has been reported that in association with a

zeaxanthin binding protein, pi-isoform of glutathione S-transferase, *meso*-zeaxanthin provides slightly better protection against lipid membrane oxidation than zeaxanthin (Bhosale & Bernstein, 2005); however without the binding protein the situation is reversed. Recently, Li et al (2010) demonstrated that a mixture of lutein, zeaxanthin and *meso*-zeaxanthin (in a ratio of 1:1:1) quenches more singlet oxygen which would not be achieved by any of these carotenoids in isolation. By doing so they may reduce the levels of damaging free radicals, thus preventing the impairment of mitochondrial function and consequently avoiding the triggering of neuronal death (Chucair et al. 2007). Recent supplements on the market now contain *meso*-zeaxanthin along with lutein and zeaxanthin, because it is thought that it may be more advantageous to the macula to increase the ratio of total zeaxanthin compared to lutein.

### **3.8 The ‘Optical’ Hypothesis of MP**

Following on from the ‘protective’ hypothesis, MP’s absorptive capacity and physical location within the retina has initiated the ‘optical’ hypotheses of this pigment. The ‘optical’ hypothesis of MP was originally discussed by Reading & Weale (1974), and later by Nussbaum et al. (1981), and includes MP’s reputed ability to enhance visual performance and/or comfort by diminishing the effects of CA and light scatter, via its light-filtering properties. The theory that filtering defocused SW light could enhance VA (and/or contrast sensitivity) by attenuating the effects of CA and light scatter goes back as far as Schultze 1866, however empirical evidence has yet to be established to support this theory. Importantly, the effect of MP on retinal, ocular and cortical health may also translate into indirect (non-optical) improvements in visual performance and comfort, and should not be excluded in the assessment of the overall role of MP on vision (Loughman et al. 2010). In

addition to its role as a SW blue light filter, it has been established that MP is a powerful and effective free radical scavenger, which supports the view that MP may mitigate the damaging effects of ROS on the physiological function of photoreceptors and their axons. These factors combine to enhance visual performance.

The 'optical' hypothesis of MP may be related to at least one of the following properties: MP may reduce visual discomfort by alleviating the effects of glare and dazzle; it may enhance visual contrast; MP may sharpen visual detail by the absorption of 'blue haze' or it may enhance VA by lessening the effects of CA (Walls & Judd, 1932). Given its SW absorption characteristics and central location, MP retains ideal properties to improve visual performance

The illumination experienced by people in the environment continually oscillates. Although adaptation facilitates performance over a wide range of ambient illumination levels, it does not mean however that we see equally well at all levels (Loughman et al. 2010). Eyes are very sensitive under dim conditions and can detect subtle changes in luminance, but acuity for pattern details and colour discrimination are poor. Visual parameters such as threshold visibility, colour appearance and VA are different at different illumination levels, and can change over the course of the day, and over the time course of light and dark adaptation.

MP reduces sensitivity of the macular region to SW light by acting as a broad band filter (Reading & Weale, 1974). Evolution of the primate eye has ensured that almost all UVB (290-320 nm) and UVA (320-400 nm) light, is absorbed by the cornea and the crystalline lens respectively. Slightly longer wave light (400-500 nm) is then largely absorbed by MP,

which has a peak absorbance of 460nm, before reaching the macula (Snodderly et al. 1984; Bone et al. 1992). Aspects of MP location within the retina are central to the idea that it has a role to play in visual performance and many factors contribute to this assumption.

(i) Although MP is diffusely distributed throughout the retina and other ocular structures (Davies & Moreland, 2004), it is mostly concentrated at the macula and remains optically undetectable beyond 7 degrees retinal eccentricity.

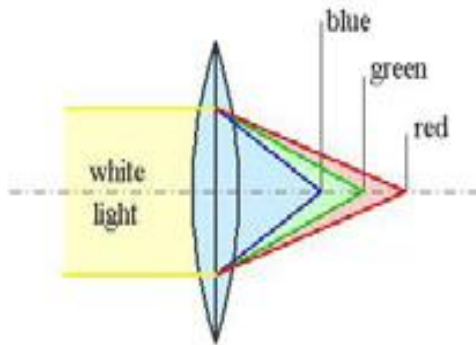
(ii) MP is located at a pre-receptor level, so that such absorptions are made prior to light stimulation of the underlying photoreceptors. The consequence of this is that the spectral distribution of light incident on the photoreceptors is altered.

(iii) MP is also distributed throughout the photoreceptor cell and therefore each photoreceptor screens other photoreceptors as well as itself because of the lateral course of the axons (Snodderly et al. 1984).

### **3.8.1 The ‘acuity’ hypothesis**

The original description of the spectral absorption characteristics of MP was made by Max Schultze, in 1866 (Schultze, 1866). He suggested that MP might improve VA in broadband illumination by filtering out SW energy before absorption by the photoreceptors. The most long-standing and unproven hypothesis deals with the possibility that MP improves VA, by reducing the effects of longitudinal CA (Wald, 1945). Because the refractive index of the human cornea differs slightly from the crystalline lens and because the refractive index within the lens is not homogenous, the cornea and lens do not provide the equivalent of an achromatic doublet. As a result visible wavelengths (400nm-700 nm) are not all perfectly focused on the retina. The effect of longitudinal CA across wavelength in terms of blur is non linear i.e. shorter wavelengths are significantly more blurred than longer wavelengths.

When an emmetropic eye is in focus for middle wavelength light (555nm) (natural daylight), it will be myopic by ~ 1.2 D for SW light (460 nm), and slightly hyperopic, ~ 0.50 D, for long-wavelength light (650 nm) (Gilmartin & Hogan, 1985; Howarth & Bradley, 1986). Considering the spectral energy of average sunlight, and the photopic spectral sensitivity of the normal observer (Wyszecki et al. 1982), an eye would be in perfect focus for daylight only at 560 nm, therefore much of the SW region would be seriously out of focus. This effect is known as longitudinal CA.



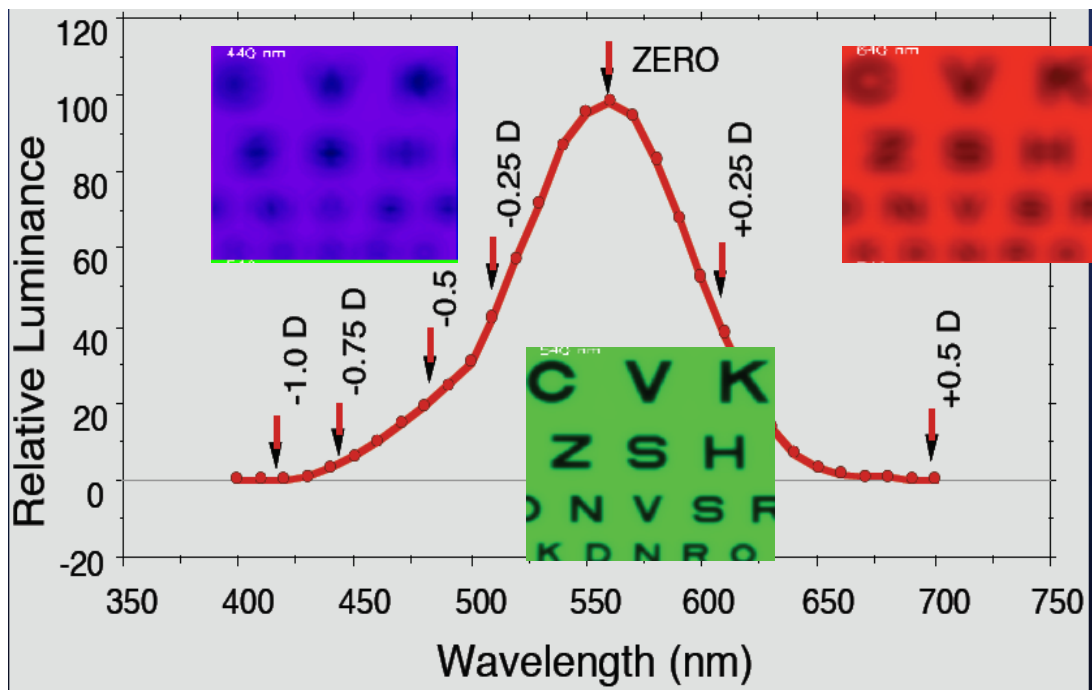
**Figure 3.3 Longitudinal chromatic aberration-focal length for incident white light varies across wavelength (Modified from Loughman et al. 2007).**

Also, the wavelength dependency of the eye's focal length means the retinal image size is proportional to wavelength, that is, the longer the wavelength the larger the retinal image. This effect is known as lateral or transverse CA. If a disc of white light is imaged on the fovea, a violet-blue penumbra will result. Together, longitudinal and lateral CA is known as CA.

Clearly both kinds of CA degrade the retinal image of any potential target. CA has been cited as possibly the most significant aberration affecting visual quality and can create up to

two dioptres of wavelength dependant optical defocus (Fig.3.4).The ultimate effect of such optical aberrations is that capacity limits are somewhat reduced. Visual acuity is also limited by diffraction and photoreceptor density in the eye (Smith and Atchison, 1997). Due to distortions created by the optics of the eye the image of a point source is distributed on the retina as a point spread function. An Airy disc pattern is formed from a point source due to the diffraction of light. Rayleigh's criteria is used to calculate the resolution of the eye for stimuli that are degraded by the optics of the eye. Retinal cone spacing can also limit VA, at least within the central 2 degrees (Green, 1970). Recent work on photoreceptor density and spatial resolution has shown that the receptor array in the human visual system can resolve in the order of 6/1 (20/3) or ~ 150 cycles per degree (Curcio et al.1990). Based on cone spacing a maximum of about 60 cycles per degree is possible, which is well above conventional clinical measures as this does not compensate for the optics of the eye and post receptor neural processing. Because VA is limited by diffraction, aberrations and photoreceptor density, the neural limits of acuity are seldom achieved even by healthy normal individuals (Loughman et al. 2010).





**Figure 3.4: Diagrammatic representation of the amount of blur induced by longitudinal CA when an eye is focused optimally for 555 nm. Note that the blue end is significantly more blurred than the red (Modified from Loughman et al. 2010 ).**

The ‘acuity’ hypothesis describes the concept that MP might improve VA for images illuminated by broadband (white) light by absorbing the poorly focused SW portion of visible light before this light is processed by the retina. In 1974, Reading and Weale presented a theoretical quantification of the absorption of SW light by MP, and concluded that its filtration of the aberrant part of the spectrum was appropriate to reduce CA to below threshold (Reading & Weale, 1974). Hence, it appears that the peak concentration of MP at the centre of the fovea is consistent with its role in minimising CA.

The yellow nature 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 (Bone et al. 1992). MP it appears, removes that component of light which has least benefit for photopic

vision from a performance perspective for a number of reasons; the number of SW sensitive (blue) cones are considerably less than long wavelength (red) sensitive cones at the macula; there is a complete absence of blue cones from the region of maximal visual performance, the foveola, and the peak retinal sensitivity lies at 555 nm. Images are not perceived to be degraded to the degree suggested by more than one dioptre of aberration because a pre-retinal filter system may reduce the amount of blue light reaching the photoreceptors. This results in a visual system, that is most sensitive to middle and longer wavelength, with minimised CA and better acuity (Wooten & Hammond, 2002).

Although individuals do not ordinarily notice the effects of CA on everyday vision, the negative effects of CA probably defines the upper limit of the eye's ability to resolve fine details (Thibos et al. 1991). The effects of foveal CA also varies widely between subjects (Howarth & Bradley, 1986), lending support to the idea that MP differences may contribute to these individual differences in contrast sensitivity. This interpretation is consistent with data showing that males have better acuity on average than females (Brabyn et al. 1979), and also tend to have higher average MPOD (Hammond et al. 1996; 2000). The 'acuity' hypothesis predicts that for targets illuminated by broadband (white) light, an individual's VA should be related to the density of their MP.

Historically spatial vision has been evaluated by acuity measures, that is, determining the finest discrimination possibly using very high contrast targets. In most clinical settings this is still the practice. Modern views of spatial vision, however, consider acuity as merely the upper limit of the more general contrast sensitivity function (CSF). In naturalistic conditions the mid and low spatial frequencies are certainly as important as the high spatial

frequencies, sometimes more so (Devalois & Devalois, 1988). Thus Schultze's original hypothesis linking MP, CA and acuity has been extended to include any measure of spatial vision where a sharpened image could improve performance. This certainly includes the entire CSF. For observers with low levels of MP, adding SW absorption with MP (either through diet and/or supplementation) or yellow lenses may improve spatial resolution as predicted by the 'acuity' hypothesis.

### **3.8.2 The 'visibility' hypothesis**

MP may also facilitate enhancement of detail by the absorption of 'blue haze'. Visible and non-visible particles, such as fog, smog, cloud, rain and haze aerosols, all contribute to light scatter. Wooten & Hammond (2002) 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 haze aerosols is the primary determinant of visual discrimination and range in the outdoors*'. This haze aerosol, as it is called, scatters SW light more than other wavelengths and results in a bluish veiling luminance. Blue haze is a major factor that degrades visibility.

It has been shown that compensation for the effects of light scatter could increase the visibility and discriminability of targets in natural settings (Wooten & Hammond, 2002). This could easily be achieved by increasing the density of MP. Problems caused by scatter are not consciously experienced by most people, but can become a significant symptom of which many patients complain. Patients with cataracts, corneal abnormalities and post laser refractive patients may all experience problems of light scatter. Scatter does have an adverse effect on the visual experience of normals and those with ocular abnormalities

alike, and any means to reduce the effects of scatter can only be of benefit. MP may improve vision through the atmosphere by preferentially absorbing SW energy produced by blue haze and thereby increasing the contrast of targets with respect to their backgrounds. This proposed role of MP is called the ‘visibility’ hypothesis.

### **3.8.3 Photophobia**

Photophobia is eye discomfort in bright light, and is continually encountered in clinical practice. Glare and dazzle are maximal in high luminance or high contrast situations. MP may however increase the threshold for photophobia under normal viewing conditions, by absorption of blue light. By removing the highest energy light component, the impact of glare on visual performance is minimised. People with retinal disease, advancing age, and cataract often complain of glare. It is a symptom that people of all ages have problems with, and very often without obvious reasons for predisposition. A common reason though may be, less than normal amounts of MP.

### **3.8.4 Optical and anatomic properties of macular pigment**

Evidence for the possibility that MP reduces glare may be inferred from its ‘optical’ properties. The optical effect of MP is evidenced by two entoptic phenomenon, which are specific to the macula, namely Haidinger’s brushes (Nussbaum et al. 1981) and Maxwell’s spot (Magnussen et al. 2004).

Because of their linear structure it is known that lutein, zeaxanthin and *meso*-zeaxanthin exhibit dichroic properties (Hemenger et al. 1982; Bone & Landrum, 1983; Bone et al. 1998). Lutein is reported to be a superior filter of SW light, compared to zeaxanthin, due to

its orientation in the cell membrane, which is both parallel and perpendicular, whereas zeaxanthin and *meso*-zeaxanthin exhibit perpendicular orientation only. Since the cone axons radiate outwards from the centre of the fovea, forming the Henle fibre layer, the preferential alignment of MP molecules within those fibres cause an entoptic phenomenon called 'Haidingers brushes' (Bone & Landrum, 1984). MP therefore has some of the same qualities as polarising sunglasses used for reducing glare. It is possible that MP could reduce glare by preferential absorption of polarised light and by absorbing forward intra-ocular light scatter, before it degrades foveal vision. The collective effect of the three carotenoids together offers optimal filtration of SW light, as these carotenoids have different absorption spectra (Sujak, 1999).

Maxwell's spot is another entoptic phenomenon which is due to preferential absorption of blue light by MP. A reddish spot can be seen in the centre of the visual field when a white surface is viewed by a normal observer through a dichroic filter transmitting red and blue lights. This phenomenon is attributed directly to the deposition of pigments at the macula. Magnussen et al (2004) have shown that the absence of short-wave-sensitive cones in the human foveola results in a blue scotoma which can be visualised as a negative afterimage. 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 (Loughman et al. 2010). MP distribution measured for the same observers corresponded closely to the lighter annular region of the afterimage.

MP is certainly reduced in individuals with age-related macular disease and this may partly explain the problems with glare in this population. Given the substantial inter-individual

variability of MP levels, it is plausible that sensitivity to glare is increased in those with low pigment levels (Werner, 1990; Hammond et al. 1998; Hammond et al. 2001).

Human visual performance tends to diminish with age. The decrease is very slow until approximately the fifth decade of life but seems to accelerate after that time. Most of the deterioration in performance can be traced to the slow degradation of the retina and lens. Wavefront-guided laser refractive eye surgery, blue filtering intra ocular lens (IOL) implants, blue filtering contact lenses and blue filtering spectacle lenses all attempt to improve or optimise visual performance.

To date there hasn't been a centralised objective to improve (or maintain) visual performance among normals, despite recognition of the optical limitations affecting vision. MP acts as an optical filter and it is believed that MP could improve visual performance through both optical effects (the 'acuity' and 'visibility' hypothesis), and by maintaining the health and functional integrity of the retina and lens (Hammond et al. 2001).

### **3.9 Visual Health Hypothesis**

The established descriptions of the optical and glare hypotheses focus solely on the optical filtration properties of MP. However, MP may have a beneficial effect on visual performance and experience because it acts as a powerful antioxidant (Khachik et al. 1997). By mopping up free radicals, MP may attenuate or prevent long-term damage to the physiological function of the photoreceptors and their axons. Along with its SW filtration capacity, MP is an efficient antioxidant and by creating a healthier retina, has the potential to impact on visual performance, including glare and photostress recovery.

There is an inverse relationship between MPOD and lens density (Hammond et al. 1997). Also, the presence of lutein and zeaxanthin in substantial concentrations in the primary visual cortex (Craft et al. 2004), and the finding of better dark adapted cone sensitivities in association with higher MP (Carboni et al. 2010), combine to suggest a key role for MP in ocular and neurophysiologic health. The majority of studies investigating the effects of MP augmentation in ocular disease, including AMD, have reported a beneficial effect in vision (Richer et al. 2004, Chakravarthy et al. 2009). These findings may be due to the neuroprotective effect, along with, the optical properties of these carotenoids.

Light absorbed by the RPE may result in the generation of phototoxic products and a slight rise in temperature which results in loss of pigment integrity. This results in a loss of blood retinal barrier with consequent leakage of systemic proteins reaching the photoreceptors, where membrane de-stabilisation of the discs in the outer segments occurs (Kennedy et al. 1995, Curcio et al. 1996). Melanin, enzymes and antioxidants (MP) probably neutralise the phototoxic products normally but with excessive light exposure these mechanisms are less efficient (Ham et al. 1978, Taylor et al. 1990, Lam et al. 1990). When RPE cells are treated with lutein, phototoxic effects are reduced greatly (Boulton et al. 1993). The macular pigments, lutein, zeaxanthin and *meso*-zeaxanthin absorb SW light and also quench singlet oxygen. Because of these effects, MP is thought to reduce the potential for auto-oxidation in the central retina, thereby resulting in a healthier and more viable photoreceptor layer. A complex interplay between optical, neurological and physiological mechanisms underlies vision which can be uniquely affected by MP (Nolan et al. 2011).

### **3.10 Measurement of Macular Pigment**

MP can be measured in donor eyes or in live subjects. *Ex-vivo* techniques include autopsy analysis using HPLC (Bone et al. 1985), and micro densitometry (Snodderly et al. 1991).

*In-vivo* techniques can be divided into two categories: Subjective psychophysical techniques and objective optical techniques.

- Psychophysical: Colour matching or heterochromatic flicker photometry (HFP) using “maxwellian” and “free-view” systems.
- Image-based: Fundus reflectometry and Auto fluorescence.
- Signal based: Raman spectroscopy.

#### Ex vivo techniques

The main limitations of MP measurements in donor eyes include the need for expensive specialist equipment, and the laborious preparation and fixation of the tissues that is required if potential postmortem alterations in the spatial profile of the pigment are to be avoided (Snodderly et al. 1984). One obvious limitation is that postmortem measurements do not allow investigators to prospectively study MP and factors that influence it such as diet.

#### In vivo techniques

##### **3.10.1 Fundus reflectometry**

In the imaging mode, fundus reflectometry measurement of MP is typically obtained at the fovea and parafoveal region using a fundus camera attached to a charge-couple device and a scanning laser ophthalmoscope (Kilbride, 1989). The method is objective and has the capability to map the spatial distribution of MP, but is based on the assumption that all the



reflected light that is detected has been attenuated by MP, however since other absorbers exist in the eye, it cannot be considered chemically specific. Imaging fundus reflectometry also requires pupil dilation in some implementations, expensive equipment and technical expertise which limit its widespread use.

### **3.10.2 Auro fluorescence imaging (AFI)**

This method measures MPOD levels by determining MP's attenuation of the fluorescence of lipofuscin in the RPE. MPOD levels are calculated from the difference in lipofuscin fluorescence intensities at foveal and extrafoveal sites (typically 7 ° eccentricity) (Delori, 2001). AFI has a number of distinct advantages in that it can map spatial variation in MP without pupil dilation, is objective, rapid, requires little training of the subject and minimises confounding scattering effects from the anterior ocular media.

### **3.10.3 Signal based techniques**

Resonance Raman Spectroscopy (RRS):

RRS measures the excitation of bond vibrations within molecules which is directly proportional to the concentration of MP compound existing in the irradiated macular area (Bernstein et al. 2002). One advantage of RRS as compared to HFP, reflectometry and AFI, is its high chemical specificity. RRS measurements of MP however have limitations. In particular absorbance or scattering of the lens can attenuate the Raman signal. Estimation of MP levels in older populations using this technology should therefore carefully take into account the status of the lens (Hogg et al. 2007). Wide pupil dilation is also generally required for measurement, and rather high levels of light are used, so an expensive laser light source is required.

### 3.10.4 Heterochromatic flicker photometer technique

The most commonly used psychophysical technique utilises HFP to establish the optical density of the pigment at the foveal centre, which is proportional to its concentration. A detailed description of this procedure may be found in chapter five (section 5.3.7). HFP used for taking measurements of MP is reproducible, exhibits good test-retest reliability (Hammond et al. 1997), and shows good agreement with absorbance spectra generated from *in vitro* preparations of liposome bound zeaxanthin and lutein (Bone et al. 1992). A spatial profile of MPOD level is also possible with this technique. Although HFP may be minimally invasive as it does not require pupil dilation, and uses advantageously low light levels, it is a psychophysical procedure, necessitating both proper training of the subject and his or her attention while performing the measurement. Also subjects must have normal corrected or uncorrected VA to fixate the central and peripheral targets.

The fact that MP can be enhanced has prompted interest in its measurement on a large scale. MP is modifiable through dietary changes and/or supplementation so accurate, repeatable and non-invasive methods of measuring MPOD are becoming increasingly important. The ability to measure MP *in-vivo* is therefore very valuable in determining the possible risk of developing AMD and in monitoring treatment. It is thought that an individual's MP level is not followed by a rapid decline following discontinuation of the modified diet, unlike serum levels of lutein and zeaxanthin. Studies have shown that it reflects long-term carotenoid consumption (Landrum et al. 1997). However, recent studies have shown that this may not be the case (Connolly et al. 2010; Loughman et al. 2011). A machine for measuring MP, which is accurate and reliable, would certainly prove useful as

it would provide a good indication of a person's overall MP status and a means of monitoring MPOD.

HFP has recently been employed in the development of desktop devices for measurement of MPOD in clinical practice and commercially available devices include the MacuScope (Macuvision Europe Ltd, Solihull, United Kingdom) and the MPS 9000 (also known as the M:Pod and the QuantifEYE, Topcon, Newbury, Berkshire, UK) and a new clinical version of the Densitometer by Billy Wooten. HFP is an important and accessible clinical means to analyse MPOD and this method is now being applied to larger populations, therefore, the effects of individual differences in MP can now begin to be considered (Curran-Celentano et al. 2002).

### **3.11 Dietary Assessment of Lutein and Zeaxanthin**

Mean daily intake of lutein and zeaxanthin combined, ranges from 0.8 mg to 4 mg per day, depending on the population studied and the method of dietary assessment used (Sommerburg et al. 1998; Landrum et al. 2001). A recent Irish study, involving 826 subjects estimated mean lutein/zeaxanthin intakes of 0.6 - 2.4mg/day (Nolan et al. 2007). Based on the mean dietary intake and serum concentration reported in the above Irish study, subjects would need a dietary lutein/zeaxanthin intake of 5-8mg/day to achieve optimal serum levels. United States Department of Agriculture (USDA), recommend 4-8mg/day, while research recommends 6-10 mg/day, both of which are achievable through dietary means (USDA, 2005). Lifestyle events and factors which indicate a need for more foods rich in these carotenoids include smoking and regular alcohol consumption, plus low intake of fruits and vegetables. Long-term inadequate intake of carotenoids is associated

with chronic disease including heart disease and various cancers (Block et al. 1992; Gaziano et al. 1995).

### **3.11.1 Evidence that macular pigment can be augmented**

From animal studies, it has been shown that animals raised on lutein and zeaxanthin free diets, form no MP (Leung et al. 2004). Malinow et al. (1980) studied the retinas of macaque monkeys fed a carotenoid-free diet for more than three years and compared results with those of control primates on a standard diet containing lutein and zeaxanthin. Colour fundus photography and fundus fluorescein angiography indicated a total absence of MP in those animals not receiving carotenoids. A normal foveal appearance was evident in the control monkeys. Plasma lutein and zeaxanthin was undetectable in the primates deprived of dietary carotenoids.

Neuringer et al. (2004) studied monkeys that were supplemented with either pure lutein or pure zeaxanthin and found that, although serum levels of lutein rose faster than those of zeaxanthin, by approximately 16 weeks both had stabilised at comparable concentrations, potentially indicating lutein and zeaxanthin had reached saturation levels. If the accumulation of lutein or zeaxanthin in the macula eventually reaches a saturation level, a slower rate of increase of optical density in subjects whose density is already high would be expected. The tendency for post supplementation optical density to remain elevated is consistent with previous studies (Landrum et al. 1997; Hammond et al. 1997), but not with others (Connolly et al. 2010; Loughman et al. 2011).

The ability to increase the amount of MP by dietary supplementation with lutein has been demonstrated (Hammond et al 1997; Landrum et al. 1997). Since lutein and zeaxanthin cannot be endogenously synthesised, a diet rich in these carotenoids is necessary to increase MP (Mares et al. 2006). Consumption of certain fruits and vegetables will increase dietary intake of lutein and zeaxanthin (Sommerburg et al. 1998). Hammond et al. (1997) reported that an average increase of approximately 20% in human MPOD was obtained after four weeks of a diet enriched in corn and spinach, but not in all subjects. Three types of response to corn and spinach supplements were identified out of 11 subjects. They reported eight retinal 'responders', where subjects showed a significant increase in MPOD and serum lutein. There were two retinal 'non-responders', in whom serum lutein increased significantly without a parallel increase in MPOD and finally there was one retinal and serum 'non-responder', in which no significant rise in MPOD or serum carotenoids was noted (Hammond et al. 1997).

In another study, two subjects who took a daily dose of 30 mg of lutein for 140 days had mean increases in MPOD of 39% and 21% in their eyes respectively (Landrum et al. 1997). The authors estimated that this increase in MPOD resulted in a 30% to 40% reduction in SW light reaching the photoreceptors, Bruch's membrane and the RPE. The persistence of raised MPOD following discontinuation of lutein supplements and return to pre-supplementation serum levels of lutein prompted Landrum et al. (1997) to postulate a low turnover of carotenoids in the retina.

The response to carotenoids however varies among individuals. Studies have suggested that individuals differ in their ability to absorb nutrients from food into their tissues. Some

individuals can have a relatively high intake of fruits and vegetables and high nutrient blood levels but low levels of retinal nutrients. Effective absorption of lutein and zeaxanthin from the alimentary tract depends on many processes including digestion of the food matrix, the formation of lipid micelles, and uptake of the carotenoids by mucosal cells and transport of the carotenoids to the lymphatic or portal circulation (Beatty et al. 2004). Hammond and coworkers measured MPOD in 88 subjects and correlated the results with serum levels of lutein and zeaxanthin and with dietary intake of carotenoids for males and females (Hammond et al. 1996). It was found that MPOD for males was 38% higher than for females and was positively and significantly related to dietary intake of carotenoids for males only (Hammond et al. 1996). In contrast, plasma lutein and zeaxanthin correlated significantly and positively with the density of MP and with dietary intake of carotenoids for both sexes. It was postulated that the greater MPOD in men may be as a result of the difference in the way carotenoids are metabolised by the male and female retina.

More recently dietary supplements have included the third major carotenoid of MP, *meso*-zeaxanthin. Landrum et al (2007) conducted a study with 10 subjects, who were given gel caps that provided 20 mg/day of *meso*-zeaxanthin and smaller amounts of lutein and zeaxanthin. The authors showed for the first time that *meso*-zeaxanthin is absorbed into serum following ingestion and that there was a significant increase in MPOD following predominantly *meso*-zeaxanthin supplementation compared to the placebo group.

Connolly et al (2010) measured MPOD and serum levels of carotenoids in a group of 10 subjects instructed to consume a formulation containing 7.3 mg of *meso*-zeaxanthin, 3.7 mg lutein and 0.8 mg zeaxanthin every day for an eight week period. The authors reported a

significant increase in serum concentrations of *meso*-zeaxanthin and lutein following supplementation with the above formula and a significant increase in MPOD after only two week of supplementation, highlighting that the turnover in the retina may not be at low as was originally thought (Landrum et al. 1997; Loughman et al. 2011).

### **3.11.2 Dietary factors affecting macular pigment absorption**

Lutein and zeaxanthin are fat soluble substances and require the presence of dietary fat for proper absorption through the digestive tract. A minimum of 3g of fat in a meal is necessary for efficient absorption of carotenoids, except for lutein esters, which require >3g of fat/meal (Roodenburg et al. 2000). As a result, carotenoid status may be impaired by a diet that is extremely low in fat. Studies show that lutein is much better absorbed from egg yolk than lutein supplements or even spinach, as it is more bioavailable. Certain medical conditions that result in a reduction in the ability to absorb fat, such as crohns disease, celiac, cystic fibrosis or gall bladder disease may effect the absorption of these carotenoids, which would result in low concentration of lutein/zeaxanthin in the macula. Serum concentrations however can be used to rule out any malabsorption problems. Sublingual sprays are now available for people with these conditions. With these sprays lutein and zeaxanthin are absorbed directly into the bloodstream under the tongue, therefore bypassing the stomach.

Dietary fibre has been shown to impair lutein absorption (Riedl et al. 1999), as it suppresses bile salt excretion, and therefore micelle formation. Other forms of dietary fibre such as wheat bran may decrease the absorption of lutein. The cholesterol lowering medications referred to as bile sequestrants also lower blood levels of carotenoids. The butter spreads

enriched with plant sterols such as 'benecol' may also decrease the absorption of lutein and zeaxanthin. High density lipo proteins (HDL's) are known to be primary carriers of lutein and zeaxanthin (Viroonudomphol, 2003), and hence an individual's lipoprotein profile may influence the transport and delivery of these carotenoids to the retina, with a consequential impact on MP.

The accumulation of MP carotenoids at the macula is variable, and affected by a multitude of previously defined factors (Snodderly et al. 1984). Apart from ocular tissue, adipose tissue is a major storage site in the body for these xanthophylls and as a result serum measurements of lutein and zeaxanthin have not been comparable to MPOD (Beatty et al. 2008). Human MPOD however can be augmented with dietary modification and/or nutritional supplementation and the protective effect of MP, if any, can be investigated through experimental observation of MP levels. By measuring MPOD on a wide scale, it will be possible to analyse the effects of augmentation of MP on disease process and prevalence.

### **3.11.3 Recommended daily allowance (RDA)**

Dietary modification (Hammond et al. 1997) or supplementation with purified supplements (Bone et al. 1988; Landrum et al. 1997) have been shown to increase MPOD. As lutein and zeaxanthin are fat soluble, they are not excreted daily from the body and hence there is an unknown potential for toxicity. Despite the absence of an RDA, set by the Food Safety Authority, lutein has been incorporated into dietary supplements since 1996. The majority of lutein supplements contain in the range of 6 - 25mg/day, well above the current average Irish intake. Toxicity levels of lutein are to date unreported. However, when supplement



dosages are compared with RDA and Daily Recommended Intake (DRI) levels, they are however well above recommended levels. Most notably the fat soluble vitamins, with quantities of up to 40 times the DRI for vitamin E (no RDA set), 12 times the RDA for vitamin A, 10 times the RDA for zinc, 8 times the RDA for vitamin C, 2 times the RDA for copper and, 1.4 times the RDA for selenium (See Appendix A). High-dose zinc can cause gastric irritation or anemia (Johnson et al. 2007), vitamin A (as beta carotene) has been associated with an increased risk of lung cancer among smokers (Omenn, 1996), and increased risk of heart failure in at-risk populations who consume high levels of vitamin E supplements (Yusuf et al. 2000). More recently, an Irish study found lutein supplementation depressed the absorption and plasma concentrations of beta-carotene (Thurnham et al. 2008), suggesting that carotenoids may compete with each other for absorption. As a result of insufficient evidence and absence of an official RDA for lutein and zeaxanthin, a food based rather than a compound based approach is recommended (Granado et al. 2003).

#### **3.11.4 Dietary sources of lutein, zeaxanthin and *meso*-zeaxanthin**

Lutein and zeaxanthin are found in a typical Western diet, in fruit and vegetables (e.g., spinach, corn, orange peppers, red grapes) (Sommerburg et al. 1998), whereas *meso*-zeaxanthin has been identified in some less commonly consumed foods including fish (e.g. salmon and trout), shrimp, and turtle fat (Maoka, 1986). A healthy diet including these carotenoids is strongly recommended as many supplements are often well in excess of the recommended levels of certain minerals and vitamins, with some dosages carrying potential adverse effects. Achieving adequate lutein and zeaxanthin intake through dietary means would be a positive first step in maintaining eye health and avoiding degenerative

consequences of age-related eye diseases such as AMD. Foods which are high in lutein and zeaxanthin are outlined in Table 3.1. This table also highlights the number of portions a person would need to incorporate into ones diet, in order to achieve the daily recommended 6 mg's of lutein and zeaxanthin. If dietary or lifestyle practices can enhance MP, improve quality of life and possibly delay or prevent vision loss, then decreased health care costs may be realised. Beneficial effects of MP may reside in its ability to protect against chronic and cumulative damage. In other words MP levels in youth and middle age are likely to determine the protection, if any, that this pigment confers against AMD. Lutein and zeaxanthin in the macula, as measured by MPOD, is affected by dietary intake more slowly, and can be used as a long term marker, over 2-3 months, of nutritional status (Granado et al. 2003).

**Table 3.1 Lutein and zeaxanthin 6 mg/day exchanges.**

Food	mg/100g	Medium portion (g)	Food (g) = 6mg exchange	Choose 1 No. of portions needed to provide 6 mg
Broccoli©	0.83	85	723	9
Brussels Sprouts©	1.29	90	465	5
Cabbage®	0.31	95	1935	20
Carrots®	0.358	60	1676	28
Corn©	1.8	146	333	2
Green beans©	0.7	90	857	10
Green peas©	1.35	70	444	6
Minestrone soup ©	0.15	220	4000	18
Spinach ©	7.043	90	85	1
Spinach ®	11.938	90	50	1
Tomatoes®	0.13	85	4615	54
Fruit cocktail	0.112	115	5357	47
Oranges	0.187	160	3209	20
Orange Juice	0.105	160	5714	36
Tangerine	0.243	60	2469	41
Eggs	0.55	50	1091	22
Kellogg's Cornflakes	0.339	30	1770	59

C = cooked R = Raw

**Table 3.1: Lutein and zeaxanthin 6mg exchanges, highlighting the number portions needed in order to achieve the recommended 6mg of carotenoids a day through dietary means (Values calculated from data used in modified WISP nutrient data base; see chapter six).**

However, individual differences in MP may also be influenced by non dietary factors such as genetics, demographics and lifestyle characteristics. Concentrations of carotenoids in human serum and tissue are extremely variable and reflect not only diet and supplement use, but factors such as carotenoid chemistry, individual efficiency of absorption, fat intake, competition among carotenoids for absorption, cholesterol and lipoprotein status, metabolic status, body composition and BMI. A holistic approach needs to be taken with regard to MP. Diet is important, but so is knowing that these carotenoids are being absorbed, therefore serum measurements are also necessary. Because of the known existence of retinal ‘non-responders’ it is important that we can measure MP in the eye to ensure people are benefiting from their protective and visual effects.

The appropriate age profile for subjects to be targeted for any protective effect that these retinal antioxidants/diet may confer against AMD is the 20 yr group and upwards, as changes are occurring in the retina on a microscopic level many years before AMD actually presents. We can subsequently relate our findings to established and putative risk factors for this condition (O’Connell et al. 2008). Studies are needed to establish the optimal daily intake of lutein and zeaxanthin to maintain a healthy retina. At present, however, there are no definitively established physiologically significant cut off point for lutein in serum above which ‘protection’ or ‘prevention’ against chronic diseases are

ensured. Short and long term studies, using different protocols, doses and sources of lutein (diet and capsules) have shown that serum concentrations of lutein in the range of 0.6-1.05 $\mu$ mol/l, achievable through diet, produce optimal beneficial effects, including an increase in MPOD upon supplementation, (Hammond et al. 1997; Landrum et al. 1997; Johnson et al. 2000), and an improvement in visual performance in subjects with compromised visual function (Olmedilla et al. 2001; Richer et al. 2004).

## **CHAPTER FOUR**

### **Macular Pigment: It's Contribution to Visual Health of the Eye and Visual Performance and Comfort.**

The current epidemic of AMD has directed researchers to investigate the 'protective' hypothesis of MP in detail. This chapter will focus on the evidence which supports the view that people with low levels of MP are at a higher risk of developing AMD (EDCCS, 1993; Beatty et al. 2001; Bone et al. 2001; Bernstein et al. 2010). It has been proposed that MP protects the macula by two processes (Beatty et al. 2000). Its presence in the photoreceptor axons (Snodderly et al. 1984), together with the range of spectral absorbance (~400 – 500 nm), provides MP with the ability to shield posterior tissues such as the photoreceptor segments and RPE from actinic blue light. Additionally its presence in the outer segments and RPE (Rapp et al. 2000), may mitigate the photo-oxidative damage caused by SW light, via its antioxidant and free radical scavenging properties. Evidence to support the view that MP enhances visual performance and comfort, by attenuating SW light, will be reviewed later in this chapter. This hypothesis however, remains largely unproven and poorly investigated.

#### **4.1 Age-Related Macular Degeneration**

Advanced AMD is the leading cause of irreversible vision loss in individuals more than 65 years of age in the Western world, with the prevalence increasing over the last 50 years (Evans et al. 1996). It accounts for 8.7% of blindness world wide (WHO, 2002), and is the leading cause of registered blindness in Ireland (Kelliher et al. 2006). AMD results in a gradual breakdown of light-sensitive cells, in the macula, which results in the loss of central vision or straight ahead vision and not total or peripheral vision loss (Rosenfeld et al.

2009). The macula accounts for only 4% of the retina, however it contains a high concentration of photoreceptor cells, which are responsible for central vision, seeing colour, and distinguishing fine detail (Forrester et al. 1996).

#### **4.1.1 Classification of AMD**

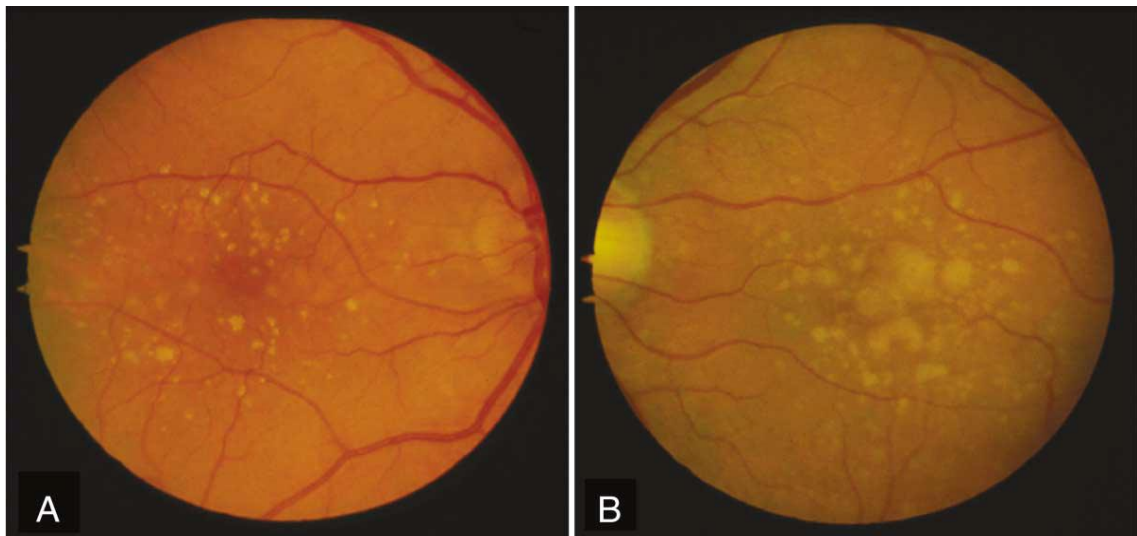
In an effort to standardise disease definition and study methodology, the International ARM published in 1995 an international classification and grading system for AMD, in the hope of producing a common detection and classification system for epidemiologic studies (The International ARM, 1995). In that article, all age-related changes are referred to as ARM.

ARM is characterised by any of the following macular findings: soft drusen; areas of increased pigment or hyperpigmentation associated with drusen; areas of depigmentation or hypopigmentation associated with drusen. Of note, hard drusen are not included in the definition ARM. AMD is reserved for the late stages of ARM. Dry AMD refers to GA and wet AMD is characterised by choroidal neovascularisation (CNV), detachment of the RPE, sub retinal hemorrhage, or retinal scarring (The International ARM, 1995).

The disease entity AMD has also been described as either: Dry or Wet; Non-Exudative or Exudative; Early or Late. This terminology is commonly used in everyday clinical practice.

#### 4.1.2 Early stage AMD

Early AMD (or early/dry ARM as it is correctly termed) is defined as the presence of soft drusen ( $> 63\mu\text{m}$ ) alone, associated RPE pigmentary changes alone or a combination of distinct/indistinct drusen with pigment irregularities and mild loss of VA (Ambati, 2003). Soft drusen precede macular degeneration, and can lead to RPE atrophy, with resultant overlying photoreceptor atrophy and vision loss (Gass, 1973; Bressler, 1990). When vision falls below or equals 20/30, the disease is termed non-neovascular or dry macular degeneration. GA is an advanced form of dry AMD.



**Figure 4.1 Comparison of the gross appearance of the two major drusen phenotypes observed at the funduscopy level. “Hard” drusen (A) tend to be smaller with relatively distinct margins, whereas “soft” drusen (B) are larger and typically have less distinct borders (Modified from Hageman et al. 2001).**

#### 4.1.3 Late/dry AMD/advanced form of non-exudative AMD/GA

In addition to drusen, there is a breakdown of light-sensitive cells and supporting tissue in the central retinal area. The dry form of AMD can be considered a typical neurodegenerative disease where a primary defect disrupts the function and viability of a

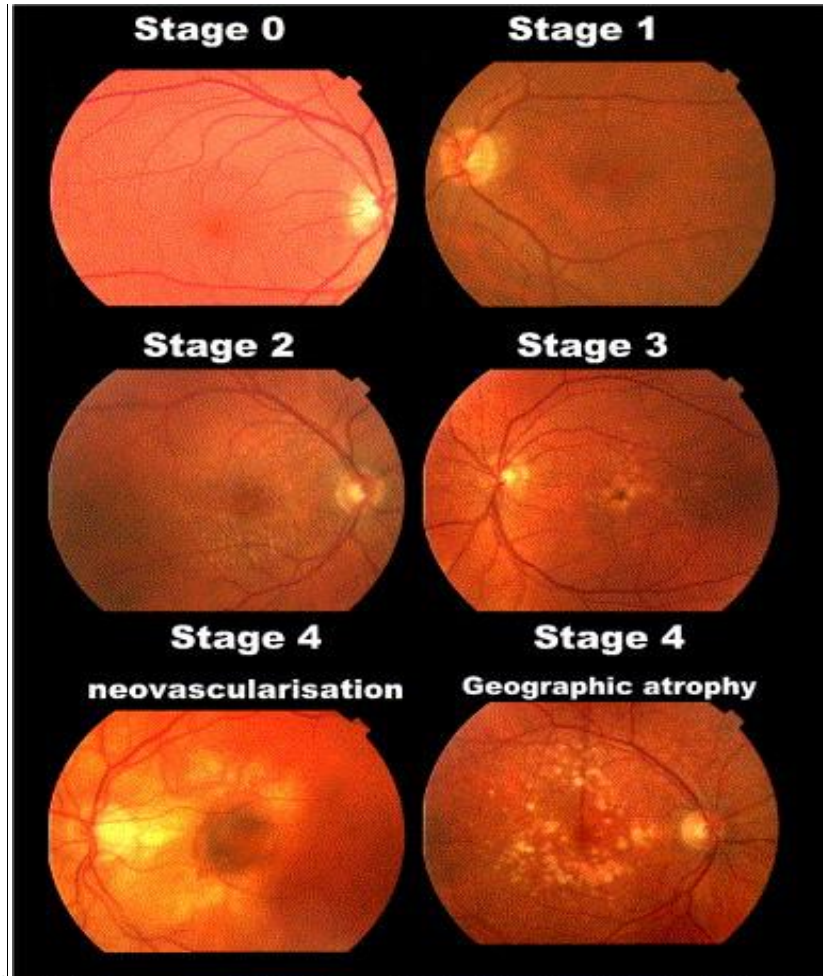


specific group of neurons: photoreceptors. The advanced form of non-exudative AMD, GA, is characterised by outer retinal and RPE atrophy with loss of choriocapillaris. As the disease progresses to a more advanced form, total loss of central vision may result (Bird et al. 1995). Focal hyper pigmentation along with the presence of five soft, large and confluent drusen is associated with an increased risk of progression of RPE atrophy and choroidal atrophy. These eyes have higher incidence of developing CNV (Sarks, 1980). Non-exudative AMD is the most common form of AMD accounting for 80% to 90% of cases overall (Kahn, 1977).

#### **4.1.4 Exudative AMD or wet AMD/CNV**

Although less prevalent than ‘Dry’ AMD, ‘Wet’ causes a more sudden and a greater central vision loss. The presence of sub retinal fluid, sub retinal hemorrhage, RPE detachment, or hard exudates indicates CNV, which heralds the onset of exudative macular degeneration (Klein, 1992). Choroidal capillaries, the mainstay of nutritional support for the outer retina, proliferate and extend through defects in Bruch’s membrane and junctional complexes of the RPE layer into the sub retinal space. Disruption of the normal retinal architecture, serous and hemorrhagic detachment of the RPE and retina, pigment remodelling and disciform scarring contribute to the death of photoreceptors in the macular region (Bressler, 1990; Green & Enger 1993). Cone loss is secondary to the abnormal growth of leaky blood vessels. CNV affects approximately 10% of the AMD patient population. CNV alone however represents 80% of the legal blindness from AMD. The loss of vision is rapid and severe and the chief method of diagnosis is angiography.

AREDS have introduced the concept of staged disease severity with ARM 1, 2, 3 representing combinations of drusen and pigmentary irregularities, and 4 when CNV or GA is seen (Fig 4.2) (AREDS, 2001; Hogg & Chakravarthy, 2010).



**Figure 4.2: Disease severity scale with ARM 1, 2, 3 representing combinations of drusen and pigmentary irregularities, and 4 when CNV or GA is seen (Modified from Hogg & Chakravarthy, 2010).**

#### **4.1.5 Geographic atrophy**

Retinal areas affected by GA involve the parafoveal and perifoveal retina early in the disease, sparing the foveal centre until late in its course (Sarks et al. 1988, Weiter et al. 1988). The central sparing of annular macular degeneration also suggests that MP plays a

protective role against certain disease processes and it refers to an annular pattern of atrophy in the perifoveal region with sparing of the fovea, seen in RP and AMD (Sarks et al. 1988). There have been reports (some dating > 50 years) suggesting visual benefit in RP from lutein-containing medications (reviewed by Nussbaum, 1981). Dagnelie et al. (2000) assessed the effect of lutein supplementation in patients with RP. They reported moderate vision improvements in response to short-term lutein supplementation.

Despite good VA, many patients with GA have difficulty in reading because of an inability to see a full enough central field as scotomas near the fovea and involving the foveal centre compromise visual performance. VA alone is an inadequate marker of visual function in patients with GA. Patients may complain that they can read small news print but not larger headline letters. For this reason, it is important to take into account that such patients may be able to read smaller letters on a Snellen chart even if they are unable to read the 20/400 letter (Sunness et al. 1997). Patients with GA may also complain of having great difficulty recognising faces stemming from their inability to assimilate all features simultaneously. Glare problems, delayed or decreased dark adaptation, loss of contrast are all common symptoms of retinal degenerations, and it has been suggested that such symptoms are due to low macular pigment levels, resulting in a failure to absorb scattered light which causes excessive photoreceptor bleaching by SW light components (Haegerstrom-Portnoy, 1988).

Because GA can be clinically visualised in many patients before the development of moderate or severe vision loss, unlike CNV, there is greater potential for medical intervention to preserve visual function. While there is currently no definitive treatment to reverse the progression of GA, there are therapies for retarding disease progression.

Supplementation with dietary antioxidants has been shown to slow the progression of ‘Dry’ AMD to the next stage over five years (AREDS, report no 8, 2001), but once dry AMD reaches the advanced stage no form of treatment, laser or other, can prevent vision loss.

## **4.2 Circumstantial Evidence**

The evidence to support the hypothesis that MP protects against AMD may be classed as circumstantial, epidemiological, experimental or clinical. The term circumstantial refers to parallels between the risk of developing ARM and factors associated with low MPOD. These parallels include light iris colour (Hammond et al. 1996), cigarette smoking (Hammond et al. 1996), female sex (Hammond et al. 1996) and increasing lens density (Hammond et al. 1997).

### **4.2.1 Iris colour**

Iris colour is a hereditary factor that may be associated with AMD (Weiter et al. 1985). Investigators have demonstrated an inverse relation between iris pigmentation and the risk for ARM (Weiter et al. 1985; EDCCS, 1992), however reports have not been unanimous, as the Beaver Dam Eye Study did not find any relationship between iris colour and the incidence and progression of AMD (Klein, 1998).

In 1996, Hammond et al. reported a significant and positive relation between MPOD and iris pigmentation. Possible explanations to account for their findings include the possible shared tendency to accumulate melanin and retinal carotenoids, as both mechanisms co-evolved in response to environmental stresses such as light and oxygen. In some studies Iris colour was used as an indicator for eye pigmentation. Blue eyed caucasians were found to

have higher retinal stray light values compared to pigmented brown eyed non-caucasians, leading to the conclusion that pigmentation is a source of variation in straylight in normal eyes (Ijspeert et al.1990, Elliott et al.1991). Van den Berg (1991) showed that this pigmentation dependence is partly caused by variations in transmission of light through the ocular wall. For dark brown eyes of pigmented individuals, transmission was found to be two orders of magnitude lower than for blue eyed individuals. MP depletion may therefore occur as a result of increased oxidative stress in those eyes with light coloured irides because of increased light transmission (Van den Berg et al. 1991).

#### **4.2.2 Cigarette smoking**

In a recent review paper by Chakravarthy et al (2010) on the clinical risk factors for AMD, current cigarette smoking showed a strong and consistent association with late AMD. Significant increases in AMD were seen in a meta-analysis for current versus never smoked (included in this meta-analysis were six prospective studies, five case-control studies and five cross-sectional studies). In 1996, Hammond et al. reported their measurements of MPOD in 34 cigarette smokers and compared the results with those in 34 non-cigarette smokers matched for age, sex, dietary patterns and overall pigmentation. It was found that tobacco users had significantly less MP (MPOD 0.16) than control subjects (MPOD 0.34). Further, smoking frequency was inversely related to MPOD ( $r=-0.448$ ). It is known that smokers have lower concentrations of carotenoids in their bloods (Handleman, 1999). One important deleterious effect of smoking may be increased oxidative stress to tissues. Therefore, as oxidative damage has been causally linked to CNV (Snodderly, 1995), it is possible that a lack of macular carotenoids among smokers may shift the oxidant/antioxidant balance in favour of neovascular AMD (Hammond et al. 1996).

Inter-individual variation in MP density is high (Bone et al. 1988; Hammond & Fuld, 1992) and cigarette smoking has been found to be one of the major factors for this large degree of variability (Hammond et al. 1997). Smoking over 25 cigarettes a day has a more pronounced detrimental effect on MPOD (Hammond et al. 2005). Smoking increases oxidative burden (Ambrose & Barua, 2004), decreases plasma and tissue vitamin C, reduces retinal capture of zeaxanthin in heavy smokers (Nolan et al. 2007; O'Connell et al. 2008) and decreases HDL compared with non-smokers. To date smoking is the most consistently established modifiable risk factor for AMD (Delcourt, 1998).

#### **4.2.3 Female sex**

In 1996, Hammond et al. investigated the sex differences in MPOD, adjusted for age and caloric intake, and found that males had an average of 38% more MP than females. It has been suggested that lutein might accumulate in the retina more readily in men than in women. Although there was a positive correlation between serum carotenoids and the density of MP for both sexes, the relation was stronger for men (males  $r=0.62$ , females  $r=0.3$ ). Such poor correlations between retinal, diet and blood carotenoids among females prompted the authors to suspect the presence of moderating variables, possibly hormonal interactions. Furthermore, higher levels of MP in men may be related to the lower level of body fat in men compared to women (Johnson et al. 2000).

#### **4.2.4 Crystalline lens density**

Hammond et al. (1997) have demonstrated an age-related inverse relation between MPOD and crystalline lens density ( $r=-0.47$ ,  $p<0.001$ ), and this supports the concept that ARM and age related cataracts share a common pathogenesis (Liu et al. 1989). Although the cause of

the inverse relation remains uncertain, it has been postulated that individuals with higher MPOD may also accumulate greater quantities of lutein and zeaxanthin in the lens, and the lenticular carotenoids may prevent or retard cataract progression through their antioxidant properties (Yeum et al. 1995). In a study by Olmedilla et al (2001), supplementation with lutein increased and maintained serum lutein levels, which was associated with an improvement in vision function in cataract and AMD patients. The authors postulated that the improvement in visual function in the patients with cataracts, may be a direct effect of the retina (macula), independent of cataract progression. Although there is no direct evidence for a shared mechanism of uptake of these carotenoids in the macula and the lens, the concept is supported by the finding that the lens and the macula both accumulate lutein and zeaxanthin to the exclusion of other carotenoids in the blood (Yeum et al. 1995; Shazia et al. 2004).

### **4.3 Epidemiological Evidence**

Epidemiology is the cornerstone method of approaches to clinical practice and for preventive medicine. These studies are helpful in providing a perspective about whether the effect of carotenoids on eye diseases suggested by short-term animal experiments or human clinical trials can be generalised to longer periods in persons with differing circumstances. Inconsistent findings however have been found to date.

The Eye Disease Case-Control Study (EDCCS, 1993) compared the frequency of possible risk factors among individuals with AMD to a cohort of control patients without the disease. They obtained personal, medical, physiological, biochemical and ocular data on 421 subjects with AMD and 615 without the disease. Of the 21 biochemical variables

analysed, only serum carotenoid and serum cholesterol were found to be significantly associated with risk of neovascular AMD. Subjects were classified by blood level of the micronutrient (low, medium and high). Multivariate analysis identified a markedly decreased risk of neovascular AMD in those subjects with higher levels of serum carotenoids, and a markedly increased risk in those with high levels of serum cholesterol. Patients with carotenoid levels in the (medium and high) groups compared with those in the low group had markedly reduced risks of developing neovascular AMD, with levels of risk reduced to one half and one third respectively. The positive correlation between serum lutein and zeaxanthin and MPOD, coupled with the findings of the EDCC, support the view that macular carotenoids are protective for neovascular AMD.

The EDCCS also evaluated dietary intake of vitamins A, C, E and the carotenoids in 356 subjects with AMD using a food frequency questionnaire (FFQ), and compared the results with a control group which was statistically similar in terms of age and sex (Seddon et al. 1994). No protective effect was found for consumption of vitamin C, E or preformed vitamin A (retinol), however a higher dietary intake of carotenoids was associated with reduced risk of AMD. After correcting for known risk factors for ARM and AMD, it was found that those in the highest quintile of carotenoid intake had a 43% lower risk for AMD, than those in the lowest quintile. Of the dietary carotenoids, lutein and zeaxanthin were found to be the most protective.

The National Health and Nutritional Examination Survey (NHANES) was designed to measure the health and nutritional status of a cross sectional sample in the United States. They used interview based questionnaires to assess dietary intake of vitamins A and C for



178 subjects with ARM and compared the results with those of 2904 controls with healthy maculas (Goldberg, 1988). It was found that consumption of fruits and vegetables rich in vitamin A was inversely associated with ARM. A diet rich in fruit and vegetables however also contains high quantities of lutein and zeaxanthin (Sommerberg et al. 1998). The findings of the NHANES and the multicentre EDCCS provide strong evidence that antioxidant status is related to the risk of ARM/AMD, and that MP may play a protective role (EDCCS, 1992; Goldberg, 1988).

Since the findings of Seddon et al. (1994), the relationship between lutein and zeaxanthin in the diet or blood and AMD has been investigated in several populations and higher intakes or blood levels of lutein and zeaxanthin have been associated with lower rates of certain types of AMD (Seddon et al. 1994; Mares-Perlman, 2001; Gale, 2003).

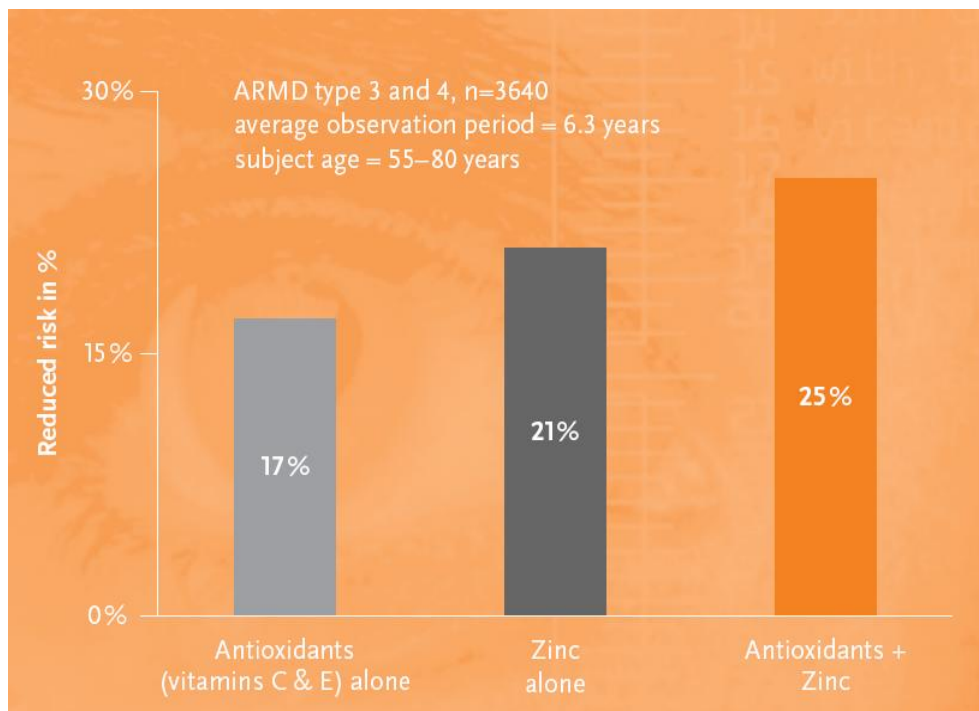
#### **4.4 Experimental Evidence**

Landrum et al. (1997) reported MP measurements using HPLC in 22 ARM and 15 control human donor eyes and found that eyes with ARM had significantly fewer carotenoids in the macula and whole retina than healthy eyes. It was noted that, 17 of the 22 diseased eyes had less MP than the mean of the control group. As the differences in carotenoid concentrations were consistent across the retina, the investigators concluded that lower MP levels are probably causally linked to ARM and not simply the result of the degenerative process of the macula.

Bone et al (2001) determined whether there was an association between MPOD in the human retina and the risk of AMD. Retinas from 56 donors and 56 controls were examined

and the amount of lutein and zeaxanthin extracted from each tissue sample was determined by HPLC. Lutein and zeaxanthin levels were less on average for AMD donors compared to controls. Results are consistent with a theoretical model that proposes an inverse association between risk of AMD and the amounts of lutein and zeaxanthin in the retina (Bone et al. 2001).

In 2001, AREDS showed that supplementation with a preparation of antioxidants and zinc slowed the progression of AMD to an advanced form by 25% over 5 years (AREDS, report no 8, 2001). AREDS, a double-masked clinical trial, enrolled 3640 patients, aged 55 to 80 yrs, who had clinical evidence of extensive small drusen, intermediate drusen, non central GA, or advanced AMD in one eye and randomly assigned them to receive daily oral supplements containing either antioxidants (vitamin C, 500mg; vitamin E, 400IU and beta-carotene, 15mg), zinc (80mg as zinc oxide and copper, 2mg as cupric oxide), antioxidants plus zinc or placebo. Average follow up was 6.3 yrs. Investigators observed that treatment with zinc alone or in combination with antioxidants significantly reduced the risk of progression to advanced AMD (AREDS, report no 8, 2001). Many retinal specialists recommend the AREDS formula, because of the overall lessening of risk of advanced AMD.

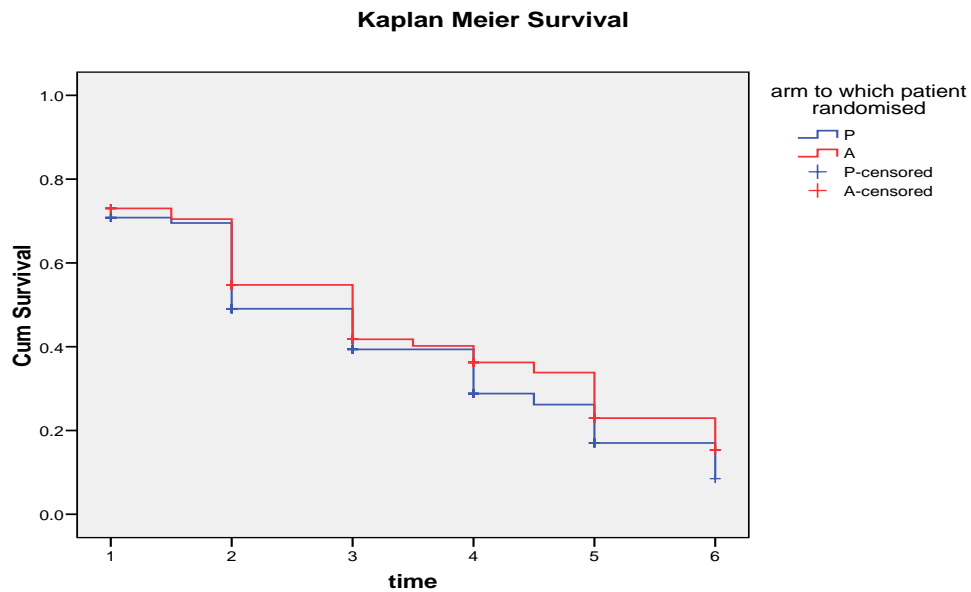


**Figure 4.3. The estimated 5-yr probability of progression to advanced AMD was reduced (AREDS, report no 8, 2001)**

The Lutein Antioxidant Supplementation Trial (LAST), was the first trial to demonstrate improvements in visual function when supplementing with 10mg of lutein alone or in addition with other antioxidants (Richer et al. 2004). Results showed an increase in MPOD in subjects with and without AMD, and improved vision in patients with advanced AMD. Improvements were also seen in contrast sensitivity and subjectively on amsler grid and glare recovery. Placebo control subjects achieved no such improvements.

The Carotenoids and co-antioxidants in Age-Related Maculopathy study (CARMA) investigated the putative beneficial effects of supplemental lutein and zeaxanthin with co-antioxidants in patients with AMD (Chakravarthy et al. 2009). It was a randomised, placebo controlled double blind study. BCVA was significantly better amongst subjects randomised

to supplementation but only at 36 months. In conjunction with existing evidence from AREDS, CARMA provides additional support for supplementation with lutein and zeaxanthin and co-antioxidants in the prevention of progression to late AMD. Figure 4.4 shows a slower rate of progression to late AMD, in the active group compared to placebo.



**Figure 4.4. Morphological progression in study eyes (Chakravarthy et al. 2009) (Courtesy of J Nolan) (Figures on the X axis represent outcomes at six monthly intervals; figures on the Yaxis represent morphological changes to the retina).**

#### 4.5 Clinical Evidence

The accumulation of lipofuscin with age within the RPE has been well documented (Feeney-Burns et al. 1984, Wing et al. 1978) and regional differences have been noted with the highest concentrations in the macular region, however there is a dip at the fovea, where

MP is highest (Wing et al. 1978). The central sparing of annular maculopathy also suggests that MP plays a protective role against certain disease processes. Annular maculopathy, also known as bull's eye maculopathy, refers to an annular pattern of atrophy in the perifoveal region with sparing of the fovea, and is seen in AMD (Sarks et al. 1988; Weiter et al. 1988). Weiter and co-workers (1988) measured the size of central sparing in 45 patients with annular maculopathy and compared results with the size of macular yellow pigment in 40 normals, using fundus fluorescein angiography and monochromatic photography. There was no significant difference between the mean diameter of the area of foveal sparing [0.34 (SD± 0.15) disc diameters] and the mean diameter of MP [0.31± (0.12) disc diameters]. The pattern of MP distribution corresponded exactly to the area of central sparing, and prompted the authors to suggest that "*the close approximation of these values suggested that macular yellow pigment contributed to the annular pattern through a photo protective mechanism*". (Weiter et al. 1988).

Topographic studies of atrophic AMD have shown that the region most vulnerable to damage lies between 2 and 4 degrees of eccentricity where the density of MP is low (Sarks et al. 1988), and that there is a focal reduction in RPE lipofuscin concentration at the very centre of the fovea where the macular carotenoids reach their peak concentrations (Wing et al. 1978). This evidence provides further support for the hypothesis that MP protects against ARM.

To learn about the long-term effects of lutein and zeaxanthin on preventing earlier stages of AMD, it is necessary to look at long-term prospective studies to better understand the other determinants of MP levels. Currently the National Eye Institute has recruited 4,000

participants in its second study (AREDS II), to assess the effects of the AREDS I nutrient formula. Lutein and zeaxanthin, along with omega 3 long-chain PUFA's (350mg DHA and 650mg EPA), will be examined in relation to the progression of AMD (not prevention), since there has been evidence to suggest that eating oily fish two times a week reduces the risk of AMD (Seddon et al. 2006). Results of this study are expected in 2012.

#### **4.6 Macular Pigment and Eye Disease Risk Factors**

Risk factors associated with the occurrence of AMD can be described as modifiable or non-modifiable. Non-modifiable risk factors include age, female gender, iris colour, race and genetic background (Hammond et al. 1996; 1996; O'Connell et al. 2008). Being aware of non-modifiable risk factors for AMD might encourage a person 'at risk' to be extra vigilant about the modifiable risk factors for this eye disease, which include smoking, diets low on xanthophyll containing foods, obesity, cardio vascular disease (CVD) and increased sunlight exposure (Mares-Perlman, 1995; Delcourt, 1999; Hammond, 2001; Richer et al. 2004; Ambrose & Barua, 2004; O'Connell et al. 2008). Smoking, previous cataract surgery and a family history of AMD are consistent risk factors for AMD. Cardiovascular risk factors are also associated with AMD (Chakravarthy et al. 2010).

##### **4.6.1 Cardiovascular disease/obesity**

There have been several studies investigating the proposed relationship between CVD and AMD, with both diseases sharing some common risk factors, including a raised blood pressure (bp) >160/95, physical inactivity, obesity and inflammation. The most compelling evidence is for obesity, as being a common denominator for risk of AMD and CVD. There is evidence of an inverse relationship between body fat and MPOD in humans (Hammond

et al. 2002; Thurnham et al. 2008), suggesting that lutein receptors in adipose tissue and retinal tissue compete for lutein. Johnson et al. (2000) reported a positive correlation between serum lutein and zeaxanthin and MPOD for males only, possibly explained by the higher percentage body fat often present in females. This may also explain the predisposition of women to AMD (Loane et al. 2007). Conversely, a recent Irish study suggested that a body mass index (BMI) >27, only affects serum and macular zeaxanthin concentration (and not lutein), however this result needs replication (Nolan et al. 2007).

HDL facilitates increases in MPOD (Viroonudomphol et al. 2003), but HDL is reduced in obese subjects. Adipocyte HDL competes with retinal HDL, which is needed to transport lutein and zeaxanthin in the body (Seddon et al. 2003), and as lutein and zeaxanthin are transported primarily by HDL, low HDL may impair transport of these carotenoids.

#### **4.6.2 Sunlight exposure**

Cumulative exposure to light may cause gradual loss of photoreceptor cells in the macula (Weiter et al. 1985). Photo-oxidative damage by ROS induced by light may promote the development of AMD (Mares-Perlman, 1995; Delcourt, 1999). In 1976, Ham et al analysed light induced retinal damage as a function of wavelength by exposing rhesus monkey retinas to laser illumination and found that sensitivity to threshold damage rose exponentially with decreasing wavelength. Investigators calculated that 100 times less power is required to produce retinal injury with blue light (440 nm) than with orange light (590 nm).

Ruffolo et al. (1984) investigated the influence of arterial oxygenation on photochemical damage of the retina of macaque monkeys, and found that elevated blood oxygen is associated with a reduced threshold for injury and more severe damage. The oxygen enhancement of blue light damage suggests that the basic mechanism of the photochemical injury is the photodynamic production of free radicals from the toxic combination of light and oxygen (Ruffolo et al. 1984).

MP protects the region of the retina involved in vision from photo-oxidative stress by filtering out the potentially damaging SW light component of light, however MPOD varies greatly from one individual to another and low MPOD is generally regarded as a risk factor for AMD. MPOD is related to genetic makeup, prior history of light exposure and lifestyle factors.

#### **4.6.3 Nutrition and macular pigment**

In recent years, much research has focused on the concept of ‘optimal nutrition’ and proponents of this idea have suggested that even though a balanced diet will provide sufficient amounts of vitamins to prevent deficiency, judicious supplementation of selected micronutrients may confer additional health benefits (e.g. avoidance of cancer, CVD). The potential role of nutritional supplements to reduce the incidence or severity of AMD has received a great deal of attention (Seddon et al. 1994; Snodderly, 1995; Bernstein et al. 2010). The lack of an effective treatment for the majority of cases of AMD, coupled with the public’s perception that over-the counter nutritional supplements are relatively harmless, creates the potential for widespread use of these supplements. Many studies have used serum levels of micronutrients to investigate the relationship of these micronutrients and AMD.



## **4.7 Macular Pigment and its role in Visual Performance and Comfort**

MP may protect against the development of AMD by defending the retina against cumulative and chronic photo-oxidative damage, however as AMD is a late onset disorder, the primary role of MP may rest more on its contribution to visual performance and comfort. The function of MP in the 'protective' hypothesis is twofold. MP protects the eye against AMD by filtering SW light and it also acts as a powerful antioxidant. The traditional view of the optical ('acuity' and 'visibility') hypothesis posits that MP's contribution to visual performance rests purely on its optical 'filtration of SW light' properties. More recently though, the effect of MP on retinal, ocular and cortical health may translate into indirect improvements in visual performance and comfort, as MP also acts as an antioxidant. Improved visual performance may be due to either MP's SW filtration effects, but also because MP scavenges free radicals, and as a consequence MP may improve visual performance because the retina is healthier. The current available evidence on the impact of MP on visual performance will now be explored.

### **4.7.1 The evidence**

The role of MP on visual performance and comfort may be as a result of its optical density and spectral absorption characteristics or due to biological effects on the retina and the crystalline lens (Loughman et al. 2010). The evidence supporting the role for MP in visual performance is largely associative and somewhat difficult to translate to a natural environment. Studies investigating the role of MP and visual performance can be divided into

- 1) Populations with established eye diseases, however it is difficult to know if these findings can transfer to a normal population.

2) Studies involving normal subjects (lacking ocular pathology).

#### **4.7.2 Studies in subjects with retinal pathology**

Hereditary retinal degenerations:

Glare and slow dark adaptation are common symptoms reported by patients with hereditary retinal degenerations. Low MP levels, which results in a failure to absorb scattered SW light, may result in excessive photoreceptor bleaching. Reduced contrast sensitivity could in part explain the symptoms of these patients. The SW filtration property of MP and its ability to mop up free radicals, suggests that MP may play an important role in retinal degenerative diseases, as it may help to preserve visual function in the longterm.

RP results in a progressive degeneration of the retina, specifically the light receptors. The rods are involved earlier in the course of the disease and cone degeneration occurs later. Peripheral vision slowly constricts and central vision is usually retained until late in the disease. Ushers syndrome is a condition which affects both hearing and vision and the vision loss in this syndrome is RP. Finally, choroideremia is a condition resulting in the progressive degeneration of photoreceptors, RPE and choroid. A number of intervention studies investigated the relationship between visual function and MPOD in these conditions.

Dagnelie et al. (2000) assessed the effect of lutein supplementation in patients with RP and reported moderate visual improvements following short-term supplementation with lutein. Subjects took 40mg of lutein for two months, followed by a dose of 20mg for four months. Mean VA improved by 0.7 dB and mean visual field area by 0.35 dB, although the largest gains were attained by blue-eyed participants. Aleman et al. (2001) investigated the

relationship between RP and Ushers syndrome and vision function in subjects supplemented with 20mg of lutein for 6 months. No significant improvement in visual function (measured as absolute foveal sensitivity) was found despite increases in MPOD. Duncan et al. (2002) investigated choroideremia. 13 subjects were supplemented for six months with 20mg of lutein and vision function was assessed, however no improvement in retinal sensitivity was noted. These studies highlight the difficulties inherent in assessing visual performance and MPOD. Dosage difference may however explain the discrepancy in findings here.

#### **4.7.3 Age-related macular degeneration**

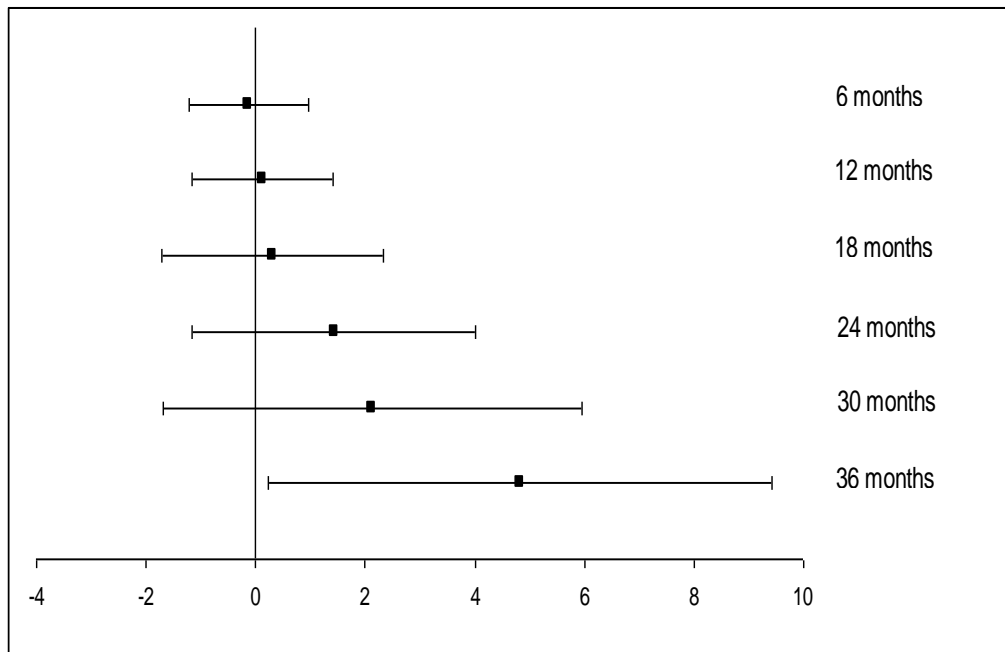
Numerous investigators have explored the relationship between dietary and serum levels of MP and risk of AMD (Nolan et al. 2007), with respect to preservation of vision rather than enhancement. A number of intervention studies have however investigated the relationship between vision function and MPOD which will be discussed here.

Richer (1999) assessed the effect of dietary modification with atrophic AMD and visual performance. 14 Subjects were put on a high lutein and zeaxanthin diet. Short-term enhancement of visual function in one or both eyes in terms of amsler grid testing, Snellen acuity, contrast sensitivity, glare recovery was demonstrated. Richer et al. (2004) went on to evaluate the effect of supplementation on visual performance in 90 subjects with atrophic AMD. It was a double blind placebo controlled trial, with two treatment groups and a placebo group. The first group supplemented with 10mg of lutein and the second group supplemented with 10mg of lutein and antioxidants. The investigators observed statistically significant improvements in VA. Snellen equivalent acuity improved 5.4 letters in the

lutein group and by 3.5 letters in the lutein plus antioxidant group. Improvements in contrast sensitivity and subjective measures in glare recovery were seen in both treatment groups but not in the control group.

Bartlett & Eperjesi. (2007) investigated the effects of supplementation with lutein combined with vitamins and minerals on contrast sensitivity among patients with ARM and atrophic AMD. The authors reported no significant improvement in contrast sensitivity using a Pelli Robson chart, with 6mg/lutein and other antioxidant vitamins and minerals. Dosage levels for this study however were quite low and MPOD was not recorded at baseline. The authors suggest that lutein dosage may be an important factor in the effectiveness of ocular nutritional supplements.

Chakravarthy et al (2009) investigated the effects of lutein and zeaxanthin supplementation with co-antioxidants on the progression of AMD. 433 subjects were randomly assigned to either the treatment or placebo group. Distance VA was found to be significantly better in the intervention group at 3 yrs follow up and progression of AMD was inversely associated with serum lutein (Neelam et al. 2008; Chakravarthy et al. 2009). Figure 4.5 illustrates visual improvement of almost 5 letters in the active group after three years.



**Figure 4.5. The mean (95% CI) differential change in BCVA in study eyes between Treatment and Placebo Group. X axis is number of letters read (Change is from baseline to each visit). The Y axis represents outcomes at six monthly intervals (Beatty et al. 2009) (Courtesy of J Nolan).**

#### **4.7.4 Cataract**

Olmedilla et al. (2003) explored the possibility that lutein supplementation might influence vision function in patients with age-related cataract. Visual performance was evaluated through VA and glare sensitivity measures. This randomised, placebo-controlled study revealed significant improvements in VA and glare sensitivity associated with increased blood serum lutein after supplementation. No such improvements were achieved in placebo controls or in those supplemented with  $\alpha$ -tocopherol. The authors postulate that such improvements were not the consequence of any change in the crystalline lens, but possibly improved retinal function. A branch of AREDS looked at the effect of antioxidants and zinc

supplementation on visual loss in people with age-related cataract but found no significant effect (AREDS, report no 9, 2001).

#### **4.7.5 Studies in normal populations**

Photophobia and glare:

Photophobia is a symptom many people complain of and it results from exposure to light that is of sufficient intensity to produce discomfort. Clinicians are often presented with this phenomenon, often without any obvious reason for these symptoms. Because of MP's absorption characteristics, the ability to determine MPOD in clinical practice will prove invaluable as it may now be possible to determine an individual's threshold for subjective complaint of bright lights (photophobia).

Photophobia involves an acute intolerance to light typically marked by some sort of behavioral aversion (squinting or closing the eyes). Thresholds for photophobia were determined at wavelengths from 440 to 640 nm by Stringham et al. (2003) for three subjects. Photophobia was assessed by means of electromyography. The authors showed that while subjects displayed a trend of increasing sensitivity with decreasing wavelength, they exhibited a notch which centered at 460 nm. A possible explanation for the pronounced notch found could be the absorption of light by MP, as the wavelength of peak absorption of MP is 460 nm (Wyszecki & Stiles, 1982). Stringham et al (2003) found that MP plays a major role in the attenuation of photophobia, substantially greater than what would be expected from spatial averaging of MPOD. For photophobia it appears that even small amounts of spatially integrated MP could prove to significantly reduce photophobia mediated by central viewing.

Such findings led to a subsequent study to test the direct relationship between MP and Photophobia. Wenzel et al. (2006) explored the relationship between baseline MPOD levels and photophobia thresholds as well as the effect of increasing MPOD on such thresholds. The authors found that individuals with higher MPOD were less susceptible to photophobia. The energy necessary to induce photophobia for a SW target relative to a long wavelength target was linearly related to MPOD. Furthermore increasing MPOD in subjects who consumed lutein supplements over a 12 week period, appeared to confer a predictable improvement in photophobia threshold.

Recently Stringham & Hammond (2007) evaluated whether MP was related to visual performance under glare conditions. They looked at baseline visual performance under glare conditions by evaluating photostress recovery and grating visibility. Visual thresholds under glare conditions were strongly related to MPOD and photostress recovery time was found to be significantly shorter for subjects with higher MP. A subsequent trial, supplementing subjects with 10mg per day with lutein and 2mg per day with zeaxanthin for six months, investigated changes in visual performance associated with augmentation of MPOD. Most subjects exhibited improved photostress recovery and glare tolerance in association with an increase in MPOD (Stringham et al. 2008). Average MPOD increased from 0.41 to 0.57 and was shown to significantly decrease the deleterious effects of glare for both visual performance tasks.

#### **4.7.6 Spatial vision**

Engles et al (2007) evaluated the acuity hypothesis by measuring MPOD, gap and hyper acuity in 80 healthy subjects. 40 subjects were assigned to the gap acuity experiment and 40

to the hyper acuity experiment. Acuity measurements for both groups were taken under two conditions. One condition consisted of mid-wavelength yellow light that is not absorbed by MP and the other consisted of a white light that was subject to CA because the blue portion would be absorbed by MP (Engles et al. 2007). No relationship between MP and resolution acuity or between MP and hyper-acuity in either illumination condition was found. Subjects employed in the Engles study however typically exhibited average to high MP levels, with few subjects exhibiting MP levels below 0.2 optical density. Reading & Weale (1974) suggested that due to the non-linear effect of CA, MPOD levels  $> 0.3$  were probably superfluous to visual performance.

In another study, the effect of lutein and antioxidant dietary supplements on visual function was assessed (Bartlett & Eperjesi, 2007). 46 healthy participants were randomised to placebo or active group (6mg lutein combined with minerals and vitamins). Visual function tests included distance and near VA, contrast sensitivity and photostress recovery time. No statistical significant difference between groups for any outcome measure was noted over 9 or 18 months. The authors suggest that daily supplementation with 6 mg lutein combined with zinc and antioxidants may not be sufficient to effect a change in vision function.

Loughman et al. (2010) in a cross sectional analysis involving some 142 young healthy subjects (COMPASS), observed statistically significant relationships between MPOD and best corrected VA, and also with photopic and mesopic contrast sensitivity at intermediate spatial frequencies. MP appeared to constitute up to 0.1 log unit refinement of high contrast VA (equivalent to 0.25 D or residual blur). Correlations between MPOD and VA and contrast sensitivity however, although statistically significant, account for only a small



percentage of the potential variability ( $r^2 = 10\%$ ), and the authors suggested that these results should be interpreted with caution.

The study investigating the relationship between MPOD and visual performance is discussed in chapter five. MPOD measurements and a battery of visual performance tests were performed on 51 young healthy subjects. This study formed one arm of a baseline study group for COMPASS. This was the first study to report an association between MPOD and contrast sensitivity in a young healthy population (not confounded by dietary supplementation or ocular pathology). Findings are consistent with those of Kvangsakul et al. (2006), who reported that MP augmentation via supplementation enhances contrast acuity thresholds under mesopic conditions. Central MPOD (i.e. at  $0.25^\circ$  and  $0.5^\circ$  retinal eccentricity) in this study was found to be positively and significantly related to both mesopic and photopic contrast sensitivity at intermediate spatial frequency (i.e. 5.7 cpd), i.e. a spatial frequency to which the visual system is more highly tuned (Campbell et al. 1968). See chapter five.

#### **4.7.7 Colour vision**

Colour vision and hue discrimination are more accurate at the fovea corresponding to increased cone density (Boyton, 1964). It would seem likely that MP influences colour vision through selective absorption of SW light, since the MP absorption spectrum ranges from about 400 nm to 500 nm and peaks at 460 nm (Snodderly et al. 1984). MP, which acts as a pre-receptorial filter, selectively absorbing SW light, thereby influencing the SW sensitive cones and as a result has the potential to alter colour vision (Davison et al (2011). Moreland & Dain (1995) reported that hue discrimination measured using the Farnsworth-

Munsell 100-Hue test (FM 100) is indeed adversely affected primarily for blue wavelengths, by simulation of high MPOD using liquid notch 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.

Another study investigating the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP and red-green or yellow-blue colour discrimination thresholds. However a marginal improvement in red-green discrimination with increased MPOD was reported, supporting the theory that increasing MP levels improve chromatic discrimination sensitivity (Rodrigues-Carmona, 2006).

Davison et al (2011) investigated the relationship between MPOD and colour sensitivity using a battery of techniques to quantify colour vision of colour-normal observers. Total error scores (TES) and % partial error scores (% PES) on the FM-100 were uncorrelated to MPOD, suggesting that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. Sensitivities on customised SWAP (cSWAP) using foveal targets were significantly inversely correlated with MPOD at both 1.75 and 3 degrees. Davison et al (2011) suggest that the association between MPOD and cSWAP may be a temporally limited effect to which the visual system normally adapts and that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity.

These studies highlight the challenges inherent in investigating the role of MP in visual performance and comfort. Methodological differences between studies and various influences of individual optical and neural architectures, all contribute to the difficulties in

establishing an association, if any, between MP and visual function. The inter individual variability of MPOD (Hammond et al. 1997), makes interpretation of such studies more challenging, and it may be the case that having an MPOD level greater than 0.3 as suggested by Nussbaum et al (1981), might be surplus to requirements with regard to visual function. As far as spatial vision is concerned the effect of MP on visual performance, if any, appears small, at least in individuals with average MPOD.

#### **4.8 Preservation of Youthful Vision into Old Age**

Visual performance tends to decline from the age of about 50. Even with the appropriate optical correction, older adults do not possess the spatial resolving power of a young adult. As people grow older pupils get smaller, there is a loss of crystalline transparency, and as a consequence there is loss of retinal illuminance. MP is a SW light filter, and as a result protects the region of maximum VA by filtering out the most destructive component of light. However, the most important role of MP may rest on the potential of lutein, zeaxanthin and *meso*-zeaxanthin to retard the ageing process through their antioxidant properties. MP acts as an antioxidant both passively, in its ability to limit photo oxidative damage by filtering SW light at a pre-receptorial level, and actively due to its capacity to quench ROS. In subjects with reduced levels of MP, the cumulative and chronic effect of increasing exposure of the retina to SW light, coupled with a weaker capacity to quench free radicals, could accelerate degeneration of the ageing retina (Loughman et al. 2010)

One of the more pronounced retinal losses with age is loss of SW sensitivity (Hammond et al. 1998). Reduced SW sensitivity is also found very early in the development of retinal disease. Hammond et al. (1998) have shown that higher MP seems to retard loss of SW and

scotopic sensitivity in the aged. The authors measured MP and visual sensitivity using psychophysical methods in 27 older (aged 60-84 yrs) and 10 younger subjects (aged 24-36 yrs) and compared the results (Hammond et al. 1998). As expected photopic sensitivity for blue and green light declined with age. For older subjects, however, photopic sensitivity was positively and significantly related to MPOD. The visual sensitivity of older subjects with higher density of MP was not significantly reduced compared with younger subjects. Subjects from the older group with lower levels of MP however had poorer sensitivity than subjects from the younger group. Better visual sensitivity and youthful levels of MP in older subjects appear to be associated in the study.

Haegerstrom-Portnoy (1988) measured the spectral sensitivity of SW sensitive cones and medium and long wavelength sensitive cones at varying degrees of eccentricity for young and aged subjects. The results showed that SW cone attenuation varies as a function of eccentricity with less occurring foveally. This observed differential loss of SW cone sensitivity across the retina, indicates that these cones may be protected centrally by the screening effect of MP, as blue cone loss is least in the central zone of densest pigment (Shaban, 2002).

Visual performance is typically much more variable in the mesopic range, where the influence of rods on spatio-temporal vision predominates and results in reduced contrast acuity. As rods are more sensitive to SW light (peak rod sensitivity lies around 498 nm in the blue-green spectrum), Kvanakul et al. (2006) postulated that SW light absorption by MP may reduce rod signals and thereby extend the superior characteristics of cone dominated vision into the mesopic range. They investigated the effect of increasing MPOD

through lutein and zeaxanthin supplementation on contrast acuity and found that regardless of whether groups were supplemented with lutein, with zeaxanthin or with a combination of the two, there was a trend towards improved performance. The improvement was statistically significant for the lutein group so that supplementation has now been shown to enhance visual performance at low illuminations.

Glare is a problem many people complain of, particularly the elderly who may have developing cataracts or retinal degenerations. The findings that MP positively impacts photostress recovery, disability glare and visual discomfort has been shown (Wenzel et al. 2006; Stringham et al. 2007; 2008), and is of huge clinical importance. However, the theory that MP can attenuate the effects of disability glare and visual discomfort, through its light filtration properties has only been demonstrated in a laboratory setting, under Maxwellian conditions. It is difficult to translate these findings to the real world and to date there are no studies which demonstrate this effect.

The photostress investigation by Loughman et al (2010) was designed to test the neuroprotective and/or optical property of MP. The blue component in the photostress lamp was more comparable to everyday settings (i.e. it lacked a strong blue component like that used by Stringham et al 2007; 2008; Wenzel et al. 2006). While the nature of the lamp may be more in tune with blue light emission in natural environments, it may not have been enough to establish a relationship in this study. However the fact that the authors failed to find an association between MP and photostress recovery, in actual fact corroborates with the findings of Stringham et al (2007; 2008), in that the associations between MP and glare are strongly wavelength dependant (Loughman et al. 2010). There is a current trend for

change to compact fluorescent and light emitting diode installations, which typically emit significantly more blue light and are therefore extending our exposure to SW light sources, which may render the role of MP for visual performance, if any, even more important.

## CHAPTER FIVE

### The Relationship between Macular Pigment and Visual Performance

#### 5.1 Abstract

##### **Purpose**

To assess whether MP optical density (MPOD) is associated with visual performance.

##### **Methods**

51 young (mean age  $29 \pm 6$  SD years) healthy subjects were recruited into Dublin Institute of Technology (DIT). The spatial profile of MPOD (i.e. at 0.25, 0.5, 1, 1.75 and 3 degrees of retinal eccentricity) 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 sensitivity, photostress recovery time (PRT), Farnsworth-Munsell 100-Hue test (FM100), and customised short wavelength automated perimetry (cSWAP) at the fovea and at 1, 2, 3, 4 and 5 degrees eccentricity.

##### **Results**

The mean ( $\pm$  SD) MPOD at  $0.25^\circ$  eccentricity was  $0.39 (\pm 0.14)$  and was positively and significantly related to mean MPOD at all other degrees of eccentricity ( $r = 0.275$  to  $0.879$ ,  $p \leq 0.05$ , for all), with the exception of mean MPOD at  $3^\circ$  of retinal eccentricity ( $r = 0.088$ ,  $p = 0.538$ ). Pearson's correlation coefficient analysis showed a statistically significant positive relationship between BCVA and MPOD at  $0.25^\circ$  and  $0.5^\circ$  retinal eccentricity ( $r = 0.345$ ,  $p = 0.013$ ,  $r = 0.317$ ,  $p = 0.024$ , respectively). MPOD was also positively and significantly related to both mesopic and photopic contrast sensitivity (at 5.7 cpd), but this

relationship was confined to the central MPOD at 0.25° retinal eccentricity ( $r = -0.394$ ,  $p = 0.004$ ,  $r = -0.313$ ,  $p = 0.027$ ) and was unrelated to MPOD at the more peripheral locations. cSWAP, PRT and blue-green colour discrimination were unrelated to MPOD across its spatial profile.

## **Conclusions**

Measures of central visual function, including VA and contrast sensitivity, are positively associated with MPOD. A longitudinal, placebo-controlled and randomised supplementation trial would however be required to ascertain whether augmentation of MP can influence visual performance.

## **Key words**

Macular pigment optical density; mesopic; photopic; photostress recovery time; visual acuity; customised short wavelength automated perimetry.

## **5.2 Introduction**

The macula is a specialised part of the retina and is responsible for high spatial resolution and colour vision (Hirsch & Curcio, 1989). The carotenoids lutein, zeaxanthin and meso-zeaxanthin accumulate at the macula where they are collectively referred to as MP (Bone et al. 1993). Lutein and zeaxanthin are of dietary origin, whereas *meso*-zeaxanthin is not normally found in a conventional diet, and is generated at the retina following lutein isomerisation (Bone et al. 1993; Neuringer, 2004).



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 (Congdon et al. 2004). It is now understood that oxidative stress (Winkler et al. 1999; Beatty et al. 2000), and associated inflammation (Hollyfield et al. 2009), which are exacerbated in part by cumulative SW visible light exposure (Algvere et al. 2006; Fletcher et al. 2008), are important in the aetiopathogenesis of AMD. MP is a SW (blue) light filter (Bone et al. 1992), and a powerful antioxidant (Khachik, 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 et al. 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 & Weale (1974) and later by Nussbaum et al. (1981) and include MP’s putative ability to enhance visual performance and/or comfort by attenuation of the effects of CA 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 VA, contrast sensitivity, glare sensitivity, photostress recovery, critical flicker fusion frequency (CFF), and colour vision, amongst others (Wooten & Hammond, 2002; Stringham & Hammond, 2004; Hammond & Wooten, 2005; Kvensakul et al. 2006; Stringham & Hammond, 2007; Engles et al. 2007; Stringham & Hammond, 2008; Bartlett & Eperjesi, 2008). However, the findings from these studies are inconsistent and, in some cases, lack validity as a result of inappropriate methodology.

In this manuscript, baseline data from the Collaborative Optical Macular Pigment Assessment Study (COMPASS) is presented, and as such represents a cross sectional evaluation of the relationship between MPOD and visual performance and comfort across a broad and refined range of functional tests.

### 5.3 Materials and Methods

#### 5.3.1 Subjects

51 healthy subjects volunteered to participate in this study, which was approved by the research ethics committees at 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 DIT vision science laboratory. Self-selected recruitment of subjects (DIT:  $n = 51$ ) was facilitated by poster and newsletter advertisement, and also by word of mouth. All subjects were aged between 18 to 41 years, in perfect general (self report) and ocular health, and with VA 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 5.1.

**Table 5.1:** Parameters assessed and their sequence for a typical study visit

DESCRIPTION	TIME (minutes)
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
Colour 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
<b>Total time:</b>	<b>260</b>

### **5.3.2 Demographic, medical history, lifestyle and vision case history questionnaires**

The following details were recorded for each volunteer using a demographic and lifestyle questionnaire: demographics; general health status; smoking (never, current or past); alcohol consumption (average alcohol consumption on a weekly basis); health exercise (in a typical week total time spent performing exercises); BMI [defined as kg body weight/height in m<sup>2</sup>]; blood pressure; ethnic background; marital status; education; occupation.

The following details were recorded using vision case history questionnaire: last eye examination; use of spectacles or contact lens use; history of ocular treatment or surgery; history of eye patching in childhood; family history of eye diseases; current problems with vision; eyestrain associated with computer use; headaches history (See Appendix H).

### **5.3.3 Spectacle refraction, visual acuity, and ocular dominance**

Each subject underwent precise spectacle refraction by an experienced optometrist to determine refractive error and 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 meters, using a Sloan ETDRS letterset. BCVA was determined as

the average of 3 measurements, with letter and line changes facilitated by the software pseudo-randomisation feature. BCVA was recorded using a letter-scoring VA rating; with 6/6 VA 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 (Roth et al. 2002), and with the dominant eye chosen as the study eye, except in cases of observed equi-dominance, in which case the right eye was selected. All subsequent tests were conducted with the subject's optimal subjective refraction in place.

#### **5.3.4 Glare sensitivity**

Glare sensitivity was assessed using a Functional Vision Analyser (Hohberger et al. 2007) (Stereo Optical Co. Inc. Chicago, IL) using the Functional Acuity Contrast Test (FACT) (Hitchcock et al. 2004; Terzi et al. 2005) and a customised 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  $-15^\circ$ ,  $0^\circ$  or  $+15^\circ$  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 ( $3 \text{ cdm}^{-2}$ ) 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 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 responses were entered into the Eye view software provided, and contrast sensitivity scores for no glare, medium and high glare conditions were determined for the respective spatial frequencies.

### **5.3.5 Visual function in normal’s questionnaire**

A 30-part Visual Function assessment in Normal’s questionnaire (VFNq30) was designed specifically for the study (See Appendix 5.1). The design was based loosely on a previously validated visual activities questionnaire (The Visual Activities Questionnaire, 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:

- (1) Situational Analysis – the subject was asked to rate their performance under specified, daily life situations.
- (2) Comparative Analysis – the subject was asked to rate their visual performance in comparison to their peers/friends/family.
- (3) Subject Satisfaction Score – the subject was also asked to rate their overall visual performance.

Each of the three metrics above is computed to give a performance score ( $x / 100$ ) for 5 different functional aspects of their vision including:

-Colour Discrimination

-Glare Disability

-Visual Acuity

-Light/Dark Adaptation

-Daily Visual tasks

### **5.3.6 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" View Sonic 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 generated luminance modulated sine gratings (Gabor patches). The orientation of the stimuli was vertical. The Gabor patches were presented on the cathode ray tube (CRT) monitor and subtended a visual angle of 4.2 degrees. 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,  $\delta x$  and  $\delta y$ .

Contrast sensitivity functions were determined under both mesopic and photopic conditions. Each subject was seated at a fixed viewing distance of 1.5m 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 monitor generated background and stimulus light). 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, U.K), which communicated with the VSG device via an infra red link. A four alternative forced choice testing system was used, with 4 possible target locations. The stimuli were randomly presented at 2 degrees 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 6 different spatial frequencies (1.0, 3.1, 4.2, 5.7, 7.7 and 11.5 cpd) under both mesopic and photopic conditions, all at a mean luminance of  $3\text{cdm}^{-2}$  (mesopic) and  $100\text{cdm}^{-2}$  (photopic).

A log scale 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 5 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.

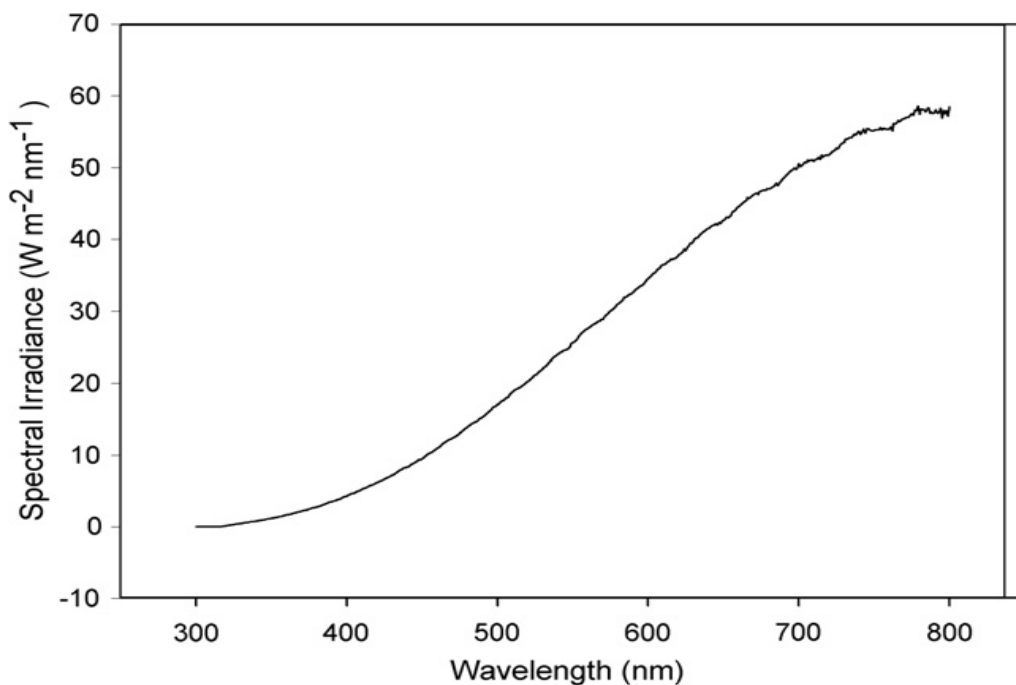
For each subject, the Metropsis software plotted the inverse of the contrast threshold against the range of spatial frequencies tested to provide a contrast sensitivity function under both mesopic and photopic conditions.

### **5.3.7 Photostress recovery**

PRT was calculated using a macular automated photostress (MAP) test (Dhalla et al. 2007; Dhalla & Fantin, 2005). MAP is a novel photostress method for the evaluation of macular function using the Humphrey® field analyser (Model 745i Carl Zeiss Meditec Inc. Dublin, CA, USA). The foveal threshold feature of the field analyser 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 0.1log 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 meter. The spectral irradiance in the wavelength range, 300 nm to 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 meter was obtained by using the photopic weighting function. The spectral irradiance at 1 meter fixation distance from the photostress lamp is presented in Figure 5.1.



**Figure. 5.1.** Spectral Irradiance at 1 m fixation distance from Arri 300 photostress lamp (Modified from Loughman et al 2010).

Immediately post-photostress, a continuous and timed cycle of foveal sensitivity measurements were conducted and recorded for each subject. The reduction in foveal

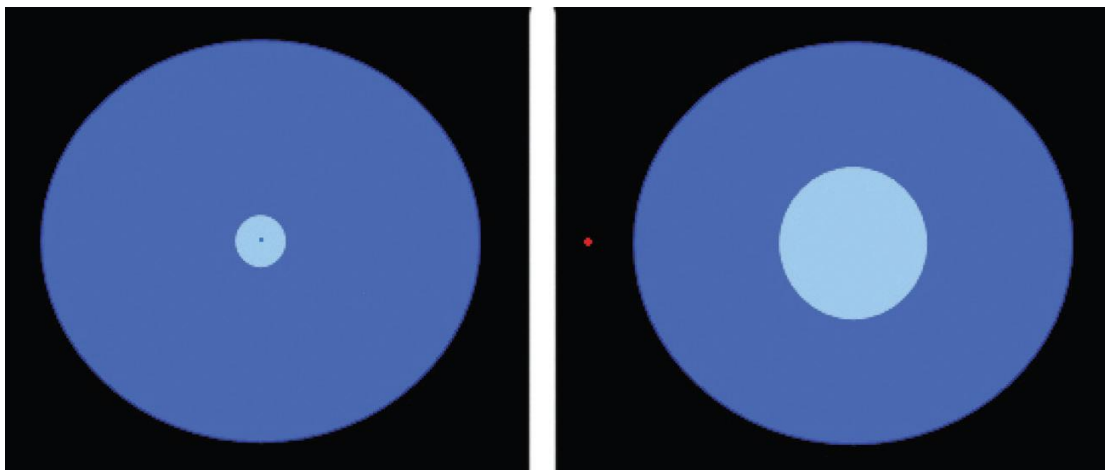
sensitivity from baseline, along with the time taken to recover to baseline foveal sensitivity, was recorded.

### **5.3.8 Macular pigment optical density**

We used the Macular Densitometer™ (Macular Metrics II, 12 River St, Rehoboth, MA 02769, USA), a device developed and originally described by Wooten et al. (1999). The Macular Densitometer™ uses HFP to obtain a valid measure of MPOD at a given retinal location (Hammond et al. 2005). This method has recently been refined and is now referred to as customised HFP or cHFP (Loane et al. 2007; Stringham et al. 2008; Nolan et al. 2009) and is slightly modified from the device described by Wooten et al. 1999. The Densitometer uses light emitting diodes (LEDs) as light sources, but the luminance of both the green (550 nm) and the blue (460 nm) LEDs are varied in a yoked manner. This avoids any change of overall luminance. The illumination of the blue and green LEDs are alternated in square-wave counter phase. A stimulus of blue light close to the peak absorbance of the MP (460 nm) alternates with a green light which is not absorbed by MP (560 nm). This flickering stimulus is presented to the foveal centre where MP reaches its maximum concentration, and then to the parafovea, where MP is optically undetectable. The luminance of one light source (usually the blue light) can be adjusted by the subject, and the flicker can therefore be eliminated if the two wavelength components are matched in luminance. When viewing the flickering stimulus centrally, the intensity of the blue light must be increased to compensate for its attenuation by MP, if the end point of no flicker is to be reached. The average intensity of the blue light aimed at the foveal region at minimal flicker ( $B_{fov}$ ) is recorded. The test is then repeated with the stimulus aimed at an eccentric fixation point where it is assumed that the MPOD is negligible ( $B_{ref}$ ). The central MPOD

level is then calculated with the equation:  $MPOD = \log(B_{fov}/B_{ref})$ . For the purpose of this study we measured MPOD's spatial profile across the retina (i.e. 0.25, 0.5, 1.0, 1.75 and 3 degrees of retinal eccentricity).

The target used to measure MPOD at 0.5 degree retinal eccentricity is a centrally loaded circular stimulus of 1 degree diameter, with a central fixation spot, at which the subject is encouraged to fixate. The 7 degrees reference target 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 disk. Both the central and reference targets are presented on a blue background test field. The wavelength of the blue background test field is 468 nm in the Densitometer. The Densitometer has the option to adjust the flicker frequency. This enables the investigator to customise the optimal flicker frequency (OFF) for each subject, which results in a more discrete end point for the test, thus minimising the variance between readings. Densitometer recordings are made under conditions of dimmed light (ambient illuminance: 4 lux, as measured with an Iso-Tech ILM 350 Lux Meter) at a viewing distance of 18.5 inches (47 cm) (Loane et al. 2007).



**Figure 5.2 Densitometer; showing the foveal test field on the left and the parafoveal test field on the right (for illustration only, not drawn exactly to scale) (Modified from Loane et al. 2007).**

### **5.3.9 Farnsworth-Munsell 100-Hue test (FM100)**

The FM100 test (X-Rite UK, Poynton) 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  $94 \text{ cd.m}^{-2}$  reflected from each colour sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was  $12 \text{ cd.m}^{-2}$ . Colour temperature is rated at  $6400^\circ \text{ K}$ . 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).

### **5.3.10 Customised short-wavelength automated perimetry (cSWAP)**

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard SWAP routine on a Humphrey Field Analyser 2i (Carl Zeiss Medetec, Jena, Germany). Yellow (530nm) background luminance was  $100 \text{ cd.m}^{-2}$ . Goldmann size V targets of 440nm and 200msec duration subtending 1.7 degrees at the eye were presented at 0, 1, 2, 3, 4 and 5 degrees eccentricity (centered at these eccentricities; i.e. 5 degree target extended from 4.15 to 5.85 degrees eccentricity) from a fixation target. The number of

targets at each 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 eccentricity was the mean of values for all targets in the group at that eccentricity.

### **5.3.11 Fundus photography**

Fundus photographs were obtained in both eyes using a NIDEK non-mydratic fundus camera (AFC-230).

### **5.3.12 Statistical analysis**

The statistical software package SPSS (version 18) was used for analysis. All variables investigated exhibited a typical normal distribution. Mean  $\pm$  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.

## **5.4 Results**

The demographic, medical, lifestyle, anthropometric, and vision-related data of the 51 subjects recruited into the study are summarised in Table 5.2. No subject was excluded from the study on the basis of fundus findings. The mean ( $\pm$  SD) age of the sample was 29 ( $\pm$  6) and ranged from 18 to 41 years. The mean ( $\pm$  SD) BMI was 25 ( $\pm$  3.3) and ranged from 19.8 to 37.55.

**Table 5.2:** Demographic, medical, lifestyle, anthropometric, and ocular related data for the entire study group.

<b>Characteristic</b>	<b>n</b>	<b>Characteristic</b>	<b>n</b>
<b>Sex</b>		<b>Family History of</b>	
Male	16	<b>Eye disease</b>	
Female	35	AMD	9
<b>Medical History</b>		Cataract	6
Diabetes	1	Glaucoma	14
High blood pressure	2	Retinal problems	1
High cholesterol	3	None	27
Angina	0	<b>Smoking habits</b>	
Stroke	0	Never smoked	32
<b>BMI</b>		Ex smoker	9
Desirable weight (BMI <25)	29	Current smoker	10
Overweight (BMI 25-30)	19	Exposed to second hand smoke	0
Obese (BMI >30)	3		
<b>BCVA</b>		<b>Ocular dominance</b>	
< 100	2	Right	28
100 - 105	5	Left	33
>105-109	33	Equi-dominant	0

>110	11		
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\*n = sample size; †smoking habits: ex-smoker = smoked  $\geq$  100 cigarettes in lifetime but none in last 12 months; current smoker = smoked  $\geq$  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

#### 5.4.1 Macular pigment optical density

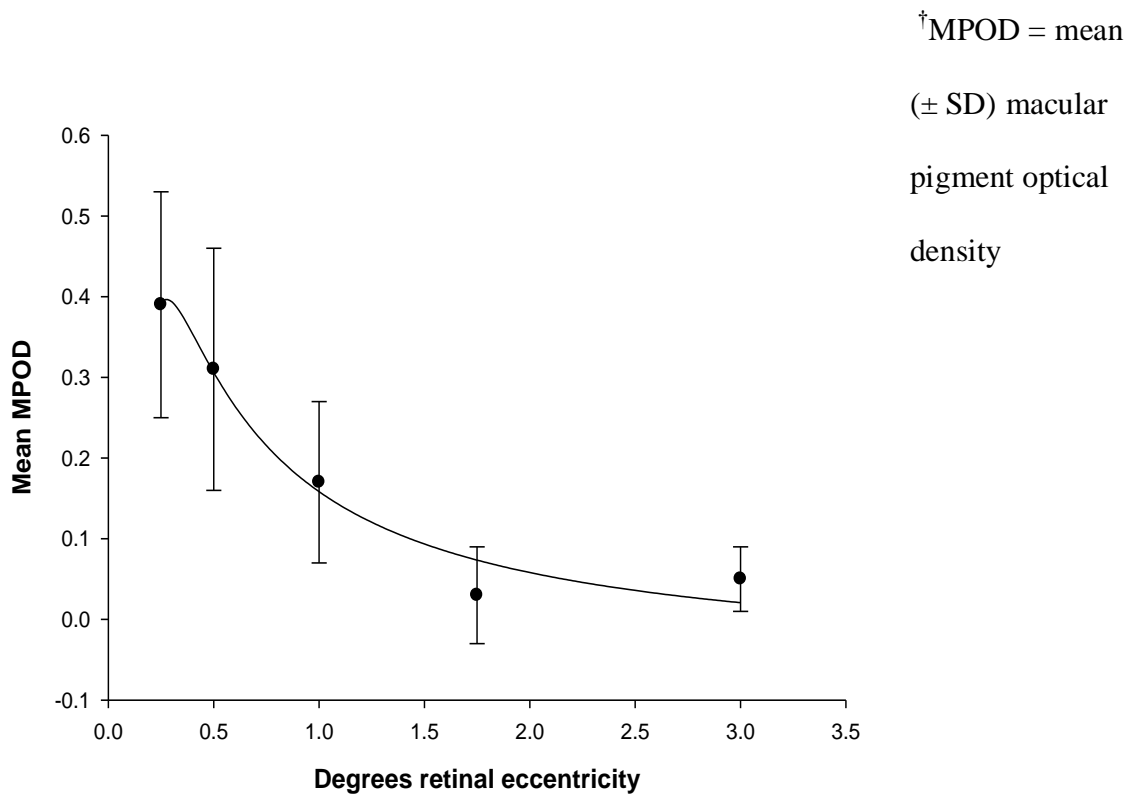
The mean  $\pm$  SD MPOD at each degree of eccentricity and averaged across the retina is presented in Table 5.3 and Figure 5.3. The mean ( $\pm$  SD) MPOD (for the entire study group) at 0.25° eccentricity was 0.39 ( $\pm$  0.14) and was positively and significantly related to mean MPOD at all other degrees of eccentricity ( $r = 0.275$  to  $0.879$ ,  $p \leq 0.05$ , for all), with the exception of mean MPOD at 3° of retinal eccentricity ( $r = 0.088$ ,  $p = 0.538$ ).

**Table 5.3:** Mean and SD's of MPOD at all retinal eccentricities:

<b>MPOD Eccentricity</b>	<b>Mean</b>	<b>SD</b>
<b>MPOD 0.25°</b>	0.39	$\pm$ 0.14
<b>MPOD 0.5°</b>	0.31	$\pm$ 0.15
<b>MPOD 1°</b>	0.17	$\pm$ 0.1
<b>MPOD 1.75°</b>	0.03	$\pm$ 0.06
<b>MPOD 3°</b>	0.05	$\pm$ 0.04
<b>Average MPOD</b>	0.19	$\pm$ 0.08

n = 51

\*° = degrees retinal eccentricity



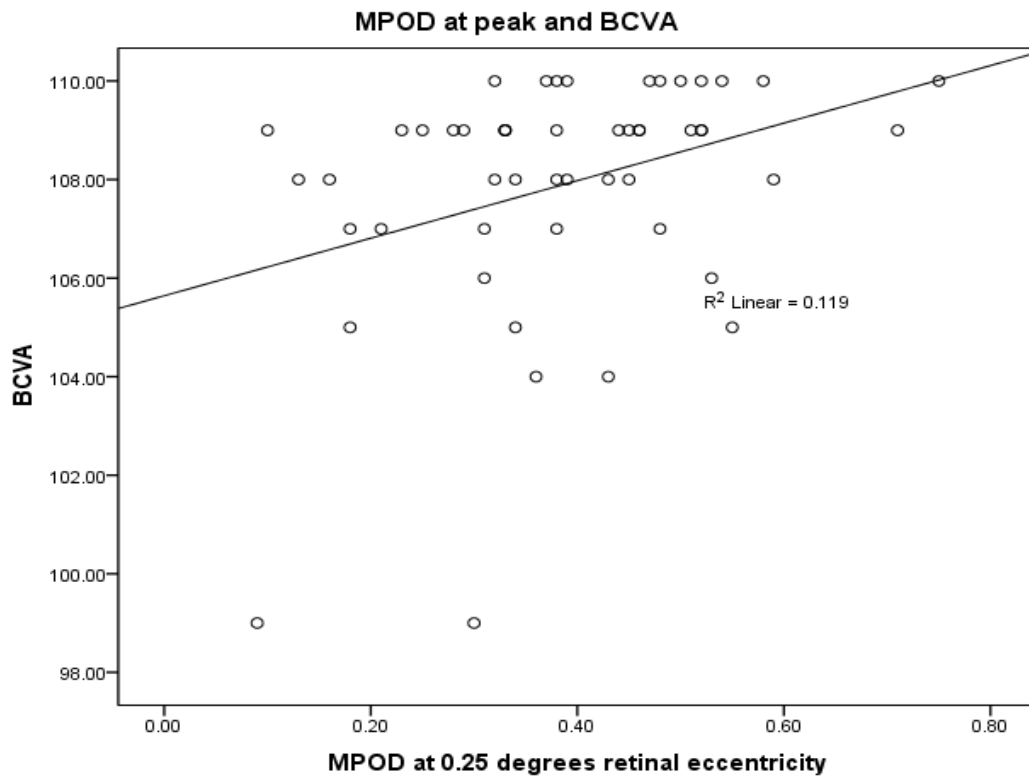
**Figure 5.3:** A second-order decreasing exponential function fit the averaged subjects' profile data well. The curved line indicates the exponential best fit. Inverse second order fit;  $R^2 = 0.97$ .

There was no statistically significant association between MPOD at any degree of retinal eccentricity [or mean MPOD for the entire study group] and demographic and lifestyle factors (e.g. age, sex, BMI and smoking habits) [MLR: dependant variable = MPOD;  $p > 0.05$  for all; Pearson correlations:  $p > 0.05$  for all correlations tested]. This finding is unsurprising given the small sample analysed and the narrow age spread of our sample (20 to 40 years).



### 5.4.2 Best corrected visual acuity and MPOD

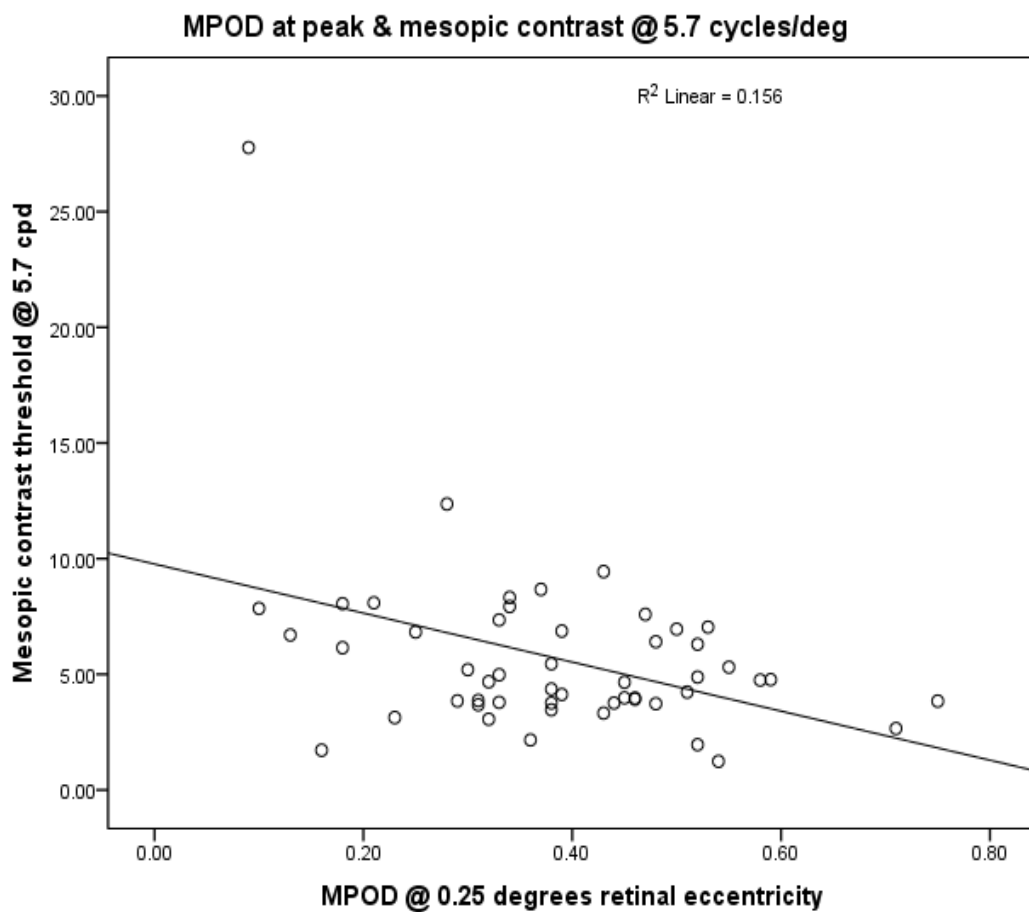
Pearson's correlation coefficient analysis showed a statistically significant positive relationship between BCVA and MPOD at 0.25° and 0.5° retinal eccentricity ( $r = 0.345$   $p = 0.013$ ,  $r = 0.317$   $p = 0.024$ , respectively), and also with average MPOD across the retina ( $r = 0.331$ ,  $p = 0.018$ ). MPOD at all other degrees of retinal eccentricity and BCVA were not significantly correlated ( $p > 0.05$ , for all). The questionnaire derived VA index (VAI) also correlated significantly with MPOD at 0.25° ( $r = 0.281$   $p = 0.046$ ). The relationship between MPOD at 0.25° of eccentricity and BCVA is presented in Figure 5.4.



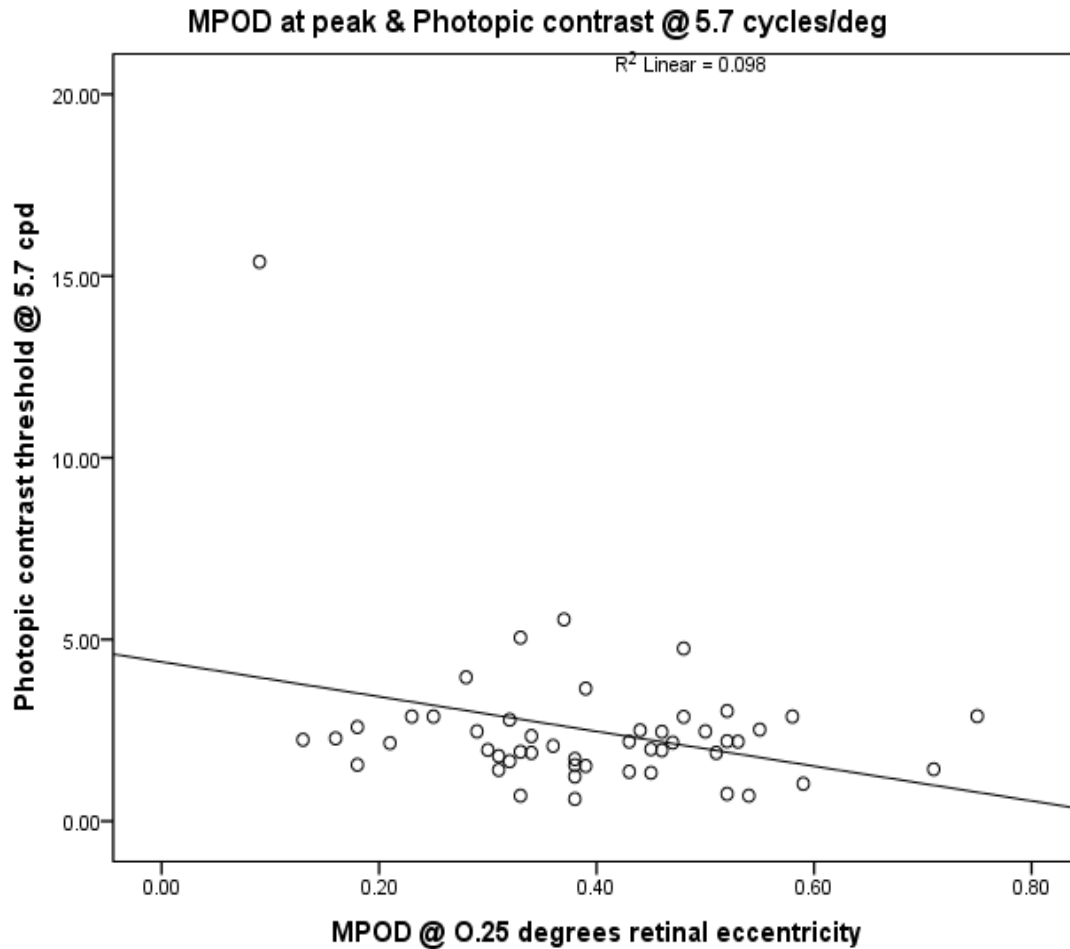
**Figure 5.4:** The relationship between MPOD at 0.25° and BCVA

### 5.4.3 Contrast sensitivity and MPOD

Mesopic and photopic contrast thresholds were inversely and significantly correlated with central MPOD [e.g. mesopic contrast threshold @ 5.7 cycles/deg and MPOD at 0.25°:  $r = -0.394$ ,  $p = 0.004$ , Figure 5.5; and mesopic contrast threshold @ 7.7 cycles/deg and MPOD at 0.25°:  $r = -0.343$ ,  $p = 0.014$ ]; [photopic contrast threshold @ 5.7 cycles/deg and MPOD at 0.25°:  $r = -0.313$ ,  $p = 0.027$ , Figure 5.6], but were unrelated to MPOD at the more peripheral locations [Pearson correlation matrix, Table 5.4]. Higher levels of MP were therefore associated with better contrast sensitivity at the spatial frequencies to which we are most finely tuned at both photopic and mesopic light levels.



**Figure 5.5:** The relationship between MPOD at 0.25° and log contrast sensitivity at 5.7 cpd for mesopic conditions.



**Figure 5.6:** The relationship between MPOD at 0.25° and log contrast sensitivity at 5.7 cpd for photopic conditions

**Table 5.4:** The Pearson’s correlation coefficient (Pearson’s r) for MPOD at each eccentricity measured and log mesopic and photopic contrast thresholds at different spatial frequencies are shown in Table 5.4.

Spatial Frequency Mesopic	MPOD @ 0.25°	MPOD @ 0.5°	MPOD @ 1 °	MPOD @ 1.75 °	MPOD @ 3 °	Average MPOD
1.0	0.033	0.103	0.179	0.324*	-0.086	0.134
3.1	-0.031	0.014	0.094	0.171	0.168	0.064
4.2	-0.288*	-0.272	-0.156	0.046	0.145	-0.217
5.7	-0.394**	-0.377*	-0.27	-0.069	0.105	-0.345*
7.7	-0.343*	-0.329*	-0.242	0.041	0.156	-0.279*
11.5	-0.226	-0.183	-0.073	0.149	0.15	- 0.126

Spatial Frequency Photopic	MPOD @ 0.25°	MPOD @ 0.5°	MPOD @ 1 °	MPOD @ 1.75 °	MPOD @ 3 °	Average MPOD
1.0	-0.024	0.04	0.167	0.498**	0.068	0.134
3.1	-0.19	-0.221	-0.202	0.218	0.05	-0.16
4.2	-0.194	-0.172	-0.101	0.367**	0.154	-0.082
5.7	-0.313*	-0.3*	-0.284*	0.039	0.148	-0.269*
7.7	-0.267	-0.272*	-0.197	0.252	0.007	-0.205
11.5	-0.115	-0.121	-0.065	0.214	0.102	-0.056

\*\*correlation is significant at the 0.01 level, indicates  $p \leq .01$  without Bonferroni correction.

\*correlation is significant at the 0.05 level, indicates  $p \leq .05$  without Bonferroni correction.

$P \leq .001$  with Bonferroni correction.

MP = macular pigment

#### **5.4.4 Colour vision and MPOD**

There was no significant relationship between total error score (TES) obtained from the FM-100 hue test and MPOD at any degree of retinal eccentricity ( $r = -0.181$  to  $0.111$ ,  $p > 0.05$  for all), with the exception of the positive and significant relationship found between TES and MPOD at 1.75-degrees of eccentricity ( $r = 0.389$ ,  $p = 0.005$ ).

There was no significant association found between MPOD at any degree of retinal eccentricity and blue/green (FM-100 hue caps no. 36 – 54 error scores) colour discrimination ( $p > 0.05$ , for all). However, MPOD at 1-degree of retinal eccentricity was inversely related to blue/green colour discrimination and approached statistical significance ( $r = -0.261$ ,  $p = 0.064$ ). Likewise, there was no relationship observed between MPOD at any degree of retinal eccentricity and blue (FM-100 hue caps no. 50 – 68 error scores) colour discrimination ( $p > 0.05$ , for all).

There was no significant relationship between the subjects colour satisfaction score derived from the VFQ and MPOD at any degree of retinal eccentricity ( $r = -0.110$  to  $0.033$ ,  $p > 0.05$  for all).

#### **5.4.5 Customised short wavelength automated perimetry**

There was no significant correlation observed between cSWAP at any degree of retinal eccentricity with the most central MPOD's at 0.25, 0.50 or 1 degrees of retinal eccentricity

( $p > 0.05$ , for all, Table 5.5). However, blue light sensitivity as assessed by cSWAP, was inversely and statistically significantly correlated with MP at 1.75 degrees of retinal eccentricity.

**Table 5.5:** cSWAP and MPOD

cSWAP	MPOD @ 0.25°	MPOD @ 0.5°	MPOD @ 1°	MPOD @ 1.75°	MPOD @ 3°	Average MPOD
cSWAP fovea	0.012	-0.08	-0.205	-0.32*	-0.159	-0.144
cSWAP 1	-0.095	-0.11	-0.149	-0.403**	-0.198	-0.196
cSWAP 2	-0.005	-0.043	-0.206	-0.424**	-0.183	-0.156
cSWAP 3	0.094	0.072	-0.073	-0.238	-0.215	-0.021
cSWAP 4	0.041	0.029	-0.048	-0.286*	-0.224	-0.057
cSWAP 5	0.027	0.009	-0.088	-0.229	-0.26	-0.075
cSWAP Total	0.014	-0.007	-0.118	-0.332*	-0.224	-0.104

\*\*correlation is significant at the 0.01 level, indicates  $p \leq .01$  without Bonferroni correction.

\*correlation is significant at the 0.05 level, indicates  $p \leq .05$  without Bonferroni correction.

$P \leq .001$  with Bonferroni correction.

MP = macular pigment

cSWAP = Customised short wavelength automated perimetry

#### **5.4.6 Photostress recovery time and MPOD**

There was no significant association between photostress recovery time (PRT) (seconds) and MPOD at any degree of retinal eccentricity ( $r = -0.239$  to  $0.066$ ,  $p > 0.05$ , for all).

#### **5.4.7 Photostress sensitivity reduction**

There was no significant association between photostress percentage reduction and MPOD at any degree of retinal eccentricity ( $p > 0.05$  for all), with the exception of a significant inverse association at 3-degrees of retinal eccentricity (MPOD at 3-degree of retinal eccentricity and photostress recovery time:  $r = -0.332$ ,  $p = 0.017$ ).

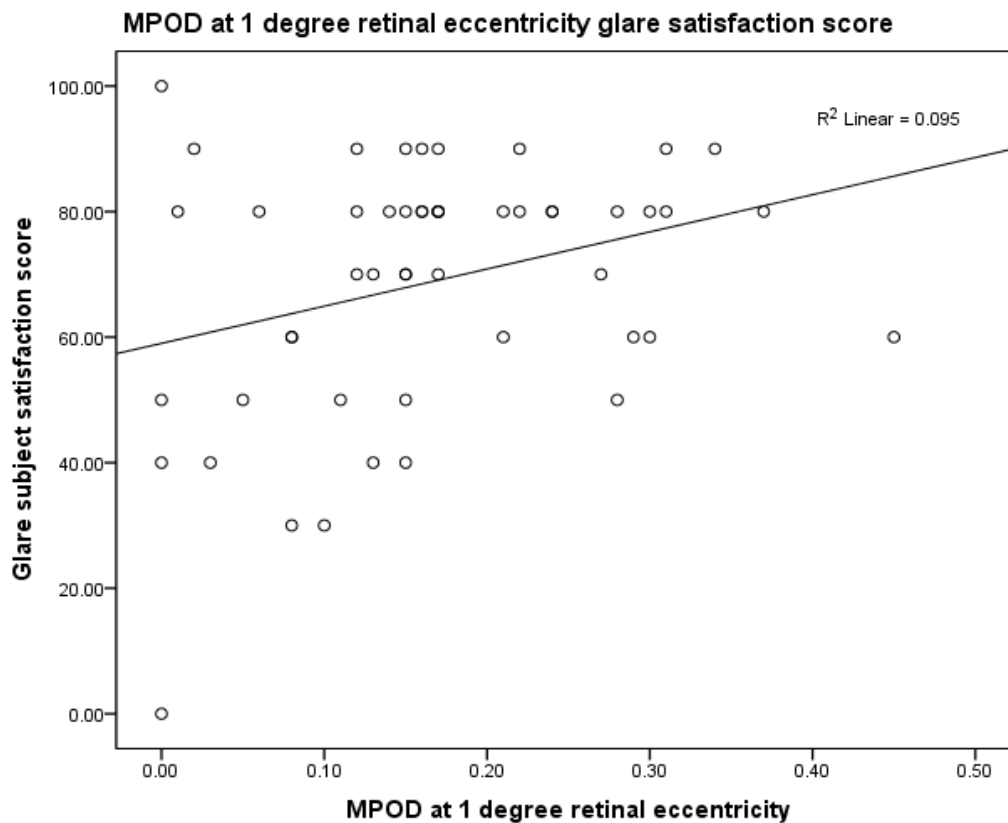
Questionnaire based assessment of visual performance as affected by glare showed no correlation with either photostress recovery time or percentage visual loss immediately post-bleaching ( $p > 0.05$ , for all).

The absence of a strong blue light component in the photostress lamp (Figure 5.1), employed in this study may partially explain the absence of any association between MP and photostress recovery time. While the nature of the lamp may be more in tune with blue light emission in natural environments, the SW component perhaps, may not have been strong enough to establish a relationship in this study.

#### **5.4.8 Subjective glare assessment and MPOD**

There was a positive association between the questionnaire based subjective glare assessments (i.e. questionnaire situational analysis, comparative analysis and overall subject satisfaction score) and MPOD at the more central locations, with positive

associations found between MPOD at 0.25-degrees and comparative analysis ( $r = 0.288$ ,  $p = 0.041$ ), MPOD at 0.25-degrees and overall subject satisfaction score ( $r = 0.279$ ,  $p = 0.047$ ). Positive and statistically significant associations were found between MPOD at 1-degree and average MP with overall subject satisfaction score for glare ( $r = 0.307$ ,  $p = 0.028$  [Figure 5.7], and  $r = 0.286$ ,  $p = 0.042$  respectively). Higher MP levels were therefore associated with better subjective glare performance.



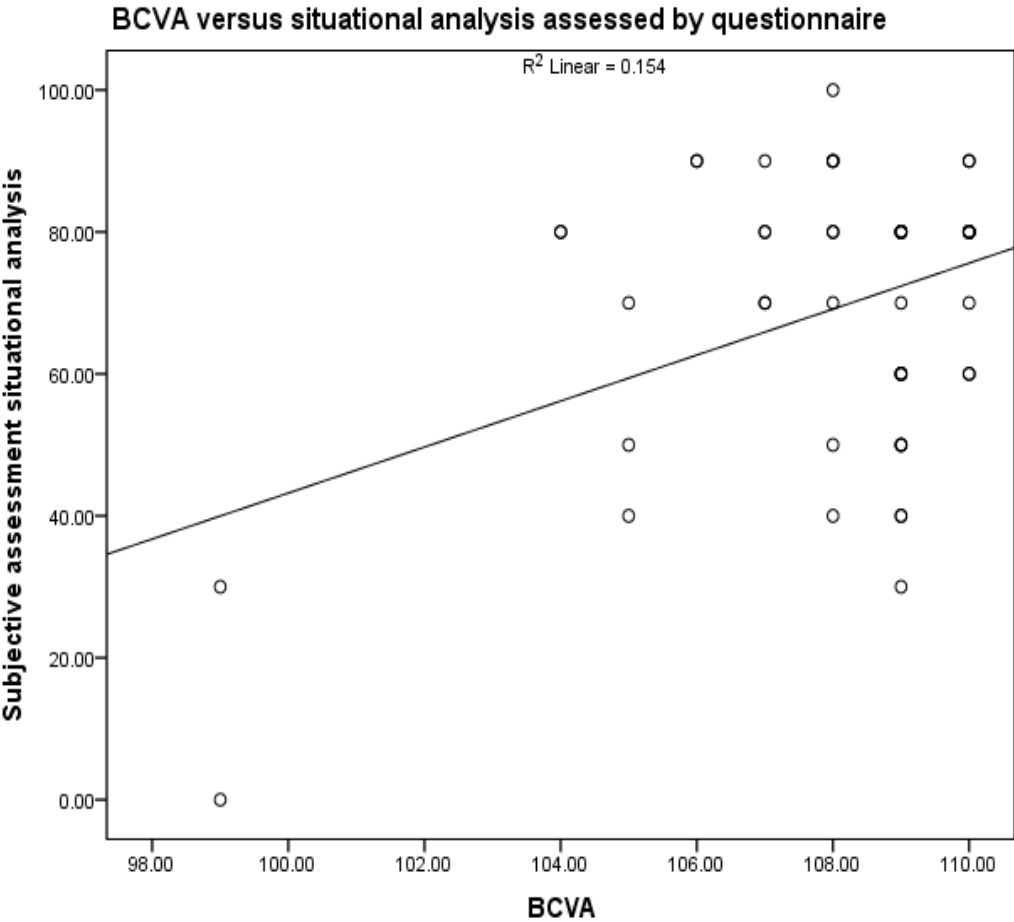
**Figure 5.7:** Glare subjective satisfaction score and MPOD at 1 degree of retinal eccentricity

#### 5.4.9 Validation of visual function questionnaire in normal's (VFNg)

The correlations between BCVA and acuity assessed by questionnaire (i.e. situational analysis, comparative analysis, overall subject satisfaction score) were positive, with a



significantly significant correlation found between BCVA and both situational analysis and subject satisfaction score acuity ( $r = 0.490$ ,  $p = 0.000$  [Figure 5.8] and  $r = 0.274$ ,  $p = 0.05$ , respectively).



**Figure. 5.8:** Relationship between BCVA and visual acuity assessed by questionnaire

## 5.5 Discussion

Given the central, pre-receptor location (Snodderly et al. 1984; Trieschmann et al. 2007), and the optical and antioxidant properties of MP (Bone et al. 1992), it is reasonable to hypothesise that MP would impact on visual performance, via its potential to attenuate CA and light scatter (Walls & Judd, 1933; Reading & Weale, 1974; Nussbaum et al. 1981; Wooten & Hammond 2002), or through its capacity to improve retinal function (Richer et al. 2004; Chakravarthy et al. 2009). 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 degrees 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 0.25 and 0.50 degree retinal eccentricity) is positively associated with BCVA in our study population, which suggests that MP plays a role in the optimisation of VA under photopic conditions in the more central locations. This finding however is all the more provocative given that subjects in the current study were young, free from ocular pathology, and uniformly demonstrated high VA. It is important to point out that extensive efforts were made by the study investigators to probe the limits of VA, 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 20/8) and recruitment of an experienced optometrist to perform functional evaluations. BCVA among the study participants ranged from a minimum of 99 (6/6-1) to a maximum of 110 (6/4).

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.  $17\text{cd/m}^2$  for the achromatic condition, and  $15.7\text{cd/m}^2$  for the chromatic condition), and were less appropriate for evaluation of photopic visual function. 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. Indeed, one might argue that the assessment of vernier acuity, a form of hyperacuity subserved by mechanisms other than basic ocular optics, was perhaps not the most appropriate methodology to assess the impact of a purely optical filter (i.e. MP) on visual performance. 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, as has been reported by Loughman et al. (2010).

We also report that central MPOD (i.e. at 0.25 degrees and at 0.5 degrees of eccentricity) is positively and significantly related to both mesopic and photopic contrast sensitivity at intermediate spatial frequency (i.e. 5.7 cpd) and MPOD at 0.25 degrees was also positively and significantly related to mesopic contrast sensitivity at 7.7 cpd but not photopic contrast sensitivity, although it did approach significance. Central MP appears to influence sensitivity at spatial frequencies to which the visual system is highly tuned Campbell et al.

(1968). However, in the current study there were some obvious outliers which weakened the relationship once they were removed. Although the association is weak, there is a trend and for photopic conditions, this finding might be attributable to the attenuation of the effects of CA and light scatter, whereby image refinement potentially cause lateral inhibitory surround responses to be dampened, and the resultant ganglion cell response optimised (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 SW's than cones (explaining the Purkinje shift in peak retinal spectral sensitivity towards blue under mesopic conditions). The pre-receptor absorption of SW light by MP might, therefore, serve to attenuate rod activity and allow cone-mediated vision (which typically exhibits better contrast sensitivity) (Puell et al. 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 Kvensakul et al. who reported that MP augmentation, via supplementation, enhances contrast acuity thresholds under mesopic conditions (Kvensakul et al. 2006).

Finally, we found that MPOD was not related to either glare sensitivity or photostress recovery, as assessed here. These findings 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 et al. 2003), photophobia (Wenzel et al. 2006), veiling glare (Stringham & Hammond, 2007), and photostress recovery (Stringham & Hammond, 2007; 2008). The cited series of experimental analyses are consistent with the rationale whereby MP attenuates the effects of SW (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 poses different analytical problems in 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 SW (blue) light component. Again, there is an obvious rationale for doing so, as MP predominantly absorbs SW 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 SW light than those employed in cited studies. Tungsten and tungsten-halogen filament lighting systems, in fact, contain a minimal SW light component. The absence of a strong SW light component in the photostress lamp, employed here, may partially explain the absence of any association between MP on PRT observed in our study (see Figure.5.1). It is worth noting, however, that the current trend for change to compact fluorescent and light emitting diode installations, which typically emit significantly more SW light (unpublished data from our laboratory suggests a two-fold increase in SW (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 VA and contrast sensitivity measures, appears to be associated with MPOD. However, photostress recovery and visual performance under glare conditions were unrelated to MPOD using the stimuli and tests employed here. 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.

VA 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, randomised, lutein-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 visual performance hypotheses of MP.

## **CHAPTER SIX**

### **Validation of Two Food Frequency Questionnaires to Assess Dietary Lutein and Zeaxanthin in Irish Adults**

#### **6.1 Abstract**

##### **Purpose**

The McCance and Widdowsons nutritional database (WISP) does not include values for lutein and zeaxanthin. This study aimed at modifying the database to include nutritional data for these two carotenoids. The database was then used to assess the accuracy of two self-administered Food Frequency Questionnaires (FFQs), to estimate dietary lutein and zeaxanthin intake in an Irish population. The study also aimed at assessing the comparability of this database with an existing nutrient database.

##### **Methods**

This cross-sectional study included 3 men and 19 women from DIT. Participants were aged 18-54 years and were all of Irish origin. Dietary intake of lutein and zeaxanthin was assessed by two different FFQs, the first of which was previously validated to assess overall habitual dietary intake in a Scottish population, and the second a brief nutrient specific FFQ, validated to assess lutein and zeaxanthin dietary intake in an Italian cohort of women. Dietary data was analysed using two different lutein and zeaxanthin databases. Serum concentrations of lutein and zeaxanthin were quantified by HPLC. MPOD was measured psychophysically using HFP. Demographic data, lifestyle data, and general health status, were also recorded by questionnaire, with particular attention directed toward



factors affecting bioavailability and absorption of dietary lutein and zeaxanthin along with risk-factors (established and putative) for AMD.

## **Results**

When dietary intake of lutein and zeaxanthin was assessed using the Scottish Collaborative Group (SCG) FFQ and modified Italian FFQ and analysed using modified WISP, differences of up to 4.88mg/day indicated a poor level of agreement. A similar lack of agreement was found between the modified WISP nutrient database and the Medical Research Council (MRC) nutrient database, with dietary intake differing by up to 4.22mg/day depending on the database used for dietary analysis. Dietary intake of lutein and zeaxanthin measured with the SCG FFQ and analysed using the modified WISP and MRC nutrient database correlated significantly with serum nutrient values ( $r=0.597$ ,  $P=0.024$ ), ( $r=0.590$ ,  $P=0.026$ ) respectively, but there was no significant correlation between the Italian FFQ and serum ( $r=0.288$ ,  $P=0.317$ ). A strong correlation was found between serum nutrient values and MPOD ( $r=0.734$ ,  $P=0.003$ ).

## **Conclusion**

A poor lack of agreement found between the dietary assessment tools and nutrient databases reinforces the limitations and difficulties inherent in dietary assessment and the lack of comparability between previous studies which differ in study design. The strong and statistically significant correlation found between serum nutrient values and MPOD would suggest that serum biomarkers are a more comparable and accurate alternative from dietary intake assessment, when investigating risk of AMD.

## **Key words**

Scottish Collaborative Group FFQ; modified Italian FFQ; Medical Research Council nutrient database; lutein; zeaxanthin; macular pigment.

## **6.2 Introduction**

To date there has been a lack of treatment options for dry AMD resulting in a growing interest in the potential role of lutein and zeaxanthin intake in the pathogenesis and treatment of AMD (Nolan et al. 2007; O'Connell et al. 2008). Accurate assessment of lutein and zeaxanthin is fundamental for advancing research in this area. Valid measurements of retrospective lutein and zeaxanthin intake, as well as ability to track lutein and zeaxanthin intake longitudinally, are crucial components for elucidating the role of lutein and zeaxanthin in health and disease. The methods that can be employed to measure dietary lutein and zeaxanthin will depend upon whether lutein and zeaxanthin is the only nutrient to be studied, or whether lutein and zeaxanthin intake will be evaluated together with assessment of energy and/or other nutrient consumption.

In clinical settings, practitioners can use estimates of usual lutein and zeaxanthin intake to determine appropriate treatment for individuals. These recommendations may involve providing dietary guidance and/or advice on lutein and zeaxanthin supplementation. Methods are therefore needed to estimate lutein and zeaxanthin intake of Irish adults accurately and efficiently, using approaches that are not overly burdensome for respondents, researchers, or clinicians.

Dietary intake is typically self-reported and only crudely quantified, even with the best developed methodologies, and as a result is subject to both bias and error. Owing to their relatively lower administrative costs and time and the ability to assess usual and longer term intake, FFQs have historically been the method of choice for collection of dietary intake data for epidemiologic studies (Cade et al. 2002). More recently, their use in other research and clinical settings has become more common as FFQs have been used to measure usual lutein and zeaxanthin intake (Nolan et al. 2007; O'Connell et al. 2008). These instruments are however commonly criticised for imprecise and biased estimates (Briefel et al. 1992) which may contribute to the failure of epidemiologic studies to show significant associations between diet and disease (Liu et al. 1987).

Few lutein and zeaxanthin specific FFQs have been developed and validated to date. As a result general FFQs have been used in their absence (Nolan et al. 2007). However FFQs designed for assessing total diet, such as those of Block and colleagues (Willet et al. 1985; Block et al. 1990) although validated, might not be suitable for all circumstances. Often these FFQs require optical scanning, making them impractical when estimates of intake are needed promptly for study enrolment or treatment decisions. In addition, total diet FFQs can be unnecessarily long when used for estimating only one nutrient, and they might not be as valid for assessing intake of a single nutrient as a trade-off for estimating intake of numerous nutrients in epidemiological studies (Cade et al. 2002). Also FFQ's developed for assessing lutein and zeaxanthin specifically may not be appropriate for estimating these carotenoids in Irish adults for a number of reasons. FFQs that were designed for particular populations (e.g. Italian women) might not be valid for use with other populations (Ward et al. 2004). FFQs developed in other countries might not reflect food eaten by adults in

Ireland (Cena et al. 2007). Finally, the high variability in food content and the limited quality and quantity of the food and nutrient composition data for lutein and zeaxanthin, also affects the ability to accurately interpret findings (Holden et al. 1999). It has been hypothesised that lutein and zeaxanthin concentration in food varies with time and according to geographical origin, however studies have demonstrated conflicting results. If the hypothesis is true, the carotenoid database used to analyse dietary intake would need to be up to date and developed using foods from the country of origin of the population being studied. This is not always possible due to the limited availability of carotenoid databases. Those that do exist are often not comparable and hence reducing the comparability with previous studies (Granodo et al. 1996; Scott et al. 1996; O'Neill et al. 2001).

Accurate assessment of individual intakes of lutein and zeaxanthin are crucial in the evaluation of the relative roles in eye health and disease. It was only in 1993, with the release of a new database on carotenoids by the USDA (Mangels et al. 1993) that dietary carotenoid intake, for the five major carotenoids found in human plasma, was compared with their respective values from food sources for the first time (Forman et al. 1993). Before that, due to the absence of databases for carotenoid fractions, carotenoid intake was based on conversion of vitamin A values to estimated carotene intake (Coates et al. 1991; Bolton-Smith et al. 1991; Ascherio et al. 1992). In addition, the nutritional composition of many of the more traditional foods has changed. The introduction of new cultivars, adoption of new feeding practices, and technological changes in food processing are all capable of altering the composition of food. An example includes the recent development of more highly coloured yellow vegetables, with a resultant increase in carotene content. Therefore, the most up to date databases should be used. Accurate assessment is also

difficult given that current databases generally report these carotenoids as a combined value. Xanthophyll carotenoid databases often consist of foods based on their xanthophyll content and not on their frequency of consumption. When lutein and zeaxanthin are considered together it makes it difficult to determine their respective roles in eye health.

Some researchers currently examining the relationship between dietary intake of lutein and zeaxanthin and risk of AMD are using a general FFQ, which was not developed with lutein/zeaxanthin specifically in mind (Nolan et al. 2007). A more specific brief Italian FFQ (Cena et al. 2007) has more recently been developed, which correlated well with plasma levels and with intakes assessed by 7 day records. In the current investigation, the comparability of the two FFQ's is examined. Dietary lutein and zeaxanthin intake is subsequently analysed using two different databases. The validity of the questionnaires and nutrient databases are determined using biomarkers; serum lutein and zeaxanthin and MPOD. As both questionnaires previously performed well when compared with dietary records, the hypothesis would be that both questionnaires would perform well when compared with each other, regardless of the nutrient database used.

## **6.3 Materials and Methods**

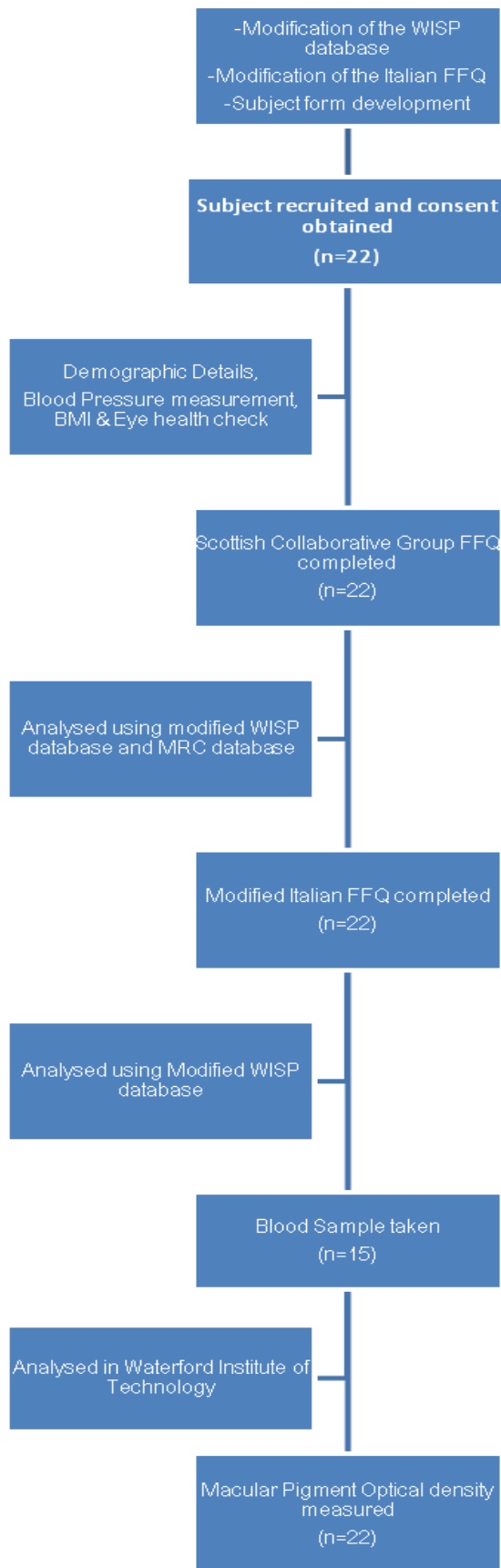
### **6.3.1 Subjects**

Subjects were recruited as a result of an information sheet circulated around DIT or through word of mouth. 19 women and 3 men from an Irish student population in DIT, volunteered to participate in the study, which was authorised by the Research Ethics Committee of DIT. Written information mapping the principles of the study was provided to each subject and informed consent was subsequently obtained from each subject prior to enrollment in the

study. Subjects in the age range between 18 and 60 years were included.. There were no other exclusion criteria. All 22 volunteers recruited were included in the study.

### **6.3.2 Study design**

The study design is illustrated in Figure. 6.1. This cross sectional study entailed the modification of a nutrient database and a brief Italian FFQ, completion of two FFQs, a demographic questionnaire, measurement of MPOD, obtaining a blood sample, blood pressure and BMI calculation. Completion of all parts of the study took approximately one hour 20 minutes per subject. During their visit, subjects were invited and instructed to complete two FFQs, the first of which was previously designed and validated to assess habitual dietary intake and the second, a modified version of a brief FFQ previously designed and validated to assess specifically lutein and zeaxanthin intake among the Italian female population aged 20-25years (see section 6.3.5 and 6.3.6). Subjects also underwent a short clinical examination, checking blood pressure, weight, height and visual health. A blood sample was collected from 15 subjects. Blood samples were not taken from 7 subjects due to extraction difficulties. MPOD measurements were taken using the Macular Metrics Densitometer<sup>TM</sup>, which is a validated psychophysical method for measuring MP. As carotenoid intake has been shown to have a wide seasonal variation, the biomarker information was collected on the same day as the FFQ administration and completion.



**Figure 6.1:** Study Design

### **6.3.3 Demographic details questionnaire**

Demographic data, lifestyle data, and health status, were recored by questionnaire, with particular attention focused towards risk factors for AMD. The risk factors investigated included age, sex, blood pressure, cholesterol, personal and/or family history of eye disease, cigarette smoking history, including frequency and quantity of cigarettes smoked, along with exposure to second hand smoke; alcohol intake including frequency and quantity, physical activity, BMI (calculated by  $\text{kg/m}^2$ ) and blood pressure (mmHg).

### **6.3.4 Food frequency questionnaire**

Before the analysis criteria for acceptable data quality, including limitations of FFQ's, were carefully considered. Common limitations of FFQs include questions left blank and over estimation of dietary intake. Overestimation of intake was minimised, particularly in the SCG FFQ, by encouraging subjects to read all foods listed in each food group before deciding frequency and quantity of consumption. All questionnaires were immediately reviewed with the subject to ensure no spaces were left blank.

### **6.3.5 Scottish collaberative group (SCG) FFQ**

A self-administered, semiquantitative FFQ developed by the SCG was used as the first tool for dietary assessment. The questionnaire which was previously validated (Masson et al. 2003), was designed to estimate a subjects habitual diet over the previous 2 to 3 months. The questionnaire comprised 166 commonly eaten food types or drink, grouped into 19 selections with three additional sections labelled 'Other foods and drinks' 'vitamin, mineral and food supplements' and 'other'. Subjects were asked to specify two things; (1) frequency of consumption of each food, on a weekly or monthly basis. Less than monthly



consumption was denoted by ‘rarely’ and nutritional contribution was considered negligible

(2) Quantity consumed by specifying how many portion ‘measures’ per day they consumed of each food. A ‘measure’ was designed to be a small portion so that a single standard portion of a food would often be more than 1 measure. A coloured photograph, depicting examples of food measures equal to 1 measure accompanied each questionnaire, along with detailed written and verbal instructions and an example of how to fill in the questionnaire. Questionnaires were completed in 23-35 minutes (See Appendix D).

### **6.3.6 Modified Italian FFQ**

An interview assisted quantitative 32-item FFQ, originally developed and validated (Cena et al. 2007) to assess dietary intake of lutein and zeaxanthin in Italian women, was modified to suit the Irish diet. Modification included the exclusion of three foods originally listed from the FFQ (collards, green turnip and tangerine juice) which did not feature commonly in an Irish diet, and the inclusion of six important lutein and zeaxanthin food sources (green peppers, turnip, eggs, kelloggs cornflakes, celery, herbs/spices) which featured more regularly (O’Neill et al. 2001). The questionnaire was originally designed as a quantitative questionnaire to assess lutein and zeaxanthin intake over the previous month, involving an interview. Brief written and verbal instructions were again provided. Subjects were asked to answer two questions; (1) frequency of consumption, either as ‘never consumed’ or on a daily, weekly or monthly basis and (2) number of standard portion measures consumed. It is well known that FFQ’s and food records may have common sources of errors such as recall bias of portion sizes and over/under reporting (Cade et al. 2002). In order to minimise this, the same food photograph was used to illustrate the quantity of food equivalent to one

portion measure for both FFQs. However, this might bias the results of the Bland Altman Plot and reinforce Pearson's/Spearman's correlation (See Appendix E).

### **6.3.7 Macular pigment optical density measurement**

MP was measured psychophysically using the Macular Densitometer™ (Macular Metrics II, 12 River St, Rehoboth, MA 02769, USA), a device developed and originally described by Wooten et al. (1999) to measure MPOD. This technique utilises the principle of HFP and for this study MP measurements were taken centrally (0.25 degrees retinal eccentricity) and peripherally (7 degrees retinal eccentricity) (See section 5.3.7 for a detailed description of this procedure).

### **6.3.8 Analysis of serum samples**

Blood samples (6-8 ml) were collected from 15 patients on the same day as MPOD assessment. Serum was separated from blood by centrifugation at 15,000 revolutions per minute (rpm) for 10 minutes, and then aliquoted into two light-sensitive micro centrifuge tubes and stored at minus 70° Celcius until time of analysis. The following method is a procedure used by Nolan et al (2007). A 400µL aliquot of serum was pipetted into a light-sensitive micro centrifuge tube (1.5 mL total capacity). Ethanol (300 µL) containing 0.25 g/L butylated hydroxytoluene (BHT) and 200µL internal standard ( $\alpha$ -tocopherol acetate) were added to each tube. Heptane (500µL) was then added and samples were vortexed vigorously for 2 minutes followed by centrifugation at 2000 rpm for 5 minutes (MSC Micro Centaur, Davison & Hardy Ltd. Belfast, UK). The resulting heptane layer was retained and transferred to a second labeled light-sensitive micro centrifuge tube, and a second heptane extraction was performed. The combined heptane layers were immediately

evaporated to dryness under nitrogen. These dried samples were reconstituted in 200 $\mu$ L methanol (containing 0.25 g/L BHT), and 100  $\mu$ L was injected for HPLC analysis. Agilent 1200 series was used (Agilent Technologies Ltd. Dublin, Ireland) system with photodiode array detection. A 5 micron analytical/preparative 4.6 x 250 mm 201TP specialty reverse phase column (Vydac, Hesperia, CA) was used 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 minutes. DSM nutritional products (Basel Switzerland) provide total lutein (TL) and total zeaxanthin (TZ) standards to operate response factors which were used to calculate serum concentrations of TL and TZ. An internal standard:  $\alpha$ -tocopherol acetate was made up in ethanol (0.25 mg/L) was used to standardise all extractions for HPLC analysis and was also used for quantification purposes. 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 (Nolan et al. 2007).

### **6.3.9 Data input and coding**

Each subject was given a reference number for anonymity purposes. Completed personal detail questionnaires, were entered into a Microsoft Excel spreadsheet. Categorical data was coded, and open ended questions were entered directly. The Excel spreadsheet was exported into the SPSS software package, version 18, and after some manipulation of the data, the final database was created.

Dietary information obtained from the SCG FFQ and modified Italian FFQ were coded and entered into Microsoft Excel. Daily nutrient intakes were determined from weekly

estimates and data entered into a food and nutrient software package (WISP V 3, Tinviel Software, 2006) and output was obtained for each method and for each subject. The SCG FFQ was analysed using a second nutrient database developed by the MRC Human Nutrition Research (Cambridge, UK), which was designed specifically for analysis of this questionnaire.

#### **6.3.10 Modification of WISP**

Every effort was made to ensure that the most up to date information on lutein and zeaxanthin was used. An extensive search of the literature and existing carotenoid databases was conducted, to identify estimation of lutein and zeaxanthin content of the 569 individual foods, which were designed to make up the 166 commonly eaten food types in the SCG FFQ and the 32 individual foods in the Modified Italian FFQ. Sources of data used included the United States National Nutrient Database (USDA) (1998); Perry et al. (2009); O'Neill et al. (2001) and Nutrientdata.com (2009). The information in Nutrition Data.com is derived from the USDA's National Nutrient Database for Standard Reference and is supplemented by listings provided by restaurants and food manufacturers, such as Kellogg's. Since only O'Neill et al. derived data from Ireland, and since it is thought that carotenoid content varies geographically, this database would have seemed like the most suitable to use. However, total carotenoid intake estimated by O'Neill et al. (2001) using this database tended to be 3 to 4 times higher than that calculated by previous investigators. This was explained in part by the potential inaccuracy and the dietary assessment tool used (Granado et al. 1996; Scott et al. 1996; O'Neill et al. 2001). Despite this however, in the absence of other Irish or European carotenoid databases, Irish data from O'Neill et al. (2001) was included in the modification of the database. Confusion as to which analysis

uses cis/trans carotenoid information makes it difficult to make a comparison between databases and to determine the most reliable and accurate database to use. Therefore, one database alone cannot be deemed the most reliable and a combination of all four were used for this study.

All four databases were used to estimate the lutein and zeaxanthin combined contents of the 569 and 32 foods included in the SCG and modified Italian FFQ. 75 foods were deemed to be sources of lutein and zeaxanthin using this approach. The WISP database was subsequently modified for lutein and zeaxanthin as a combined nutrient value, as the majority of published databases to date contain only information on lutein and zeaxanthin combined. The 44 FFQs in total were analysed using the modified database and dietary data were exported to a SPSS database.

#### **6.3.11 Data cleaning of the SCG FFQ**

The ratios of energy intake to basal metabolic rate (BMR) were calculated to identify mis-reporting (Goldberg et al. 1991). As only the SCG FFQ assessed total energy intake, under/over-reporting could not be evaluated for the modified Italian FFQ. The cut off ratio was set at 1.35, as  $1.35 \times \text{BMR}$  is the minimum energy expenditure compatible with a normally active lifestyle (Goldberg et al. 1991). However, as this would result in the exclusion of 11 subjects (50%), all subjects were included in the study. It does serve however, to reinforce the difficulties inherent in retrospective FFQ analysis.

### 6.3.12 Statistical analysis

The statistical software package SPSS (version 18) was used for analysis. The mean and SD's are given for normally distributed data. The following variables were tested as potential effect modifiers for the correlation between dietary lutein/zeaxanthin intake and corresponding biological markers: age, sex, BMI category (Kg/m<sup>2</sup>: <25, 25-29.9, ≥30), smoking status (never, former, current), alcohol intake per week, physical activity, and fruit and vegetable intake.

- (1) Preliminary analyses at all phases were performed to ensure no violation of the assumptions of normality. Concurrent agreement of the SCG FFQ, the modified Italian FFQ, the WISP database and the MRC database, along with the validity of both FFQs, with biological markers was assessed using several methods: To determine overall association: Pearson Product-moment correlation.
- (2) A paired samples t-test was performed to compare means and test for statistical significance.
- (3) To determine agreement: Bland-Altman plots including limits of agreement and coefficients of repeatability (Bland et al. 1986) were calculated for the SCG FFQ and the modified Italian FFQ. The limits of agreement define the limits within which 95% of these differences are expected to fall (mean of difference  $\pm$  1.96 standard deviations of the difference). The differences between the two FFQs were plotted against the average of the two FFQs creating a Bland Altman Plot. This was repeated to assess agreement between the two nutrient databases.
- (4) Joint classification of nutrient intake assessed by the two FFQ was assessed using quintiles of intake for lutein and zeaxanthin from the SCG FFQ and the modified Italian FFQ, respectively. The proportion grossly misclassified applied when one

dietary assessment method classified the individual's intake into the lowest quintile and the other method classified it into the highest quintile. Quadratic weighted Kappa values were calculated comparing quintiles of intake for each nutrient from the SCG FFQ and modified Italian FFQ.

## **6.4 Results**

### **6.4.1 Demographic data**

The demographic profile of the study population is presented in Table 6.1. Because only 3 subjects were males, the data was not split by gender. The study population consisted of 19 females and 3 males aged 18-54 years. Only one subject reported a previous diagnosis of high cholesterol. No previous history of eye disease was reported. Mean daily intake of lutein and zeaxanthin varied from 1.68mg/day (SD±1.16) to 1.76mg/day (SD±1.87), depending on the dietary assessment tool and nutrient database used for dietary assessment and analyses. Mean serum concentrations of lutein and zeaxanthin were (1.02ug/ml (SD±0.33) and mean central MPOD was 0.59 (SD±0.19); mean total MPOD was 1.77 (SD±0.676).

**Table 6.1** Demographic characteristics of sample population (n=22).

	<b>Mean</b>	<b>% SD</b>		<b>Mean</b>	<b>% SD</b>
<b>Age</b>	24.5	9.49	<b>BMI (Kg/m<sup>2</sup>)</b>		
<b>Sex</b>	<b>n</b>		18.5-24.9	15	68.2
Male	3	13.6	25-29.9	6	27.3
Female	19	8.4	>30	1	4.5
<b>Exercise (mins/wk)</b>	212.7	144.5	<b>Smoking Status</b>		
<b>MPOD Total</b>	1.775	0.676	Never	18	81.8
<b>MPOD Central</b>	0.591	0.19	Current	3	13.6
			Former	1	4.5
<b>L and Z intake (mg/day)</b>			<b>Alcohol Consumption</b>		
SCG FFQ (WISP database)	1.68	1.16	None	0	0
SCG FFQ (MRC database)	1.76	1.87	1 unit	2	9.1
Modified Italian	1.51	1.41	6-10 units	4	18.2
			>10 units	10	45.5
<b>Fruit Intake (g/day)</b>			<b>Vegetable Intake (g/day)</b>		
SCG FFQ	352	188	SCG FFQ	183.9	118
Modified Italian	61	49.9	Modified Italian	135.9	75.6



L and Z = Lutein and Zeaxanthin.

BMI = Body mass index; MRC = Medical research council.

Mean and Standard Deviation (SD) Number (n) and %.

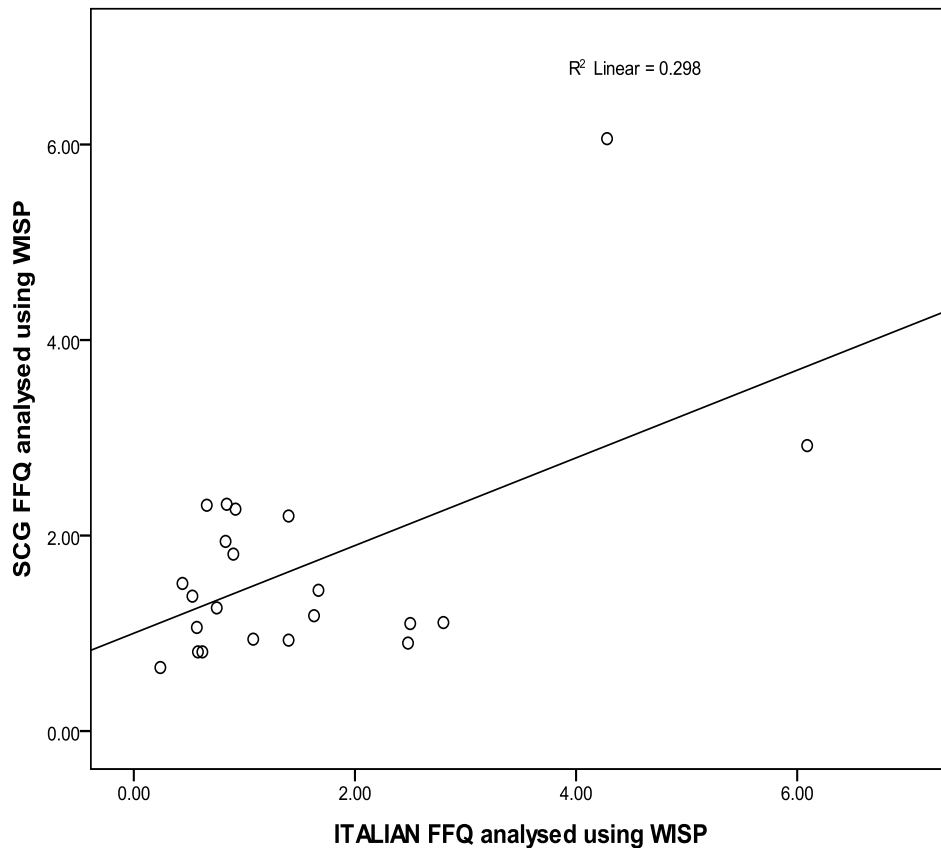
#### **6.4.2 Comparability of the two FFQ's**

Comparison of group means

The mean daily intake of lutein and zeaxanthin estimated by the SCG FFQ and the modified Italian FFQ were 1.68mg/day (SD±1.16) and 1.51mg/day (SD±1.41) respectively (analysed using modified WISP database). In order to see, to what extent both FFQs were in agreement, data were plotted in a scatter diagram [Fig.6.2 (a)]. The mean difference in intake by the two methods was 0.168mg (SD±1.2). A paired samples t-test revealed no statistical significant difference in mean intake estimated by the two FFQs, ( $t= 0.634$ ,  $P = 0.533$ ).

#### **6.4.3 Correlation between both FFQs**

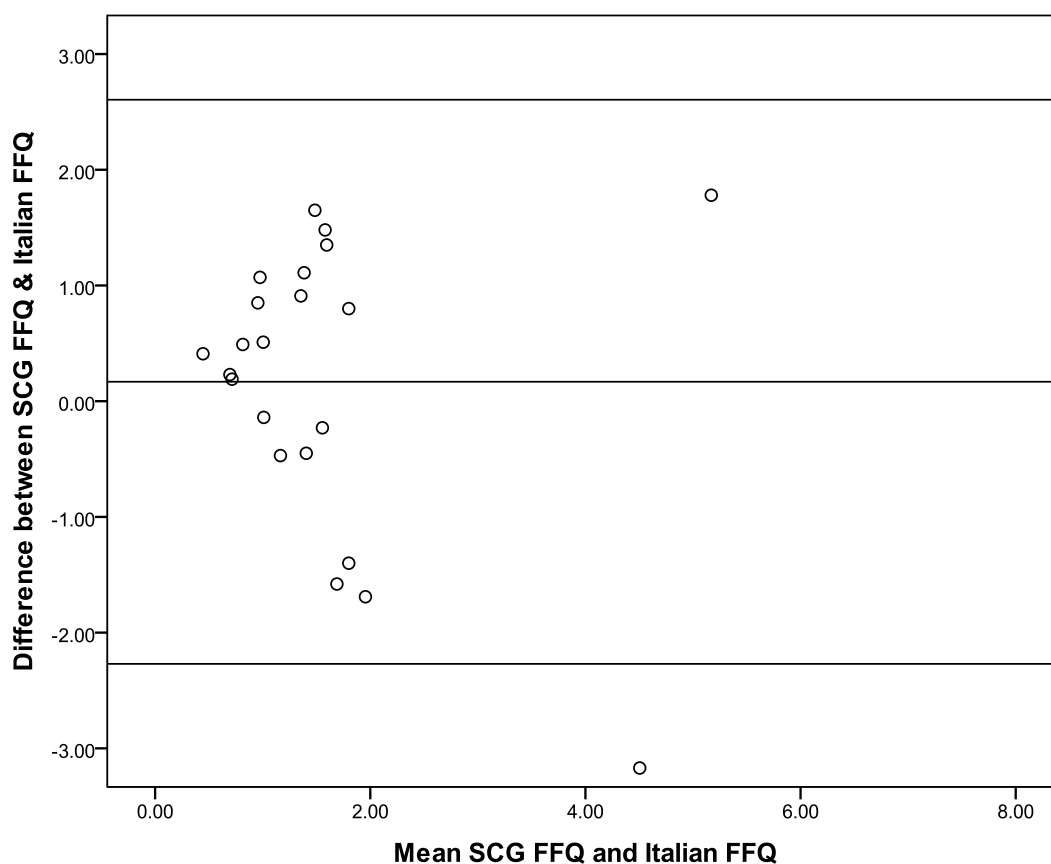
In order to see to what extent the two methods were associated, Pearson's product moment correlation was computed. There was a positive significant association between both FFQs [SCG FFQ and modified Italian FFQ analysed using modified WISP], ( $r = 0.546$ ,  $p=0.009$ ).



**Fig.6.2(a)** Correlation between lutein and zeaxanthin intakes assessed by the SCG FFQ (analysed using modified WISP) and the modified Italian FFQ.

#### **6.4.4 Assessment of agreement between FFQs: Bland-Altman Plot**

Figure 6.2(b) illustrates how well the two methods were likely to agree on an individual basis. The difference between lutein and zeaxanthin intake assessed by the two FFQs were plotted against the average of the two measurements. The 95% limits of agreement were calculated. The mean difference is close to zero, so there is little evidence of overall bias. Wide limits of agreement indicated that both methods differed by up to 4.88mg for some subjects. From the Bland Altman plot it can be seen that as the lutein and zeaxanthin intake of individuals increased, so did the magnitude of error between the modified Italian FFQ and the SCG FFQ.



**Fig.6.2(b)** Differences between lutein and zeaxanthin intakes assessed by the SCG FFQ (analysed using modified WISP) and the modified Italian FFQ plotted against the average of the two measurements (Bland Altman Plot).

#### 6.4.5 Comparability of the two nutrient databases

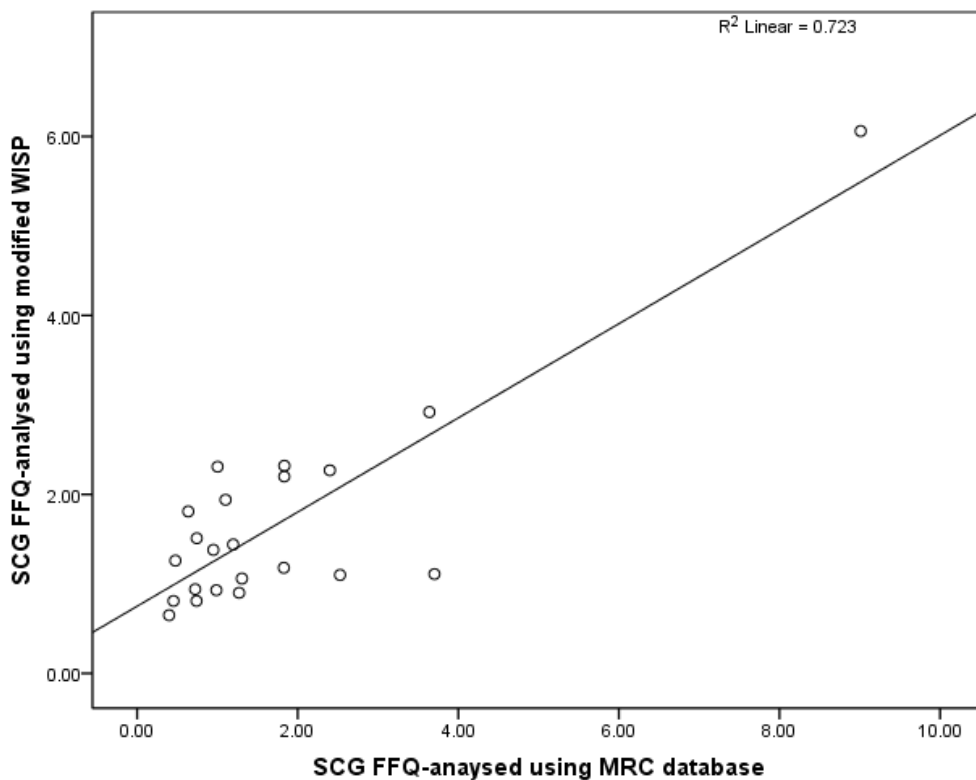
Comparison of group means:

The mean daily intake of lutein and zeaxanthin estimated by the SCG FFQ, using the modified WISP nutrient database was  $1.68 \pm 1.16$  and using the MRC nutrient database was  $1.76 \pm 1.87$ . Fig. 6.3(a) shows a scatter diagram, representing the association between dietary intakes assessed by the SCG FFQ and analysed using two different databases. On average the mean difference between the two databases was 0.08mg

(SD±1.076). A paired samples t-test revealed no statistically difference in means between the two databases, (t=-0.365, P=0.719).

#### 6.4.6 Correlation between the nutrient databases

A pearsons product moment correlation was calculated, (r=0.85, p=0.001), indicated a significant, strong, positive correlation between the two databases.

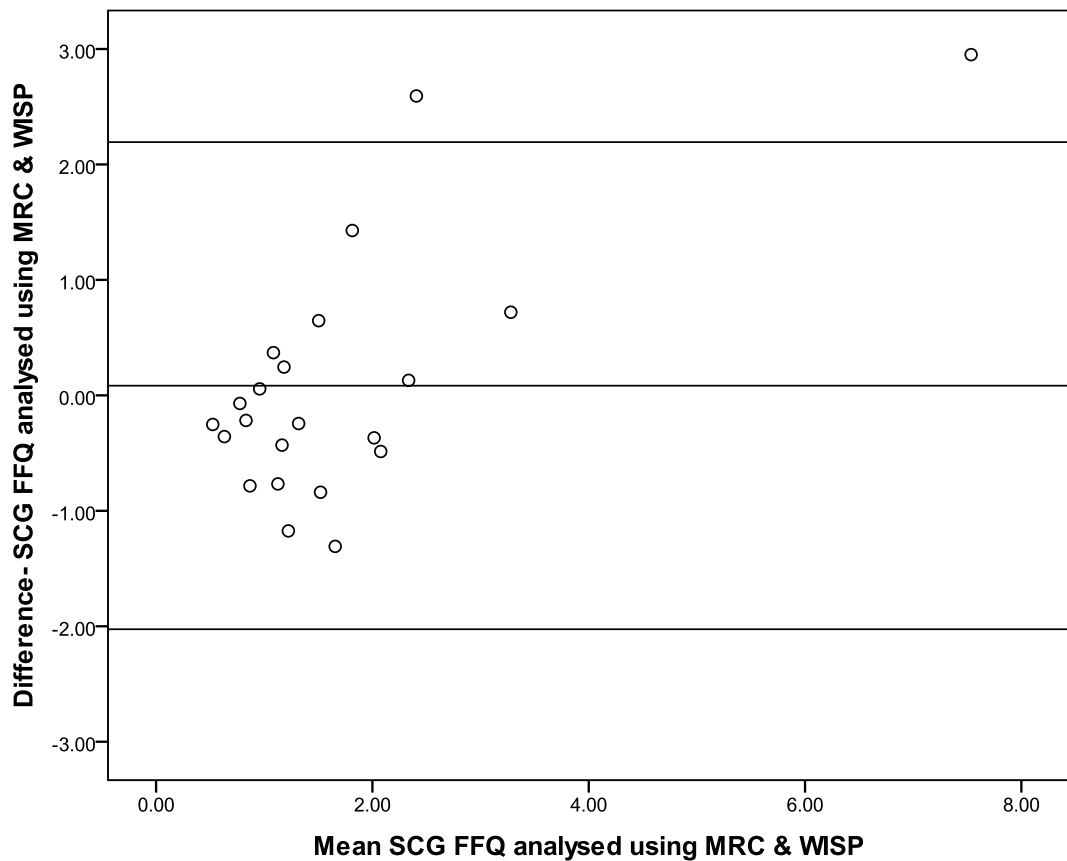


**Fig 6.3(a):** Correlation between lutein and zeaxanthin intake using the SCG FFQ analysed using WISP database and the MRC database.

#### 6.4.7 Assessment of agreement between databases: Bland Altman Plot

Figure 6.3(b) presents a Bland Altman plot, which illustrates how well the two databases were likely to agree on an individual basis. The mean difference between lutein and zeaxanthin intake analysed by the two databases were plotted against the

average of the two measurements. The mean difference is close to zero, so there is little evidence of overall bias. Wide limits of agreement represented a potential variation of up to 4.22 mg/day depending on the nutrient database used. From the Bland Altman Plot it can be seen that as the lutein and zeaxanthin intake of individuals increased, so did the magnitude of error between the modified WISP nutrient database and the MRC nutrient database.



**Fig. 6.3(b)** Differences between lutein and zeaxanthin intakes analysed using the modified WISP and the MRC database plotted against the average of the two measurements (Bland-Altman Plot).

**Table 6.2** Shows the relationships between the study parameters for the entire study group.

Table 6.2. Pearson's Product moment correlation Matrix showing relationships between the study parameters for the entire study group

	SCG FFQ (WISP)	SCG FFQ (MRC)	Italian FFQ (Modified)	Serum L+Z	MPOD
SCG FFQ (WISP)	1				
SCG FFQ (MRC)	0.850**	1			
Italian FFQ (Modified)	0.546**	0.730**	1		
Serum L+Z	0.597*	0.590*	0.288	1	
MPOD	0.205	0.350	0.161	0.734**	1

\*Correlation is significant at the 0.05 level (Pearson's correlation: two tailed)

\*\*Correlation is significant at the 0.01 level (Pearson's correlation: two tailed).

#### 6.4.8 Classification into quintiles of consumption

In this study, the number of subjects correctly classified within one quintile category by both FFQ's for estimated lutein and zeaxanthin intake analysed using the WISP database were 4 (18%) respectively. There was no gross misclassification. Weighted kappa values were poor (0.32) representing poor agreement. Despite no gross

misclassification between the two FFQ's, the relatively low proportion of subjects similarly classified, would suggest a poor capacity for both FFQ's to classify individuals into the same quintile of consumption. When comparing dietary lutein and zeaxanthin analysed using the two different databases, only 9 (41%) were correctly classified into the same quintile of consumption with no gross mis-classification.

#### **6.4.9 The relationship between dietary lutein and zeaxanthin and serum**

Mean serum lutein and zeaxanthin values was 1.01 $\mu$ g/ml (SD $\pm$ 0.33). Table 6.2 demonstrates that serum lutein and zeaxanthin concentrations were significantly correlated with the dietary intakes obtained from the SCG FFQ only. The modified WISP database showed similar correlations ( $r=0.597$ ,  $p=0.024$ ) to the MRC nutrient database ( $r=0.590$ ,  $p=0.026$ ). No significant relationship was found between dietary intake analysed by the modified Italian FFQ and serum concentration of lutein and zeaxanthin ( $r=0.288$ ,  $p=0.317$ ). The strength of the relationship was increased between the three sets of dietary data and serum lutein and zeaxanthin when BMI, alcohol intake (g/day) and smoking status were controlled for.

#### **6.4.10 The relationship between dietary lutein and zeaxanthin and MPOD**

Mean total and central MPOD calculated was 1.775 (SD $\pm$ 0.676) and 0.59 (SD $\pm$ 0.19). Table 6.2 demonstrates non significant correlation coefficients for both questionnaires, analysed using the modified WISP database and MRC database with MPOD. Similar, to the relationship with serum, correlations were strengthened when controlling for BMI, alcohol intake (g/day) and smoking status.

#### **6.4.11 The relationship between serum lutein and zeaxanthin and MPOD**

The relationship between serum values and MPOD total yielded a strong correlation of ( $r=0.734$ ,  $p=0.003$ ) (Table 6.2). The strength of the relationship was improved after adjusting for BMI, alcohol intake (g/day) and smoking status.

#### **6.4.12 Analysis of sources of lutein and zeaxanthin in the Irish population**

Lutein and zeaxanthin dietary intake, estimated from the SCG FFQ, was split into 17 different food groups and % of lutein and zeaxanthin contributed by each food group was calculated. As expected food groups contributing most substantially to lutein and zeaxanthin intake, included vegetables (44%), fruit/ fruit juices (20%), breakfast cereals (8%), potatoes (5%), breads (4%) and miscellaneous (3%). Vegetable intake estimated by both, the SCG and modified Italian FFQ, was positively and significantly correlated with estimated lutein/zeaxanthin intake ( $r = 0.712$ ,  $p=0.001$ ;  $r = 0.693$ ,  $p=0.001$ ), but not MPOD, although it did approach significance for the SCG FFQ ( $r=0.419$ ,  $p=0.052$ ). Fruit intake from the SCG FFQ and the modified Italian FFQ was not significantly correlated with lutein and zeaxanthin intake, but fruit intake from the Italian FFQ was significantly related to MPOD ( $r=0.436$ ,  $P = 0.042$ ), but not for the SCG FFQ. Mean weights of foods consumed in these food groups were also calculated. The average consumption of fruit and vegetables estimated by the SCG FFQ were 352g ( $SD\pm 188$ ) and 184g ( $SD\pm 118$ ) respectively. This was approximately 3 times greater than that estimated by the modified Italian FFQ (Table 6.1).



## 6.5 DISCUSSION

The purpose of this study was to assess dietary intake of lutein and zeaxanthin in a small sample population, using two different FFQs, and two different nutrient databases, and subsequently to assess the validity of the dietary assessment tools and nutrient databases, using nutrient biomarkers; blood serum and MPOD. A small sample size was a major limitation of this study, given that a sample size of >50 subjects is needed to allow the limits of agreement calculated in the Bland Altman plot to be estimated precisely (Cade et al. 2002). Therefore, as with any study, interpretation and extrapolation of results must be done so in a cautious and educated manner. Instead, results should be used as a guide to help direct future research in the area.

Results of this study showed a positive relationship between lutein and zeaxanthin in the diet, serum and macula. These findings are consistent with previous intervention studies, which have shown that dietary lutein and zeaxanthin fortify the MP in the eye, forming a layer of tissue that protects the macula from age-related damage (Bone et al. 1997; Johnson et al. 2005). There was a strong positive relationship between serum nutrient concentration and MPOD. Again this is supported by previous findings which indicate that the extent of MP enhancement is dependent upon the lutein and zeaxanthin in the blood serum level achieved (Hammond et al. 1997; Bone et al. 2002). However, the nature of the relationship between lutein and zeaxanthin in the diet, blood, and macula is not a simplistic one but is confounded by many variables including processes which influence digestion, absorption, and transport of the compounds in question, and accumulation and stabilisation of the carotenoids in the tissues. Regardless of the confidence in the method for dietary assessment, it is not possible to control for the extent to which interpersonal variation of these variables will influence the relationship

between lutein and zeaxanthin in the diet and its consequent accumulation in the blood and macula, whereas biomarkers, serum and MPOD are independent, to a certain extent of these influences.

Mean dietary intake of lutein and zeaxanthin assessed by the SCG FFQ and the modified Italian FFQ, and analysed by modified WISP, was  $1.67 \pm 1.16$ mg/day and  $1.51 \pm 1.41$ mg/day respectively which fell approximately 3-5mg short of the daily recommended intake of 6mg/day, however results were consistent with previous Irish findings (Nolan et al. 2007). Despite the use of several different dietary assessment tools and nutrient databases, previous researchers have found a high level of consistency in estimated intakes of lutein and zeaxanthin, suggesting comparability between studies and hence a good level of agreement between the different dietary assessment tools and nutrient databases. For example, the estimated dietary intake in the Irish and Italian population cohort, using the SCG FFQ and the brief Italian FFQ have been analysed using separate nutrient databases (Nolan et al. 2007; Cena et al. 2007). Estimated dietary intake ranged from 1.1mg/day to 1.6mg/day, differing by a negligible 0.5mg/day. Both studies found significant correlations between dietary intake and serum concentrations, suggesting validity of their chosen dietary assessment tools and nutrient databases. However, Wirfalt et al (1998) highlighted a limitation compromising the comparability of studies. Both FFQs differ in terms of design and neither have been validated in an Irish population [validation studies are not necessarily transferable to another population (Plummer et al. 2003)]. The modification of the Italian FFQ here, makes previous validation invalid (Castenmiller, 1999) so neither of the FFQ's can be considered a validated dietary assessment tool for use in an Irish population. Additionally, the fact that the SCG FFQ was previous designed and validated as a

general FFQ and not specifically with lutein and zeaxanthin in mind, would leave results yielded by its use questionable.

On average, the SCG FFQ and the modified Italian FFQ performed well against each other, with a negligible mean difference in estimated lutein and zeaxanthin intake of 0.168mg/day. Although there was a positive and significant correlation between the two FFQ's, the two FFQs demonstrated poor agreement. The wide limits of agreement calculated in the Bland Altman Plot Figure. 6.2(b) indicate that on an individual level the two methods differ in estimation of lutein and zeaxanthin intake by up to 4.88 mg/day, with differences increasing for highest consumers of lutein and zeaxanthin. Given that the range within which most differences lie is approximately 3 times the average Irish daily intake and 3 times larger than those calculated by previous researchers (Cena et al. 2007), it is possible to conclude that any false assumption of good comparability between the two FFQs, may lead to false associations between dietary factors and disease or disease related markers. As limits of agreement must be interpreted clinically and not statistically, more studies are needed to establish a reference range within which limits of agreement should lie when establishing the comparability or validity of FFQs. In this instance analyses using the Bland Altman plot served to highlight the potential danger of using mean difference or correlation coefficients alone to determine comparability of two methods. Where one method may produce a consistently higher level of output than another, a strong correlation would persist, despite a lack of agreement at an individual level. In the absence of such information, correlation coefficients continue to be the main method of comparison between studies, with >90% of studies having calculated correlation coefficients and <10% calculating limits of agreement (Cade et al. 2002). The lack of association is also

reinforced by the failure of the two FFQs to classify 18 (81%) subjects into the correct corresponding quintile of consumption, which has been identified as the most important quality of the FFQ (Hodge et al. 2009).

On average, the SCG FFQ analysed using modified WISP and the MRC nutrient database performed well against each other, with a negligible mean difference in estimated lutein and zeaxanthin intake of 0.084 mg/day. There was a significant strong positive correlation between the two FFQ's when analysed using the different nutrient databases, however, this result again is misleading, as on an individual level the two databases did not agree. The wide limits of agreement calculated in the Bland Altman Plot Fig. 6.2(b) indicate that the two methods differ in estimation of lutein and zeaxanthin intake by up to 4.22 mg/day, with differences increasing for highest consumers of lutein and zeaxanthin. The lack of agreement found between the two nutrient databases is unnecessarily reducing comparability of studies even further. While FFQs are well known for their limitations, and, as in the current study, have been found to vary substantially in accuracy (Sebring et al. 2007), the high degree of variability between databases is less appreciated. According to O'Neill et al. (2001) '*the validity of all food intake data in terms of nutrient intakes is based on the availability and accuracy of food composition data*'.

In addition to the absence of a 'gold standard' lutein and zeaxanthin dietary assessment tool, there is, similarly an absence of a 'gold standard' database. This renders the assessment of the validity of questionnaires difficult. Inaccurate information may lead to false associations between dietary factors and disease or disease related markers. Validation of the questionnaires against a 'gold standard' is necessary to determine

which method is providing the most accurate information. Studies prior to 1993 used a database developed based on limited analytical data and extrapolation of values for similar foods (Coates et al. 1991). The more recent studies have used the database originally developed by the USDA and the National Cancer Institute by Mangels et al. in 1993 and updated in 1998, either alone or in combination with other data. Several studies have examined individual associations between dietary carotenoids, and plasma carotenoid concentrations (Brady et al. 1996; Coates et al. 1991; Forman et al. 1993; Peng et al. 1995; O'Neill et al. 2001; Nolan et al. 2007; Cena et al. 2008). Previous researchers have found when databases have been compared they yielded different quantitative estimates, but similar correlations with blood concentrations (Ritenbaugh et al. 1996; Vandenlangenberg et al. 1996). Correlations reported by these studies range from 0.09-0.76 for lutein and zeaxanthin. The variation in databases leading to such differences in quantitative estimates but good agreement with blood concentrations may be explained by an absence of consistency in reporting cis/trans form of the carotenoid, bioavailability from the food, cooking method, ripeness, or country of origin.

Given that most dietary assessment methods carry considerable limitations and error and are subject to several forms of bias, with the possible exception of extensive diet diaries (e.g.14-21 days in length) or direct behavioural observation, no dietary assessment method can be deemed a gold standard (Briefel et al. 1992). In the absence of such a gold standard, this study used two nutritional biomarkers, [serum concentration of lutein and zeaxanthin and MPOD (reflecting lutein/zeaxanthin status in the eye)], to determine the validity of the two FFQs. As carotenoids are not homeostatically regulated, and the only source of these in serum and macula is diet, MPOD and serum biomarkers are assumed to be independent of respondent bias and

they represent a useful measure of the relative validity of the intake measure. Determining the threshold of acceptable validity coefficients between self-reported diet and biomarkers is often difficult i.e. deciding on how strong a correlation is needed to establish validity, since MPOD and serum lutein and zeaxanthin are influenced by factors other than food intake. Accurate interpretation of data correlating dietary intake of lutein/zeaxanthin and serum concentration of lutein/zeaxanthin in epidemiologic and clinical studies requires knowledge of biological and non-dietary factors influencing the relationship between lutein and zeaxanthin in foodstuffs, blood, and macula. Rock et al. identified demographic characteristics (age, sex, race/ethnicity, education), BMI and lifestyle factors (exercise, sun exposure, smoking and alcohol consumption) to be significantly associated with dietary lutein and zeaxanthin intake, while processes which influence digestion, absorption, and transport of the compounds in question, and accumulation and stabilisation of the carotenoids in the tissues will influence serum (a short term marker of dietary intake) and macular concentrations (a long term marker of dietary intake) of lutein and zeaxanthin (Rock et al. 2002).

Mean serum nutrient concentration ( $0.101 \pm 0.33\mu\text{g/ml}$ ) was consistent with previous Irish findings ( $0.102 \pm 0.045\mu\text{g/ml}$ ) (Nolan et al. 2007). As recommended intake of lutein and zeaxanthin based on serum levels, results would indicate that on average, this study population would require an additional 3.8 - 6.6 mg/day to achieve optimal serum levels in the range of 0.350 - 0.600  $\mu\text{g/ml}$  (Appendix B). This study found significant correlations for dietary intake assessed by the SCG FFQ only with blood concentrations, consistent with correlations found by previous researchers (Rock et al. 2002). The strongest correlation with serum lutein and zeaxanthin was for dietary intake analysed using the modified WISP but there was also a moderate correlation with the MRC

database. Assuming that higher correlations of dietary variables with serum levels can be interpreted as an indication of greater validity, it appears that the SCG FFQ is more accurate than the modified Italian FFQ and the modified WISP database produces somewhat more valid estimates of lutein and zeaxanthin intake than the MRC database when estimating intake in an Irish population. The inclusion of more up to date data (Perry et al. 2009), and the inclusion of population specific data (O'Neill et al. 2001) represent likely explanations for the increased validity of the modified WISP database.

As expected, given that fruit and vegetables are lutein and zeaxanthin rich sources, their intake contributed most significantly to lutein and zeaxanthin intake. Previous studies also reported a strong correlation between both fruit ( $r = 0.54$ ) and vegetable ( $r = 0.61$ ) consumption and blood serum concentrations of lutein and zeaxanthin (Cena et al. 2007), and were confirmed in the current study, with serum concentration correlating with vegetable consumption estimated by the SCG FFQ, although not for fruit consumption. This may be explained in part by the potential over-reporting of fruit consumption by the SCG FFQ, which estimated an average consumption of 4.5 servings of fruit/day, 3.5 portions more than that found to be the average intake in an Irish population subgroup studied by Thurnham et al. (2008). Given the semi quantitative design of the SCG FFQ, where frequency of consumption is based on standard portion size rather than open questions on quantity of food consumed, it is vulnerable to overestimation of food consumption (Molag et al. 2007). Additionally, the number of fruits listed in the questionnaire may in part account for the overestimation of intake as it has been shown that the longer the list of detailed food items belonging to a given main food group (e.g. many different types of vegetables), the higher the total level of consumption reported (Haraldsdottir, 1993). When actual consumption of fruit and

vegetables is monitored, very few account for most of the carotenoid intake, suggesting that the modification of the SCG FFQ, to include a shorter list of fruits would be sufficient. However, as Ireland is quickly becoming a multicultural nation, it may be argued that such an extensive list is needed to provide a common questionnaire for all cultures and dietary habits when assessing nutrient intake in an Irish population.

Similarly, fruit and vegetable consumption estimated by the Italian FFQ showed no significant correlation with serum. This may be explained, in part, by the brief, nutrient specific design of the questionnaire. The food list consists of 32 rich sources of lutein and zeaxanthin, commonly eaten by the Italian population. Those listed tend to be eaten only occasionally and by a relatively small proportion of the Irish population e.g. vegetable juice, tomato juice, papaya and watermelon. In addition a limited list of foods does not permit recipes or specific ingredients to be imputed for analysis. Despite this however an estimate of approximately two portions of fruit and vegetables was reported, which would seem, to be an overestimation of actual intake, which would comply with previous findings indicating, as found with the SCG FFQ, that when subjects are presented with a long list of items in a specific food group, the risk of over-reporting is increased.

While the crude quantification of dietary intake, and the inaccuracies of nutrient databases are predictive of the relationship between dietary intake and serum concentration of lutein and zeaxanthin, it is important to consider other components of the diet such as dietary fat (Seddon et al. 2003), fibre (Riedl et al. 1999), food source (Hof, 1999), and food preparation or processing (Castenmiller, 1999), can influence lutein/zeaxanthin bioavailability or serum response. These factors could potentially



influence the observed relationship between dietary intake and tissue concentrations of lutein and zeaxanthin. Certain medications such as statins can lower blood levels of carotenoids. Butters and margarines enriched with plant sterols such as 'Benecol' and 'Flora Pro Active' may decrease the absorption of carotenoids. Given the relatively young mean age of this subject population, consumption of these products was not controlled for, however, given their increased popularity and frequency of consumption among the Irish aging population (Hearty et al. 2008), their potential to confound the relationship should not be ignored. Never the less, Rock et al (2002) found that, neither fat, fibre nor cholestra were predictive of serum lutein/zeaxanthin concentration in the general population. Unfortunately, other than the recording of reported known digestive or absorptive idiosyncrasies, the scope of this present study did not permit for the control of potential confounders affecting the relationship between dietary intake of lutein and zeaxanthin and serum concentrations, and therefore, it could be expected that controlling for factors affecting the bioavailability of lutein and zeaxanthin, along with the transport of lutein and zeaxanthin in the serum (LDL/HDL), would have strengthened the correlation (Rock et al. 2002; O'Connell et al. 2008). Smoking status and alcohol consumption, due to potential direct effects on metabolism and turnover of these carotenoids (Handleman et al. 1999), were controlled for, strengthening the relationship between dietary intake and lutein and zeaxanthin concentrations in the serum and macula. In previous studies associations between circulating lutein and zeaxanthin concentrations and MPOD or risk for eye disease, determinants have sometimes (Mares-Perlman et al. 1995, O'Connell et al. 2008) but not always (Bone et al. 2001) been considered in the analysis, and again may contribute to inconsistencies in results.

Both FFQ's failed to show a significant relationship between MPOD and dietary intake. Previous researchers have showed inconsistent results; however the majority have found a significant relationship (Bone et al. 2000). The absence of significance may in part be explained by small numbers of subjects ( $n < 50$ ), which was deemed to be the most likely explanation for a lack of significant findings in previous studies (Hammond et al. 1995; Beatty et al. 2001). Two other factors, that might help explain the modest diet-macula correlations, some of which have already been mentioned, include vagaries in dietary and biochemical assessment, and a limited understanding of carotenoid metabolism. Despite previous findings of no seasonal variation in dietary intake of these carotenoids, the inclusion of a significantly younger population group than that used in previous Irish studies (mean age 24yrs), and the knowledge of a lack of dietary routine in younger population groups, may have biased the relationship between dietary intake and MPOD. It has been previously suggested, that interpersonal variation in terms of efficiency of metabolism, absorption, transport and stabilisation, may be the primary determinant of MPOD and not dietary intake of lutein and zeaxanthin (Bone et al. 2000).

With a highly significant correlation coefficient of ( $r = 0.734$ ), serum concentrations of lutein/zeaxanthin were found to be more strongly related to MPOD than to dietary intake, which is consistent with previous findings by other researchers (Hammond et al. 1996; Bone et al. 2000; Mares et al. 2006). Thus, more direct assessments of dietary intake and lutein/zeaxanthin status, such as serum concentrations of lutein/zeaxanthin and MPOD, may provide a clearer picture of the relationship between lutein and zeaxanthin and risk of AMD. Individualised dietary intake requirements (Appendix B) based on serum concentrations, would serve to eliminate vagaries inherent with dietary assessment and analyses, while automatically controlling for digestive and absorptive

idiosyncrasies. Unfortunately lutein and zeaxanthin levels in serum are not routinely determined in clinical settings, constituting an important gap in relation to the dietary requirements and distribution of lutein and zeaxanthin in serum in patients at risk of AMD.

While this study would favour the use of the SCG FFQ for assessing dietary lutein and zeaxanthin intake, it appeared to severely under-report energy intake, with over half the study population falling below the Goldberg cut-off for under-reporting. However, the fact that fruit and vegetable intake was estimated to be 3 portions more than that reported in previous Irish populations, would imply that the SCG FFQ underestimated non lutein and zeaxanthin rich foods such as protein and fat, a common limitation of FFQs (Schaefer et al. 2000), rather than fruit and vegetables, the main providers of lutein and zeaxanthin, which seemed to be over-reported.

When choosing a dietary assessment tool, practical issues related to the administration of FFQs should be examined. The length of time needed for an individual to complete each FFQ should be considered: about 35 minutes for the SCG FFQ and <10minutes for the modified Italian FFQ. Analysis times and required resources also vary among the two FFQs. The SCG FFQ requires 40 minutes for analysis, or alternatively optical scanning in Scotland, which may take several days, where as the modified Italian can be analysed in 10 minutes. However, while a nutrient specific questionnaire, such as the modified Italian FFQ, would seem to be the optimal choice in terms of practicality, its apparent lack of accuracy found in this study would indicate that the number of items on a FFQ should not be reduced just because of the length of the food list, as doing so might reduce the validity of the FFQ (Cade et al. 2002). In contrast, Wirfalt et al

reported that '*less detailed information may be sufficient when categorising individuals on nutrients present in a few foods consumed, providing that the questionnaire includes sufficient number and quality of food sources*' (Wirfalt et al. 2007). Therefore, with the correct modifications to the questionnaire, to make it population specific, as well as administering the questionnaire with interview assistance, as originally intended by Cena et al, the modified Italian FFQ, has the potential to be the optimal choice of FFQ, both practically and in terms of accuracy (Cena et al. 2007).

A potential limitation of the modified WISP nutrient database relates to the reporting of lutein and zeaxanthin as a combined value. However, this in part, was due to the limited availability of data which report the nutrients as separate values. Additionally, as both serum and MPOD provided a reading of both nutrients together, it was sufficient, for the scope of this study to report the nutrients combined as a single value. An important point worth mentioning is that considering *meso*-zeaxanthin is now believed to be the more potent antioxidant of the three carotenoids, no tool exists to assess dietary intake of this carotenoid (as it is not commonly found in many foods).

The development of the carotenoid database, modified WISP, contains, to our knowledge the most up to date information available on lutein and zeaxanthin, while being population specific. Additionally this study provides evidence for the potential danger of using dietary assessment methods for purposes other than those identified through the validation procedures. Most importantly this study highlights the lack of comparability between studies using different dietary assessment tools and nutrient databases (Granado et al. 1996; Scott et al. 1996; O'Neill et al. 2001), and may provide,

in part an explanation for inconsistent results found by researchers investigating the relationship between dietary intake of lutein and zeaxanthin, serum values and MPOD.

In summary, dietary intake of lutein and zeaxanthin assessed using the SCG FFQ and the modified Italian FFQ was not comparable. Similarly dietary intake of lutein and zeaxanthin analysed using the modified WISP database and the MRC nutrient database was not comparable. While the evidence from this study would favour the use of the SCG FFQ and the modified WISP database, the short modified Italian FFQ would appear to have the potential to provide accurate estimates of intake, if limitations identified were addressed through revisions in administration and a food-list update. While dietary assessment methods may be appropriate and useful for estimating lutein and zeaxanthin exposure on a community or group basis, evaluation of nutrient exposure by dietary means is ultimately reliant on the availability of reliable data on food composition. Therefore, concerted efforts should be directed toward improving data, with the aim of developing a ‘gold standard’ lutein and zeaxanthin database which would enhance the validity of estimates of dietary intake of lutein and zeaxanthin. Such a milestone, within the field of AMD would provide researchers with a higher degree of precision needed to assess their respective roles in eye health.

None of the available resources are currently adequate for research or clinical practice so alternatives are required. In the meantime, serum lutein and zeaxanthin, when assessed repeatedly, and prospectively on a long-term basis, may be a good indicator of healthy eating habits related to AMD. On a community level, screening of lutein and zeaxanthin concentrations in serum would allow the identification and establishment of appropriate dietary requirements, both in the lower (risk factor) and the upper levels

(preventive factor) of requirements, and to determine the adequacy and efficacy of dietary intake, or nutritional interventions, with clinical impact on disease prevention.

## **CHAPTER SEVEN**

### **An Evaluation of a Novel Instrument for Measuring Macular Pigment Optical Density: The MPS 9000**

#### **7.1 Abstract**

##### **Purpose**

Of the antioxidants found in the human retina, only the macular carotenoids can be quantified non-invasively (albeit in a collective fashion), thus facilitating study of their role at the retina. The aim of this study was to evaluate concordance between MPOD values recorded on a commercially available instrument, the MPS 9000, with those of an already validated HFP instrument. Also, we assessed and compared test-retest variability for each instrument.

##### **Methods**

MPOD at 0.5° retinal eccentricity was measured using two different heterochromatic flicker photometers, the MPS 9000 and the Densitometer™ in 39 healthy subjects. Test-retest variability was evaluated separately for each instrument by taking three readings over a one-week period in 25 subjects.

##### **Results**

In terms of MPOD at 0.5 degrees eccentricity, there was a strong positive correlation between the MPS 9000 and the Densitometer ( $r=0.68$ ,  $p<0.001$ ); however, a paired samples t-test showed a significant difference in terms of mean values, with a bias towards lower MPOD values being yielded by the MPS 9000 ( $t= -4.103$ ,  $p<0.001$ ).

Bland Altman analysis indicated only moderate agreement between the two instruments.

Test-retest variability, expressed in terms of the coefficient of repeatability, ranged from 0.18 to 0.21 for the MPS 9000 and from 0.11 to 0.12 for the Densitometer.

## **Conclusion**

These results show that the MPS 9000 consistently yields MPOD readings which represent an underestimate of actual values, and are subject to a substantial amount of test-retest variability.

## **Keywords**

Age-related macular degeneration; MPS 9000; MPOD; densitometer, heterochromatic flicker photometry; macular pigment.

## **7.2 Introduction**

AMD is the most frequent cause of blindness among individuals  $\geq 55$  years in developed countries, (Leibowitz et al. 1980; Attebo et al. 1996; Friedman et al. 2004) and with increasing longevity the incidence of AMD is rising. The therapeutic options for AMD are limited, although improving. Treatments, however, tend to be exclusively aimed at the neovascular, or wet, form of AMD, which until recently included only laser photocoagulation (Macular Photocoagulation Study Group, 1993), with or without photodynamic therapy, and macular translocation surgery (Ciulla et al. 1988). In the recent past, however, substantial progress has been made in the management of the neovascular form of the disease using intra-vitreous injections of anti-vascular endothelial growth factor (or anti-VEGF) therapy (Avery et al. 2006). Although vision loss with wet AMD is more sudden and severe, the non-neovascular form, including the atrophic type, is more prevalent and accounts for approximately 90% of cases (Richer et al.



2004). At present, there is no consensus with respect to the management (including risk analysis and/or prevention) of these more common non-neovascular forms of the condition, which may, at least partly, reflect our incomplete understanding of AMD's aetiopathogenesis.

In the absence of effective treatment strategies for non-neovascular AMD, interest has focused on prevention and/or retardation of progression. The Age-Related Eye Disease Study (AREDS) has shown that the risk of vision loss in cases of early AMD can be reduced with antioxidant supplements. Further, MP composed of lutein and zeaxanthin, two hydroxycarotenoids, which are entirely of dietary origin, and the retinal metabolite of lutein: *meso*-zeaxanthin, (Bone et al. 1997; Johnson et al. 2005) is believed to be associated with reduced risk of development and progression of AMD. MP can be augmented, not only by eating food rich in these carotenoids, such as spinach, but also by dietary fortification with one of the many commercially available food supplements (Bone et al. 2003; Murray & Carden, 2008). Epidemiological studies have observed an inverse association between the prevalence of AMD and a diet rich in lutein and zeaxanthin, (Seddon et al. 1994; EDCCS, 1993) and furthermore, eyes with AMD have consistently been shown to have significantly lower levels of MP when compared to those without AMD (EDCCS, 1993; Beatty et al. 2001; Bone et al. 2001; Bernstein et al. 2010).

The evidence in support of the view that MP plays a role in preventing or retarding the progression of AMD rests on its ability to limit photo-oxidative injury in the inner retina through its pre-receptor absorption of SW light, (Snodderly et al. 1984; Snodderly et al. 1984) and/or the antioxidant properties of these carotenoids as they act as free radical

scavengers in the retina (Snodderly, 1995). Although all humans have some MP in the retina, the optical density and spatial distribution of MP have been shown to vary dramatically between individuals, (Pease et al. 1987; Bone et al. 1992; Hammond et al. 1995) with consequential large inter-individual variation in pre-receptor SW light absorption and antioxidant activity in the retina.

Several methods for measuring the optical density of MP have been developed, thereby enabling the investigator to detect changes in MP concentration and distribution over time, and therefore able to study the response to dietary modification or fortification. Unsurprisingly, there is a growing demand for a valid, reproducible, user-friendly instrument that measures MPOD.

HFP was the first, and remains the most widely used, technique for measuring MPOD in vivo (Snodderly et al. 1984; Pease et al, 1987; Hammond et al. 1997; Hammond et al. 2005; Nolan et al. 2008; Rougier et al. 2008; Stringham et al. 2008). HFP is a psychophysical method, which requires the subject to make iso-luminance matches between green and blue flickering lights, which are typically perceived as the point of cessation of flicker. 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 optically undetectable), gives a measure of the individual's MPOD. This method (using the Densitometer) has been validated against the absorption spectrum of MP in vitro (Bone et al. 1992; Hammond et al. 2005). The MPS 9000 is a relatively new HFP instrument that has been developed for clinical use (Van der Veen et al. 2009; Schechtman & Karpecki, 2008). It is evident from the literature, however, that while based on the same basic optical principles of HFP, significant design and

methodological differences do exist. We report a concordance study between the newly available commercial instrument, the MPS 9000, and the validated and conventional research instrument for measuring MPOD, the Densitometer. We also measured and compared test-retest variability for the two instruments.

### **7.3 Materials and Methods**

This study was conducted at DIT, Dublin, Republic of Ireland. Subjects were recruited by word of mouth. Informed consent was obtained from each volunteer after the provision of a detailed information sheet. Ethical approval was granted by the research ethics committee at DIT, and the experimental procedures adhered to the Declaration of Helsinki. Inclusion criteria required participants to be aged 18 years or older, have no clinical signs of ocular pathology, and log MAR VA of better than 0.2 in the study eye.

The study eye was selected on the basis of corrected distance visual acuity (CDVA), the eye with the better CDVA being selected, (Stringham et al. 2008) and in cases of equal CDVA, the dominant eye was selected. A computer generated Log MAR test chart (Test Chart 2000 Pro; Thompson Software solutions) was used to determine CDVA at a viewing distance of 4 meters, using a Sloan ETDRS letterset. Subjects were requested to wear non-tinted normal distance correction spectacles if required. An ocular health examination was conducted by ophthalmoscopy to rule out any ocular pathology.

MPOD was measured at 0.5 degrees eccentricity on each instrument, on the same day, in 39 subjects to determine instrument concordance. In order to assess test-retest variability for each instrument, 25 subjects had MPOD measured (again at 0.5 degrees eccentricity) on three occasions over a one-week period on each instrument, 50 subjects

were recruited for this part of the investigation, 25 were randomly assigned to the MPS 9000 and 25 were randomly assigned to the Densitometer. All data were collected by a single operator.

The instruments used in this study were the MPS 9000, (Tinsley Precision Instruments Ltd, Croyden, Essex, UK), and the Macular Metrics Densitometer (Macular Metrics II, 12 River St, Rehoboth, MA 02769, USA). The MPS 9000 procedure is described in section 7.3.1. It is also described in Van der Veen et al. (2009). A detailed description of the Densitometer is given in section 5.3.7. The instrument used first in the concordance arm of the study was randomly selected on a case-by-case basis in order to minimise the risk of introducing bias attributable to a learning effect or a fatigue effect from either instrument.

### **7.3.1 MPS 9000**

The MPS 9000 is a small, portable HFP instrument, capable of measuring MPOD at a single retinal locus ( $0.5^\circ$  retinal eccentricity). The Instrument uses a foveal target of  $1^\circ$  diameter (edge located at  $0.5^\circ$  retinal eccentricity) with the reference location at  $8^\circ$  retinal eccentricity (parafoveal target of  $1.75^\circ$ ) (Van der Veen et al. 2009).

Testing was carried out according to the manufacturer's instructions. Prior to the first session, a short practice test was carried out to familiarise the participant with the technique. Once the subject successfully completed the practice run, the subject's sensitivity to flicker was determined by a built in pre-test routine, that enabled the appropriate initial luminance contrast of the two light sources to be established. This short (30 sec) pretest flicker sensitivity routine was used to ensure that participants were

in the middle of their flicker sensitivity range when performing the main task, as flicker sensitivity may vary between individuals.

During the main test, the frequency of the blue (460nm) and green (540nm) light sources were automatically ramped down from 55Hz for a series of luminance ratios of the two light sources. Initially the observer viewed the target centrally and pressed a button when flicker was detected. This sequence of obtaining a flicker threshold for each blue-green ratio continued until a curve was obtained, where the minimum represents the equalisation of the blue and green luminance (Van der Veen et al. 2009). The procedure of obtaining the flicker detection for a series of blue-green ratios was repeated, after an additional short practice run, for peripheral viewing, with the subject fixating a red 1.75° disc at a reference point of 8° horizontal eccentricity. The central and peripheral minima were used to calculate a single MPOD reading. The formation of the central and peripheral curves was monitored by the examiner throughout the course of the examination to ensure reliability of the results.

### **7.3.2 Densitometer**

The Densitometer is a validated MPOD measurement instrument capable of determining a spatial profile of MP, by measurement of MPOD at various retinal eccentricities between 0.25° and 3° (Wooten et al. 1999). For the purpose of this study, readings were taken centrally at 0.50° using a 1° disc (commonly used as it has been shown to have the highest repeatability of results (Snodderly et al. 2004) matching that used in the MPS 9000, and a reference location at 7° using a 2° target (Van der Veen et al. 2009).

Prior to using the Densitometer, all subjects were shown an explanatory video describing the method for recording null flicker matches. The subject's CFF was then measured, and the OFF determined using a defined test algorithm designed to minimise variance between readings, in a process that has become known as customised HFP (Stringham et al. 2008). The subject performed the test by turning a dial to adjust the ratio of blue to green light until, using a method of adjustment, a position of null, or minimum, flicker was defined. 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 5% of the mean null flicker radiance at that test locus. Subjects were required to perform at least five null flicker matches for each target (foveal 0.5° and parafoveal 7°). The Densitometer was calibrated daily prior to its use, in keeping with the manufacturer's instructions.

### **7.3.3 Statistical analysis**

SPSS version 18 for windows was used for data analysis. Mean MPOD for the MPS 9000 and the Densitometer were compared using paired samples t test. Bland Altman analysis and plots were used to quantify the agreement between the two instruments. Inter-session repeatability is expressed as a coefficient of repeatability, which was calculated as the standard deviation of the mean difference between measurements, and multiplied by 1.96. A one way ANOVA was conducted to assess repeated MPOD measurements on both instruments to test for a learning or fatigue effect. Coefficients of

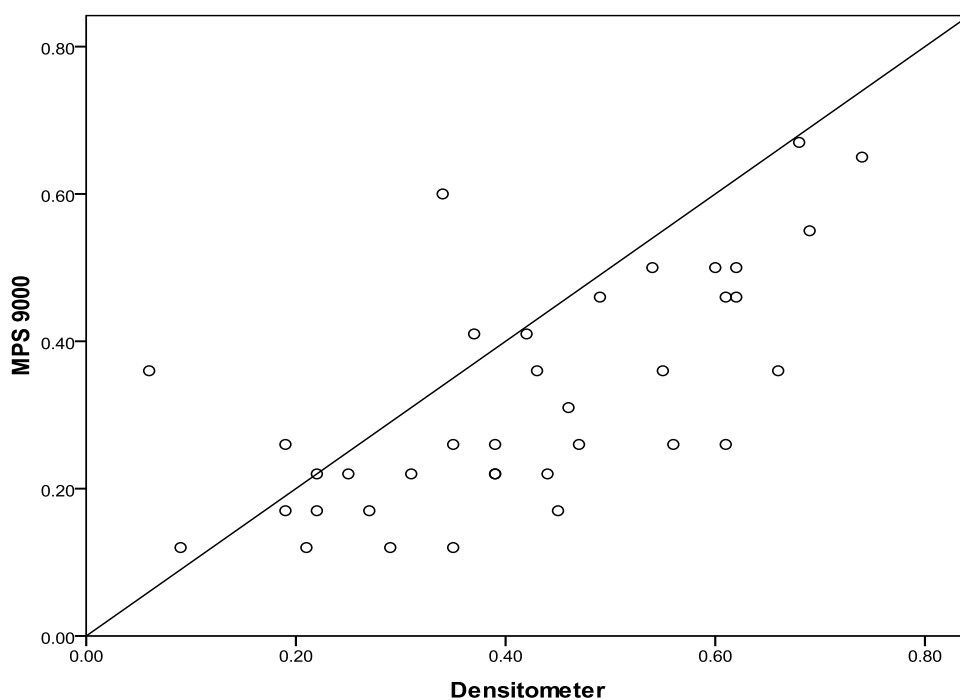
repeatability and 95% limits of agreement were calculated for (visit 1-visit 2), (visit 2-visit 3), and (visit 1-visit 3) for each instrument.

## 7.4 Results

The data were analysed (1) to compare measurements taken at  $0.5^\circ$  on the two instruments, and (2) to assess inter-sessional repeatability of each instrument. Two subjects were excluded from the instrument concordance analysis, and one subject from the instrument inter-sessional repeatability analysis, on the basis that they were deemed unable to perform the MPS 9000 task satisfactorily. Data analysis is conducted and presented for the remaining 37 subjects in the concordance analysis [mean age 29 ( $\pm 11$ )], and 49 subjects [mean age 34 ( $\pm 10$ )] in the inter-sessional repeatability analysis.

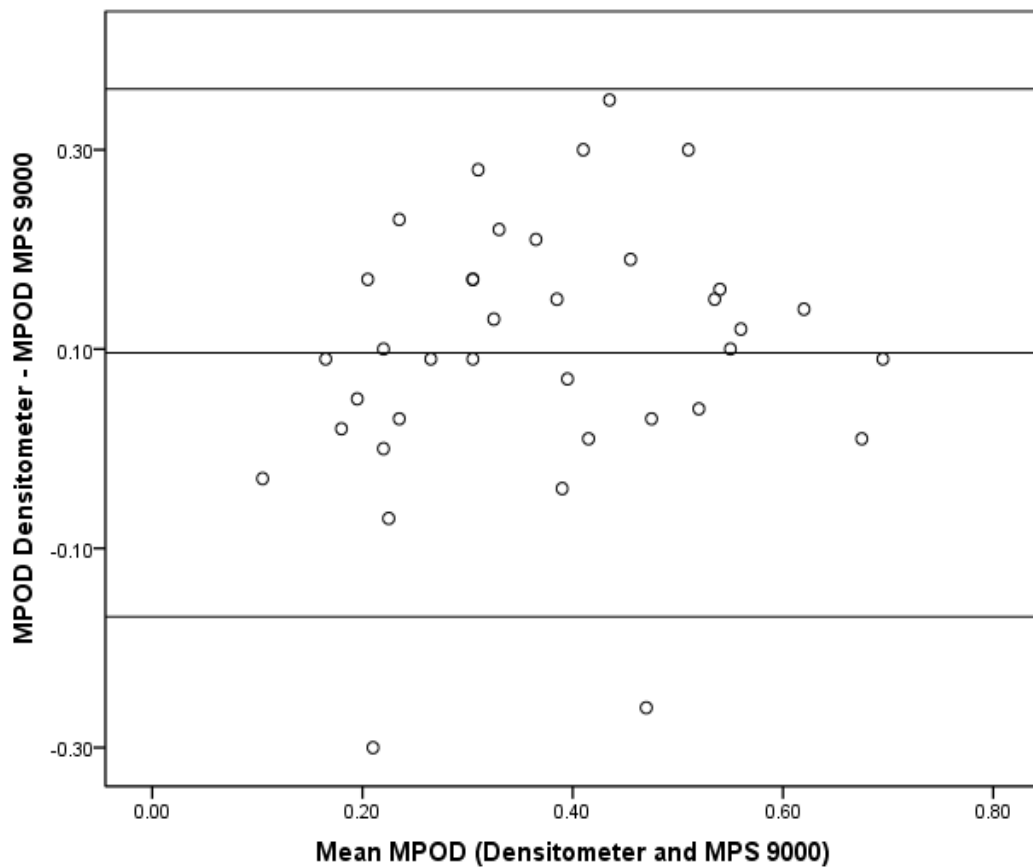
### 7.4.1 Instrument Concordance

A scatter plot comparing MPOD values at  $0.5^\circ$  eccentricity obtained with each instrument, is shown in Figure 7.1 ( $r = 0.68$ ,  $p < 0.001$ ).



**Figure 7.1.** Relationship between MPOD readings at 0.5° retinal eccentricity obtained with each instrument, with the line  $y = x$  superimposed.

A paired samples t-test comparing the mean MPOD, as measured on each instrument, yielded a statistically significant difference between instruments ( $t = -4.103$ ,  $p < 0.001$ ), demonstrating a bias of lower MPOD values obtained on the MPS 9000, reflected in an average difference in MPOD values of 0.1 log unit between the two instruments (Figure 7.2). The 95% limits of agreement between instruments were  $\pm 0.27$ .



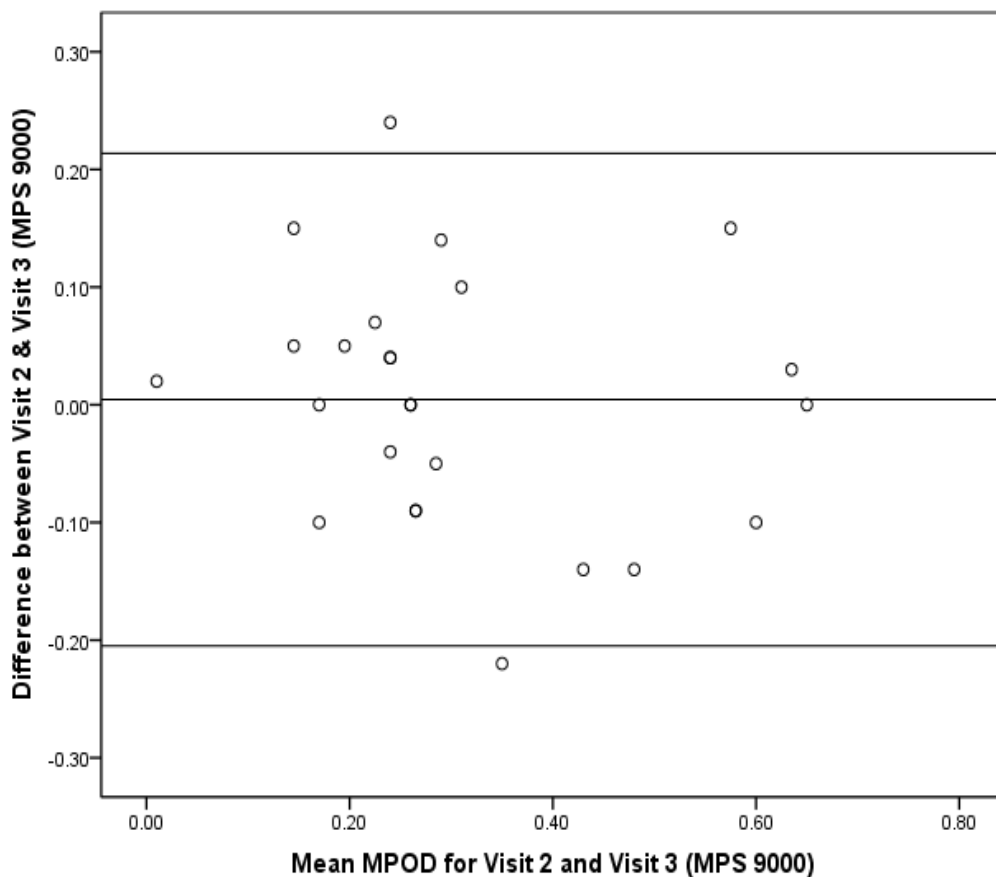
**Figure 7.2.** Bland-Altman plot for MPOD values at 0.5° retinal eccentricity, showing 95% limits of agreement between the MPS 9000 and Densitometer



### 7.4.2 Test - Retest Repeatability

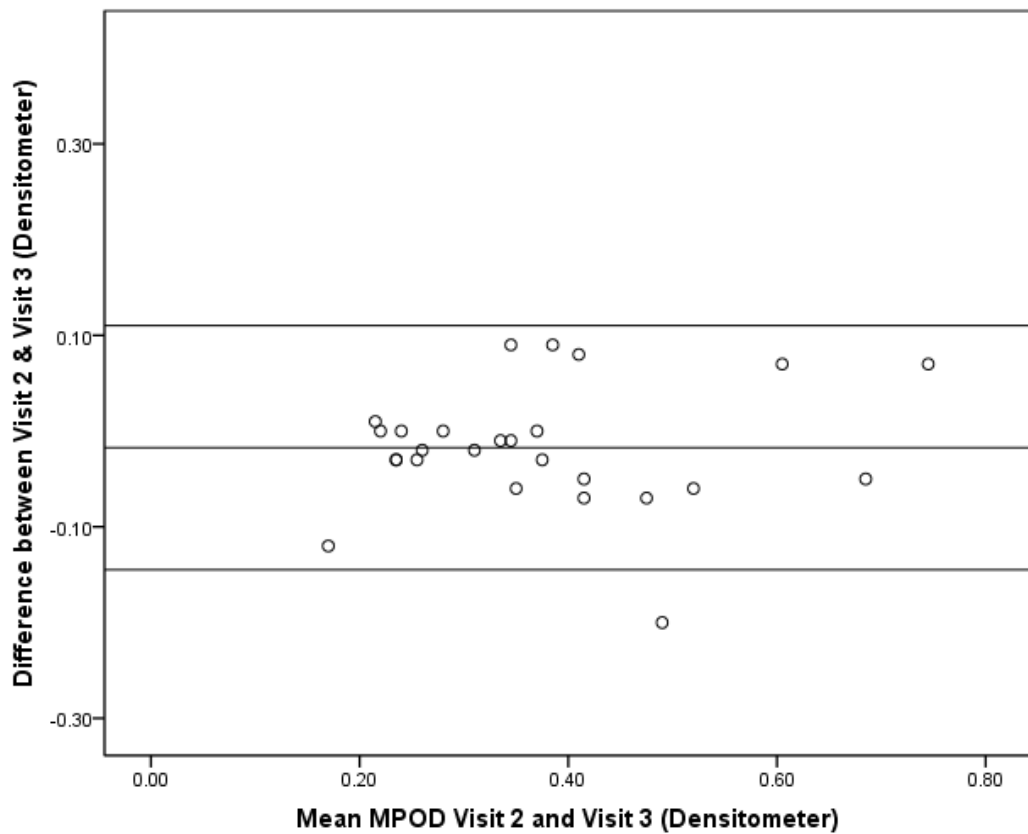
A one way repeated measures ANOVA was conducted to assess repeat MPOD measurements for a learning or fatigue effect for each instrument. Mauchly's test of sphericity was not significant ( $P > 0.05$ ) for either instrument. There was no significant difference in repeat MPOD measurements for either the MPS 9000 or the Densitometer, indicating the absence of any learning or fatigue effect [MPS 9000 ( $F = 0.09$ ,  $p = 0.92$ ); Densitometer ( $F = 2.556$ ,  $p = 0.09$ )].

A Bland-Altman plot was constructed to assess agreement between repeat measures taken on the MPS 9000 (Figure 7.3). The coefficient of repeatability for the MPS 9000 ranged from, 0.18 to 0.21 (see Table 7.1).



**Figure 7.3.** Bland Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the MPS 9000.

A Bland-Altman plot was also constructed to assess agreement between repeat measures taken on the Densitometer (Figure 7.4). The coefficient of repeatability for the Densitometer ranged from 0.11 to 0.12 (see Table 7.1).



**Figure 7.4.** Bland Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the Densitometer.

**Table 7.1.** Inter - sessional MPOD (mean  $\pm$  SD) and coefficient of repeatability for the MPS 9000 and Densitometer

Instrument	Mean ( $\pm$ SD) MPOD			Coefficient of Repeatability		
	Visit 1	Visit 2	Visit 3	Visit 1 - Visit 2	Visit 2 - Visit 3	Visit 1 - Visit 3
MPS 9000	0.31 ( $\pm$ 0.15)	0.32 ( $\pm$ 0.16)	0.32 ( $\pm$ 0.17)	0.18	0.21	0.18
Densitometer	0.40 ( $\pm$ 0.15)	0.38 ( $\pm$ 0.16)	0.40 ( $\pm$ 0.16)	0.11	0.12	0.12

### 7.5 Discussion

The Densitometer has been validated in previous studies, (Wooten et al. 1999; Snodderly et al. 2004; Stringham et al. 2008) and the HFP technique for measuring MPOD has also been validated against the absorption spectrum of MP in vitro (Bone et al. 1992; Hammond et al. 2005). The MPS 9000 is a new commercial technology designed to measure MPOD which also employs the HFP technique. The use of HFP however does not imply that the results are automatically valid and accurate, and as of yet, these important test attributes have not been adequately evaluated for the MPS 9000 instrument. Given the potential importance of MP measurement for clinical practice, the current study which assesses the accuracy and repeatability of this new commercial instrument in comparison to the current gold standard HFP instrument, the Densitometer, would seem both timely and necessary.

In the current study, the mean MPOD was 0.32 ( $\pm 0.15$ ) for the MPS 9000, and 0.42 ( $\pm 0.18$ ) for the Densitometer. These values are consistent with previous studies (Ciulla et al. 2001; Snodderly et al. 2004; Loane et al. 2007; Nolan et al. 2008; Makridaki et al. 2009; Bartlett et al. 2010). MPOD at 0.5° has been observed to range from 0.21 (Ciulla et al. 2001), to 0.42 (Snodderly et al. 2004), with studies employing the MPS 9000, where mean MPOD ranged from 0.33 to 0.35, (Van der Veen et al. 2009; Makridaki et al. 2009; Bartlett et al. 2010) and studies employing the Densitometer, where mean MPOD was recorded at 0.41 and 0.40 (Nolan et al. 2008; Loane et al. 2007). A paired samples t-test showed the difference in mean MPOD between instruments to be significant, with a bias towards lower MPOD values being produced by the MPS 9000.

Given the observed correlation between these HFP instruments (Figure 7.1), a difference between the two instruments would pose little difficulty for clinical practice, provided that the difference was systematic and predictable. In fact, a systematic difference of 0.1 might be expected between the instruments based on their respective suggested normative values, with MPOD classified as low once below 0.4 for the Densitometer, and once below 0.3 for the MPS 9000. The MPS 9000 does indeed consistently underestimate MPOD in comparison to the Densitometer (for ~85% of subjects here), and on average, the underestimation is indeed 0.10 ( $\pm 0.14$ ). This average difference of 0.10 is also replicated in the repeatability study (see Table 7.1), so it would appear that, despite both instruments using the HFP technique, there is a real, and consistent average discrepancy in MPOD values between instruments, and that the 0.1 difference in expected normative values would therefore seem appropriate.

Closer inspection of the data however reveals some surprising and problematic issues. Figure 7.2 illustrates the difference and 95% limits of agreement between the two instruments, which at  $\pm 0.27$  would seem unacceptable for clinical purpose. The central problem is not that there is a difference between the instruments, but that the difference does not appear to be systematic and predictable. The underestimation in MPOD between instruments is in the range 0.05 to 0.15 for approximately 36% of subjects only, and the difference between measurements (Densitometer – MPS 9000) ranges from 0.35 to -0.3, a 0.65 log unit range (it should be noted that all subjects were deemed to have understood and performed both measurements satisfactorily, so no cases can be dismissed as statistical outliers). This data demonstrates exquisitely that a correlation coefficient, as reported in Van der Veen et al, simply cannot be used in isolation to determine the validity and accuracy of an MPOD measurement instrument (Van der Veen et al. 2009).

It should also be pointed out however that there are a number of exceptions to the trend for lower MPOD values on the MPS 9000 compared to the Densitometer. In five cases, the MPS 9000 demonstrated higher MPOD values, and in two of these cases the difference is substantial (0.26 and 0.30 respectively). Clinically these two cases could not be discarded as both subjects were deemed to have understood and performed the MPOD measurement to an acceptable standard on both instruments. But from a statistical viewpoint however, these may be regarded as outliers. Re-analysis of the data excluding these two cases does improve the observed correlation and agreement between instruments to more acceptable levels ( $r^2 = 0.64$ , and the limits of agreement = 0.20). There are a number of significant differences between the instruments and their

respective methodologies, which may explain both the observed lack of concordance and the disparity in respective inter-sessional repeatability.

The difference in eccentricity of the peripheral target ( $7^\circ$  for the Densitometer, and  $8^\circ$  for the MPS 9000) is minimal, and in any case, if significant, would tend to lead to an underestimation in the Densitometer, which is not the case. The MPS 9000 employs a  $1^\circ$  stimulus for both the central and peripheral measurements, while the Densitometer employs a  $2^\circ$  stimulus for the peripheral measurement. Almost invariably, subjects reported significant difficulty completing the peripheral measurement using the MPS 9000, whereas no such difficulty was reported for the Densitometer. It is likely that this difference in peripheral stimulus size is the source of the increase in comparative difficulty experienced by subjects using the MPS 9000, and may explain the required exclusion of three subjects unable to complete the peripheral measurement. In this instance, the manufacturer suggests the use of an age estimate method, which computes a value for MPOD based on the central measurement alone, with a peripheral estimate predicted on the basis of age. The validity of this age estimate method is however questionable, and would be expected to present a significant source of further variation, up to an approximate 0.4 log unit range (Makridaki et al. 2009).

An additional potential source of variation lies in the fundamental difference in the respective methods for achieving the iso-luminance match. The Densitometer affords significant control to the subject, who adjusts the ratio of blue to green until a null flicker sensation is achieved. There is no significant time restriction. The subject is simply instructed to use a method of adjustment or bracketing method to define the null flicker zone. If the flicker rate is not optimal, it can be adjusted by the examiner to

facilitate the accurate determination of the point or zone of null flicker. The MPS 9000 employs a substantially different technique, where a suprathreshold flicker rate is gradually reduced at a set rate of 6 Hz per second, and the subject responds by pressing a button to indicate the point at which flicker is detected. The rate of flicker decrease is a compromise between testing time and differences in subject reaction times (Van der Veen et al. 2009). Although, reaction times are known to vary little across age, (Porciatti et al. 1991), response times are significantly more complex, and include a decision criterion which may vary substantially across individuals, and is very much age dependent, particularly in the over 60 age group (where AMD is most prevalent) (Hommel et al. 2004). There is a speed-accuracy trade off exhibited in older adults, and the extent of such a trade off will critically depend on patient age, and interestingly, on task complexity (Madden & Allen, 1995). It is therefore entirely possible that subject threshold response criteria on the MPS 9000 may change during the course of an examination, in particular if insufficient pre-test practice is given to the subject so that the task becomes apparently easier during the test, and more probably, when switching to the peripheral target where task difficulty is increased (as is noted in the product literature, and evidenced by the inclusion of an age estimation method in the instrument). Such a change in response criterion between the central and peripheral targets could not be determined by evaluating the shape of the curve produced, and could result in under or over estimation of MP.

Expanding on the issue of subject performance assessment during a test procedure, the MPS 9000 would additionally seem incapable of providing a useful measure of subject performance reliability. The MPS 9000 adopts a curve fitting technique, where measurements are repeated for a series of green-blue luminance ratios and a response

curve is formed which comprises the flicker detection points for each of the luminance ratios. The only performance check an examiner can use is to determine that a 'typical' V shaped curve is produced. The product literature describes that 'irregularities in the data' are typical, and that the shape of the curves can vary between individuals. This makes interpretation of the curve, and reliability of the result, therefore dependent on examiner skill and training, and subject to significant variation. The technique basically produces one minimum value which is used to determine MPOD by comparing the minimum centrally versus peripherally. The MPOD value determined by the Densitometer in comparison, represents the average of multiple (typically four to six measurements) endpoints determined by the subject. Variation in performance, or lack of understanding of the task, becomes immediately obvious by a large standard deviation in the radiance values produced. A low standard deviation for both central and peripheral measures is a requirement to ensure validity of the result. It is imperative that subject performance is assessed during this type of psychophysical task and the MPS 9000 simply does not achieve this to an acceptable degree.

The variation in the stimulus-background configuration between instruments is also significant, and certainly has potential to induce measurement discrepancies. For the Densitometer, the configuration is SW blue background, against which, an incremental blue target is viewed. For the MPS 9000, the blue target is viewed against a spectrally broadband white light surround. While it is likely that both configurations effectively suppress the contribution of rods and S-cones, the use of a white light surround could potentially create problems including a variation in retinal adaptation level between the target and surround (although it is stated that the luminance of the target area is "close to" that of the surround (Van der Veen et al. 2009). It may also lead to increased



perception of imperfections at the target edge due to CA caused by the spectrally broadband background. These effects could potentially be further compounded by the relatively high power +5D focusing lens employed in the MPS 9000, particularly in the peripheral viewing condition where off axis lens effects may become problematic. Other HFP methods, such as those used by Beatty et al. (2000) and Bone & Sparrock (1971), which have employed a centre surround stimulus configuration, have been shown to produce a spectral curve that is best fit with a significant rod contribution (Hammond et al. 2005). It is simply unclear as of yet, whether the target-stimulus configuration, as employed in the MPS 9000, fulfills the basic principle of any technique for the measurement of MP, that the method “*should provide spectral absorption curves that match the extinction spectra of MP*” (Hammond et al. 2005).

Measurement repeatability is an additionally important feature of MP estimation using HFP. Precise and repeatable quantification of MPOD is essential to facilitate definitive management of patients in clinical practice. This is particularly important where dietary fortification or supplementation is advised in order to augment MP in individuals with low MPOD, or predicted to be at increased risk of AMD. Practitioners need to be able to reliably determine the change (if any) in MPOD as a result of the prescribed intervention. If instrument test-retest repeatability is not of a clinically acceptable standard, the instrument is simply unsuitable for robust clinical practice. The repeatability of the Densitometer and the MPS 9000 has been separately investigated previously (Snodderly et al. 2004; Gallagher et al. 2007; Van der Veen et al. 2009; Bartlett et al. 2010). The Densitometer has been shown to demonstrate good test-retest and intraclass correlation, (Snodderly et al. 2004; Gallagher et al. 2007) and a coefficient of variation ranging from 17% to 22 %, (Snodderly et al. 2004; Gallagher et

al. 2007) all of which would indicate good test-retest repeatability. The MPS 9000 was similarly reported to demonstrate good correlation and coefficient of repeatability in a study by Van der Veen et al. (Van der Veen et al. 2009) but these results could not be replicated in a more recent study by Bartlett et al. which reported a coefficient of repeatability ranging from 0.28 to 0.33, and a coefficient of reproducibility of 0.25 to 0.26 (Bartlett et al. 2010). This substantial discrepancy between results might be explained by a significant variation in the experimental protocol adopted in the two studies.

In the Van der Veen study, only 11 subjects were included in the repeatability assessment, and the MPOD value used in analysis, represented the average of five repeated measurements conducted at each visit (Van der Veen et al. 2009). The Bartlett study recruited 40 subjects, employed two separate examiners, and used a single measurement of MPOD rather than the average value analysed by Van der Veen, (Bartlett et al. 2010). Although, there was no learning or fatigue effect noted, it could be argued that these 40 subjects were substantially less ‘trained’ than the 11 subjects who completed the MPOD examination a total of ten times. It could also be argued that the ‘averaged’ data was substantially cleaner, and therefore more robust, than the raw single measurement used by Bartlett et al. The experimental protocol employed by Bartlett et al. is however, more representative of normal clinical practice, where multiple repeat measurements are typically unfeasible, and their results are perhaps, therefore, of more clinical importance. It has been suggested that the number of subjects in the Bartlett paper with significant variation in test – retest MPOD values, represents operator error (inappropriate acceptance of low quality V- shaped functions), rather than measurement noise (Murray et al. 2010). This may be the case, but if so, this reinforces

the observations herein, that MPOD values obtained using the MPS 9000, may well be significantly affected by examiner skill level and training, and furthermore, that the limited means to determine patient performance acceptability would seem unreliable at best.

The coefficient of repeatability for the MPS 9000 in the current study ranged from 0.18 to 0.21 respectively. Although these values represent significantly better repeatability than that determined by Bartlett et al. they do, nonetheless, still suggest a significant amount of expected variability between repeat measures of MPOD (Bartlett et al. 2010).

For the purposes of comparability, the repeatability of the Densitometer was also assessed. The coefficient of repeatability for the Densitometer ranged from 0.11 to 0.12, which represents significantly better repeatability when compared to the MPS 9000. Indeed, the range of MPOD values across all three measures was less than 0.1 for 92%, and less than 0.05 for 44% of subjects using the Densitometer, compared to 54% and 25% respectively, for those using the MPS 9000.

The current study was designed to facilitate the assessment of the comparability and repeatability of MPOD measurements, as determined using the commercial MPS 9000 in relation to the gold standard Densitometer. It is important to note that the experimental protocol here was designed to be of clinical relevance. Consistent with routine clinical practice, the tests in the current study were conducted as per manufacturer guidelines to determine a single measurement of MPOD. The MPS 9000 underestimates MPOD in relation to the Densitometer and demonstrates poorer repeatability. Our analysis would suggest however, that the fundamental principles and

technique of the MPS 9000 seem generally robust, but that the increased variability observed here and elsewhere, (Bartlett et al. 2010) may largely be as a consequence of (a) the absence of a user friendly means to assess subject performance variability during the test procedure, (b) the increased difficulty associated with the peripheral task, and (c) the requirement of the user to make an instantaneous judgment as to the flicker detection endpoint. In the presence of such design features, we would recommend that best clinical practice using the MPS 9000 would require multiple measures of MPOD. Results should be discarded where large discrepancies such as those obtained by Bartlett et al. are found, and where results are more consistent, the average MPOD should be used to maximise the accuracy and validity of the MPOD value obtained.

## **CHAPTER EIGHT**

### **8.1 Discussion**

The significance of healthy MP levels is becoming more important to eye care practitioners for a number of key reasons including; a general trend towards longer life spans and aging populations, the increasing prevalence of artificial SW light sources, the contemporary prevalence of smog and haze with consequential light scatter, and the explosion in the incidence of AMD. All of these factors heighten the importance of the preservation and augmentation of MP density, which may in turn lead to improved visual performance and health during one's lifetime, and possibly lower the risk of developing age-related eye diseases, such as AMD in the long-term. Importantly, MP is modifiable, and re-pigmentation of the macula can occur in as little as 6 months and usually plateaus in 2 years ([www.macuscope.ie](http://www.macuscope.ie)).

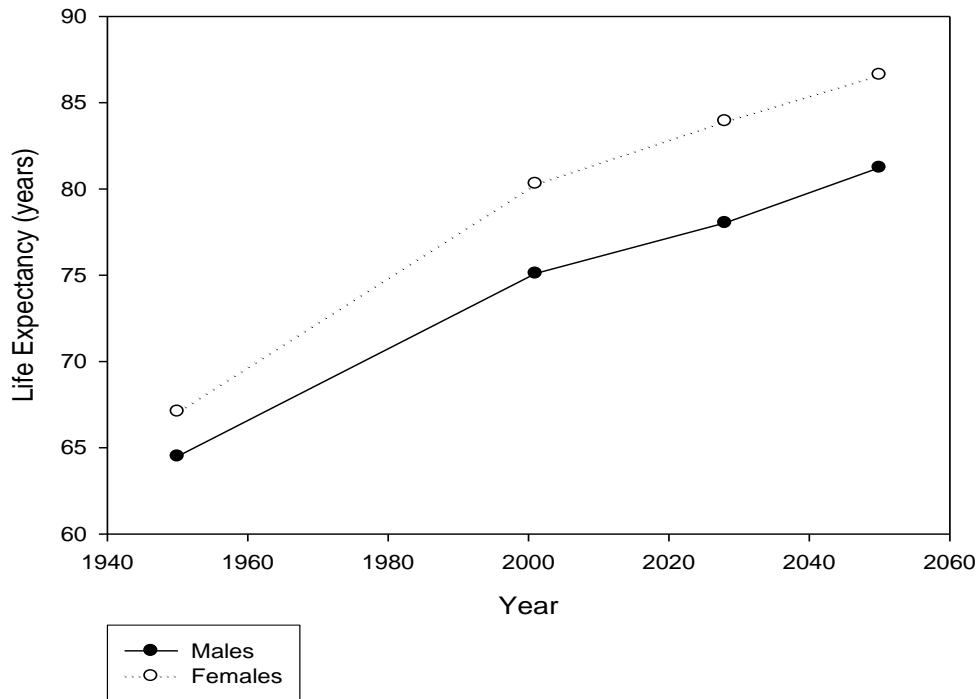
### **8.2 Macular Pigment and its Role in Clinical Practice**

#### **8.2.1 AMD**

A lot of focus has been placed on the protective role of MP and AMD. It is important to note that, although AMD is a late onset disorder, currently undetectable changes occur at the retina potentially decades before the condition presents. While signs and symptoms of AMD often present at circa 55 years of age, the condition in fact represents the end product of a lifetime of cumulative and chronic microscopic retinal changes. MP may have a key role to play in minimising the chronic effects of oxidation and consequential inflammatory response which lead ultimately to AMD development.

Researchers and clinicians have tended to focus primarily on the protective role of MP and AMD, as this eye disease is reaching epidemic proportions and is going to become a huge economic burden on society as people are living longer (See Figure 8.1).

Male and Female Life Expectancy 1950-2050



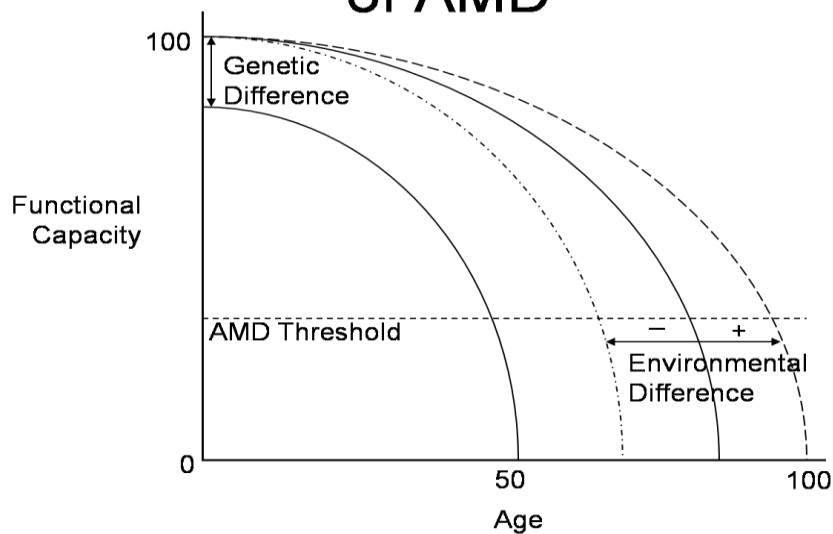
**Figure 8.1 Life expectancy of men and women between 1950 and 2050**

**(Department of Health, Ireland).**

Optometrists or clinicians need to target or identify patients ‘at risk’ for AMD and look at prevention options. Whilst blue light, oxidative stress and low MP levels are thought to contribute to the pathogenesis of AMD, other risk factors, both modifiable and non-modifiable, established and putative are thought to be involved in the aetiology of AMD. Non-modifiable risk factors include age, sex, and family history of AMD. Modifiable risk factors include smoking, a diet low in lutein and zeaxanthin, high cholesterol, BMI > 27, and raised BP. By being aware of the non-modifiable risk

factors, a person who may be ‘at risk’ can become more proactive about the modifiable risk factors.

## Genetic and Environmental Basis of AMD



Adapted from Cai *et al.*, *Progress in Retinal and Eye Research*, 2000 Mar;19(2):205-21

**Figure 8.2: Genetic and environmental influence on AMD development**

A person with a family history of AMD is already at a higher risk of developing AMD. Certain environmental stresses such as smoking, excess exposure to sunlight and low MPOD levels can increase this risk even further (See Figure 8.2). Early identification of those patients most suitable for certain interventions is essential. Appropriate advice, including the dietary modification and/or supplementation with MP carotenoids should be recommended to a person when their MPOD is low during life, rather than waiting for the first visible signs of AMD.

### **8.2.2 Visual performance and comfort**

The primary role of MP, from a Darwinian perspective, may rest on its role in visual performance. Augmented MP levels can affect visual performance and visual comfort throughout life. Most optometrists however remain blissfully unaware of this important function of MP. By increasing or maintaining MP levels, either through diet or supplementation, one can enhance a person's vision in terms of better acuity and reduced glare and/or reduced photophobia. Up until very recently however, optometrists were unable to measure MPOD in practice, so the ability to link MP levels with symptoms such as glare and photophobia wasn't possible (Stringham, 2003; Wenzel, 2006; Stringham, 2007; 2008). Higher MP levels at a young age will not only lead to better visual performance during life, but to a healthier retina where the "normal" and consequential signs of aging may be slowed or delayed. The age group that practitioners need to target is 20 years and upwards, as blue SW light damage is theoretically maximal at this age due to large pupils, clearer crystalline lens and optical media, and possible lifestyle choices such as smoking, excessive alcohol and diets which may be low in fruits and vegetables.

Many patients present in practice with symptoms such as difficulty with night driving. Historically the recommended practice was to offer an anti-reflective coating for glasses, where used. The link between glare and visual discomfort and MP has been clearly established (Stringham, 2003; 07; 08). If MPOD is low, the patient should be offered advice on how to improve the situation either through diet and/or supplementation.



Photophobia is another symptom many people complain of, and often without any obvious supporting clinical indicators. The threshold for photophobia tends to vary quite considerably and as we have seen there can be quite a dramatic variation in MP levels between individuals, which could partially explain why some people have problems with glare and others do not. Although the current study did not demonstrate a baseline correlation between MPOD and either glare or photostress recovery, this finding is largely inconsistent with studies employing SW dominated stimulus configurations, which are becoming more prevalent in modern society. Although the typical office or home environment (where the majority of us seem to spend most of our time) does not have many SW dominated light sources, the ever changing nature of internal and device lighting systems are extending our exposure to SW light, and may enhance the applicable relevance of MP for visual performance. Examples of this include the increased use of LED systems and xenon car headlights. The importance of healthy MP levels in the context of the growing prevalence of SW light is an issue which needs to be emphasised so that optometrists can give appropriate advice to their patients.

It is therefore to be recommended that practitioners investigate MP levels where problems of glare or photophobia exist, and advise accordingly in an effort to minimise such troubling symptoms.

Although MP has the potential to alter colour vision, our research, however, found no negative association between MP and colour vision. Supplementation, or dietary fortification designed to increase MPOD is unlikely, therefore, to adversely affect hue discrimination.

### **8.3 Dietary Intake of Macular Pigment Carotenoids**

Where possible a dietary approach to increasing MP should be considered first, as opposed to supplements, particularly those which may contain co-antioxidants, as some may be in excess of the RDA for certain vitamins and minerals. Patients need to be particularly mindful when also taking additional multivitamins. From a clinical point of view it appears that current FFQ's, analysed with the databases available, are not accurate enough to provide clinically useful feedback on actual intake of lutein and zeaxanthin. It is, therefore, simply more beneficial for eye care professionals to educate patients about the foods that are rich in lutein and zeaxanthin, and the number of portions one would need to take, in order to meet the recommended intake of 6 mg/day (See section 3.12.4; Table 3.1; appendix 8.1), and more importantly, to achieve a satisfactory increase in MPOD where necessary. From a practitioner's point of view, a leaflet outlining those foods high in lutein and zeaxanthin, and including information regarding the effect of bioavailability, storage, cooking of those foods, as well as the role of additional dietary fat intake on carotenoid uptake would be most useful. Such a leaflet could be used to educate patients, and could be given to such patients for home reference.

#### **8.3.1 Special precautions**

An important point to note in relation to green leafy vegetables and spinach in particular, is that many of these vegetables have a very high iron content, which is not a problem for most people but could pose a problem for someone with a condition such as haemochromatosis. This is a genetic condition which is quite common in Ireland resulting in deposition of excess iron in organs. People with this condition should avoid foods high in iron, so in this case a supplement would be more appropriate.

Additionally, it is important to note that a certain amount of dietary fat is needed to absorb these carotenoids. Some people may have a carotenoid-sufficient but fat-depleted diet and this can impair carotenoid absorption. Medical conditions which lead to fat malabsorption such as pancreatic enzyme deficiency, surgical removal of part or all of the stomach, gall bladder disease or liver disease can also lead to problems with malabsorption. Certain foods and medications can also affect dietary absorption of lutein and zeaxanthin, such as dietary fibre, margarines enriched with plant sterols such as 'benecol' and cholesterol lowering medications. HDL's are known to be primary carriers of lutein and zeaxanthin (Viroonudomphol, 2003), and hence an individual's lipoprotein profile may influence the transport and delivery of these carotenoids to the retina, with a consequential impact on MP. Therefore anyone on cholesterol lowering medication or have high cholesterol should have their MPOD routinely measured in clinical practice. Other conditions, such as cystic fibrosis, crohn's disease, and coeliac disease may cause problems with carotenoid absorption. Particular attention needs to be given to MPOD in such cases.

#### **8.4 Lutein, Zeaxanthin and *meso*-Zeaxanthin Supplementation**

A dietary approach may be impractical, unsuitable or unsuccessful for some people, so the use of carotenoid supplements should be considered. Patients who are at 'high risk' for AMD, or people complaining of symptoms of glare and photophobia might consider adding lutein, zeaxanthin and *meso*-zeaxanthin to their diet in supplement form. To date there has been no known toxic effect with carotenoid supplementation. Macushield, it appears, is the only supplement on the market that contains all three carotenoids without added co-antioxidants. Other supplements generally contain co-antioxidants, some of which are well above the recommended daily levels and can cause toxic effects if taken

to excess. High-dose zinc, for example, can cause gastric irritation or anemia (Johnson et al. 2007), vitamin A (as beta carotene) has been associated with an increased risk of lung cancer among smokers (Omenn, 1996). Supplements, therefore, should be prescribed on an individualised basis. Eye care practitioners should keep abreast of currently available supplements and their carotenoid and co-antioxidant content and advise accordingly (See Table 8.1 for list of available supplements). Given the enhanced antioxidant capacity of *meso*-Zeaxanthin, we would currently recommend the use of Macushield as the preferred supplement, and where necessary, in addition to, a routine multivitamin supplement. Sublingual sprays are now available (e.g New focus, Maxi focus). They claim to have a much greater benefit than tablets or capsules, as the carotenoids are immediately absorbed into the blood stream under the tongue. It can be difficult for some people with digestive problems to absorb capsules. These sprays would be especially important if a person has a condition such as crohns disease or cystic fibrosis.

**Table 8.1 Contains a list of some of the commercially available Visual Health Supplements**

Supplement	Lutein mg	Zeaxanthin mg	Meso-zeaxanthin	Co- antioxidants
Macushield	10 mg	2 mg	10 mg	None
Vitalux plus Omega 3	2.5 mg	6.25 mg	None	Vitamins A,C, copper, Zinc & DHA,EPA
I caps	4 mg L & Z Recommends 2 tabs daily		None	Vitamins A, C E, Zinc, copper, magnesium & selenium

Ocuvite lutein	6mg lutein	None	None	Vitamins C, E, Zinc & Copper
Ocuvite lutein complete	10 mg	2 mg	None	Vitamins C, E, Zinc, EPA & DHA

### 8.5 Measuring Macular Pigment Density in Practice

New desktop MP measurement devices now make it possible to measure MPOD routinely in clinical practice. MP is modifiable through dietary changes and/or supplementation, so accurate, repeatable and non-invasive methods of measuring MPOD are becoming increasingly important.

Ideally anyone over 20 yrs of age should have MP measured in practice. If not feasible in a busy clinic environment, then particular attention should be given to those with a family history of AMD, those complaining of symptoms of glare, night driving problems, individuals with high cholesterol and using statins, smokers, people exposed to excessive sunlight and anyone who might have a diet low in fruit and vegetables or a BMI > 30.

Given the results of the current study, if using the MPS 9000 device, we would recommend that several MPOD measurements be taken to establish the baseline and subsequent MPOD levels. Because there is good inter-individual agreement of MPOD, with mean differences of only 5% for zeaxanthin and 11% for lutein between fellow eyes (Handleman, 1991), measuring MPOD on both eyes would be a good way of double checking instrument accuracy. Large discrepancies between the two eyes should be questioned. A suggested protocol would be to measure MPOD on both eyes and

repeat readings, discarding obvious outliers, and taking the average as the value for MPOD. Anyone with low MPOD values, particularly if they are in the ‘high risk’ group for AMD, should consider modifying their diet and/or taking a supplement.

### **8.6 Macular Pigment Protocol for Clinical Practice**

Increased awareness of the dual functional role of MP, coupled with coherent protocols when recommending MP carotenoid supplementation in clinical practice, may confer long-term visual health benefits, which may translate as improved visual performance and comfort during life, and also as a reduced risk of developing AMD in later years. Targeting people at a young age will not only reap beneficial visual effects during life but may also lead to a healthier lifestyle as MPOD is a biomarker for long-term nutrient status. If MPOD is routinely measured in practice, low MP levels can be addressed a lot earlier. Supplementation should not necessarily be the first port of call, but a holistic approach needs to be taken such as improve diet (include fruit and vegetables), reduce BMI, reduce cholesterol, if exposed to excessive sunlight exposure ensure the proper sunglass protection is worn.

Anecdotally, current practice with regard to MP supplementation or dietary fortification appears highly unstructured, somewhat flippant, and most likely, therefore, not achieving maximal impact in terms of the reinforcement of the importance of the pigment for optimal and maintained visual health. A structured practice framework would seem logical to somewhat standardise the quality and appropriateness of MP related interventions. Such a structure might include:

- A detailed case history can be used to determine the presence of symptoms that

might be attributable to MPOD deficiency, the presence of factors affecting absorption/ transport of lutein/zeaxanthin, the presence of any medical conditions such as crohns, cystic fibrosis, coeliac disease or any other malabsorption problems that might impact MPOD, and further, to identify patients at increased risk of AMD.

- Repeat MPOD measurements should be incorporated into the normal eye examination routine for all patients, but in particular, for those “risk”, “carotenoid compromised”, or “symptomatic” patients identified in the case history.
- Where appropriate, dietary fortification or supplementation should be advised. It is important to note that there is no simple means to determine “normal” MPOD levels. These may very much be individualised. Therefore, there should not be a specific and universal target optical density as such. The simplest, and probably most efficient protocol, should include the augmentation of MPOD where desirable, until a plateau level is reached which is most likely optimal for that individual.
- Additional advice, regarding exposure to SW light, and additional risk factors for AMD, should be given to patients as appropriate.
- Where dietary fortification and/or supplementation have been recommended, the patient should be provided with an information leaflet such as that outlined in section 8.3 above. Where supplementation is recommended, the patient should be informed of the unknown possibility of adverse effects and interactions with multivitamin supplements, in particular when co-antioxidants are included in the MP carotenoid formulation.
- MPOD measurements should be re-assessed at routine, perhaps 6 monthly intervals. Such measurements will inform a review of the dietary or

supplementation requirements, and allow consideration for change of recommendation (e.g. change from diet to supplement based augmentation), or dose adjustment.

### **8.7 Future Research**

At present, there are no definitively established, physiologically significant cut-off points for lutein, zeaxanthin or *meso*-zeaxanthin in serum or MPOD, above which protection or prevention against chronic diseases or enhancement of vision is ensured. Reading & Weale (1974) suggested that an MPOD greater than 0.3 is superfluous to visual performance. Future studies should aim to determine optimal MPOD levels for maximal visual performance and for optimal visual health, and whether such levels are highly specific to the individual, or whether generalisations can be made about a population optimum level.

Further work is also required to determine both the optimum dosage of the three constituent carotenoids, and any associated co-antioxidants, and the optimum duration of supplementation, which can be costly to the patient who may not “see” a significant benefit for their expenditure. The question still remains as to whether supplementation needs to be continuous, or can be cycled using the assumption that once MPOD reaches a plateau, retinal turnover will be slow, and plateau levels will be sustained over a certain level of time. It may also be possible to simply reduce the dose, for example, taking a supplement every second day, once this plateau is reached. This question can certainly be easily answered on an individual basis through repeat MPOD measures over time in response to changes in supplementation protocol.



Additionally, there are newly emerging technologies available to practitioners for the measurement of MPOD. The Zeiss Visucam R200, for example, is a fundus camera with the additional capacity to measure MPOD using reflectance technology. The Wooten Clinical Densitometer is a new clinical version of the research gold standard. It would be highly desirable to initiate further research that will clarify if such instruments can accurately measure MPOD and changes in MPOD in response to supplementation. Furthermore, it would seem important to clarify whether measurements made using different techniques or devices, are in any way interchangeable.

Finally, diabetes is another condition that is on the increase and it can affect the eyes. Diabetes is linked to oxidative stress and as MP is a powerful antioxidant, future studies could examine the relationship, if any; between MPOD and diabetes as a condition that causes macular damage.

## **8.8 Conclusion**

There is now a growing body of evidence to support the value of high MPOD levels for both visual performance and visual health. Armed with such evidence, and the newly emerging MPOD measurement technology, optometrists, and primary eye care practitioners, are now keenly placed to drive a preventive health agenda that will have individual and societal relevance in an era where changing population demographics and lifestyle are causing a significant elevation in the burden of age-related disease. It has been shown here, and elsewhere, that MP can influence vision and visual health, and can reduce the burden of ocular disease. Technologies now exist to allow eye care practitioners to fully implement MP interventions in clinical practice, and to monitor their effects. Together with a structured protocol for MP assessment in clinical practice

as has been outlined here, it is now possible to define and decide the future of primary eye care practice that affords significantly increased focus and clinical assessment in relation to MP.

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## **Publications**

Loughman J, Akkalli M, Beatty S, Scanlon G, Davison P, O'Dwyer V, Cantwell T, Major P, Stack J, Nolan JM. The Relationship between Macular Pigment and Visual Performance. *Vis Res.* 2010;50, 1249-1256.

Loughman J, Nolan, J, Scanlon G, Beatty S. A Case Study in Human Macular Pigment Deprivation. Abstract submitted to Cambridge 2011

Nolan JM, Loughman J, Akkali MC, Stack J, Scanlon G, Davison P, Beatty S.

The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS, *Vis Res.* 2011; doi: 10.1016/j.visres.2010.12.016

An Evaluation of a Novel Instrument for Measuring Macular Pigment Optical Density: The MPS 9000

Davison P, Akkali M, Loughman J, Scanlon G, Nolan J, Beatty S. Macular Pigment: its Associations with Colour Discrimination and Matching. *Optom Vis Sci.* 2011;88(7):816-22.

Scanlon G, O'Dwyer V, Beatty S, Stack J, Nolan JM, Loughman, J. Comparison of 2 Heterochromatic flicker photometer machines: The MPS 9000 & the Macular Metrics Densitometer™. (Submitted to *ACTA Ophthalmologica* for publication).

## Appendix A

### Visual Health Supplement Formulations, Dosages, and Comparison with Recommended Intake Levels

No <sup>5</sup>	Vit. A (IU)	%RDA <sup>2</sup> 2334 <sup>1</sup>	Vit.C (mg)	%RDA 60mg	Vit.E (IU)	%DRI <sup>4</sup> 10.05	Zinc (mg)	%RDA 7	Cu (mg)	%RDA 1.1	Se (µg)	%RDA 40µg	L/Z (mg)
1	28640	1227	452	271%	400	3980	69.6	994%	1.6	145%	-		6
4	28640	1227	452	271%	400	3980	69.6	994%	1.6	145%	-		-
1	1000	43	300	180%	100	995	40	571%	2	181%	55	137%	2
2	-		120	200%	27	269	10	143%			40	100%	13
1	5000	214	60	100%	45	448	15	214%	2	181%	20	50%	0.25
2	20000	857	750	450%	200	1990	12.5	179%	-		50	125%	10
2	14320	614	226	377%	200	1990	34.8	497%	0.8	72%	4	10%	-
2	14320	614	226	377%	200	1990	34.8	497%	0.8	72%	-		-

- 1: No of tables needed to attain the values listed in the table
- 2: Equal to 700ug Vitamin A (retinol equivalents) McCance and Widdowson's 2004)
- 3: RDA for females only. RDA for males = 900ug/d
- 4: RDA for adult females. RDA for males = 9.5mg/d
- 5: DRI: Daily recommended intake. No RDA set in Ireland

## Appendix B

### Estimated dietary requirement range

$$\frac{(\text{Current L and Z intake (mg/day)} \times (0.35\mu\text{g/ml)})}{\text{Blood serum L and Z concentration } (\mu\text{g/ml})} \text{ to } \frac{(\text{Current L and Z intake (mg/day)})}{\text{Blood Serum L and Z concentration } (\mu\text{g/ml})} \times (0.6\mu\text{g/ml})^2$$

Blood serum L and Z concentration ( $\mu\text{g/ml}$ )

Blood Serum L and Z

concentration ( $\mu\text{g/ml}$ )

<sup>1</sup>Based on optimal serum L/Z concentration (Granado et al., 2003)

<sup>2</sup>Equation is based on dietary and blood serum L and Z concentrations in an Irish sample (Nolan et al., 2007)

## Appendix C

Food Source	Lutein and Zeaxanthin	Lutein	Zeaxanthin
Egg yolk	89	54	35
Sweetcorn	86	60	25
Kiwi	54	54	0
Red grapes (seedless)	53	43	10
Zucchini squash	52	47	5
Pumpkin	49	0	0
Spinach	47	47	0
Orange pepper	45	8	37
Yellow squash	44	44	0
Cucumber	42	38	4
Pea	41	41	0
Green pepper	39	36	3
Red grape	37	33	4
Butternut squash	37	37	0
Orange juice	35	15	20
Honeydew	35	17	18
Celery (stalks, leaves)	34	32	2
Green grapes	31	25	7
Brussels sprouts	29	27	2
Spring onions	29	27	3
Green beans	25	22	3
Orange	22	7	15
Broccoli	22	22	0
Apple (red delicious)	20	19	1
Mango	18	2	16
Green lettuce	15	15	0
Tomato juice	13	11	2
Peach	13	5	8
Yellow pepper	12	12	0
Nectarine	11	6	6
Red pepper	7	7	0
Tomato (fruit)	6	6	0
Carrots	2	2	0
Cantaloupe	1	1	0
Dried apricots	1	1	0

Lutein & Zeaxanthin = Macular Pigment (MP)

### Dietary Sources of Macular Carotenoids (mol %)

## Appendix D

Subject Code

Scottish Collaborative Group Food Frequency Questionnaire version 6.5



# Diet Questionnaire



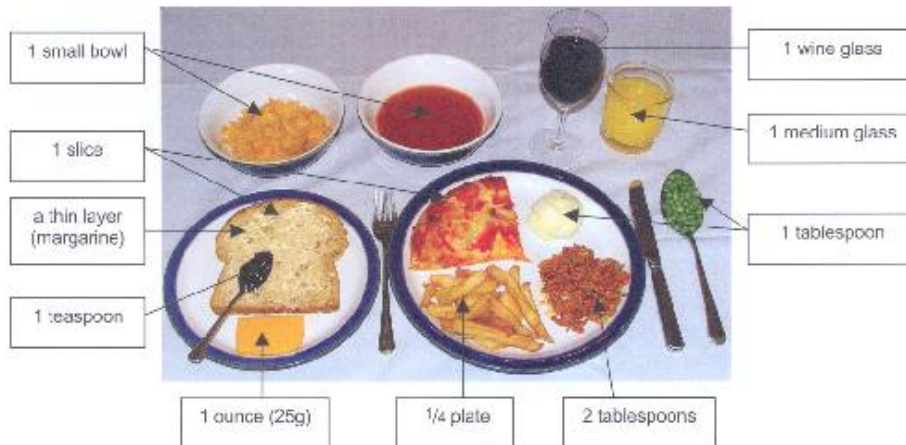
© University of Aberdeen, 2005

**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 <b>4</b> 5+	R M 1 2 3 4 5 6 <b>7</b>
b) Apples	1 medium apple	<b>1</b> 2 3 4 5+	R M 1 2 3 4 <b>5</b> 6 7
c) Chips from a chip shop or restaurant	1/4 plate	<del>1</del> <b>2</b> 3 4 5+	R <b>M</b> 1 2 3 4 5 6 7
d) Tomato juice	1/2 medium glass	1 2 3 4 5+	<b>R</b> 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.**



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### 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 <input type="checkbox"/>	Brown / granary <input type="checkbox"/>	Wholemeal <input type="checkbox"/>

### 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

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	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, casserole 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, casserole or fried)	2 tablespoons, 2 slices or 1 chop	1 2 3 4 5+	R M 1 2 3 4 5 6 7

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	Measure	Measures per day					Number of days per week									
f)	Chicken or turkey (roast, grilled, casserole 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

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### 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

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	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

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## 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

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	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

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### 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).			<input type="text"/> <input type="text"/>
e) Did you use any butter, margarine or other fat spread or oil on bread?		Yes <input type="checkbox"/> No <input type="checkbox"/>	
If yes, please give full details of the one or two types you used most (e.g. Asda Sunflower buttery spread).			
<b>If you did not spread any fat or oil on bread, please go straight on to question g.</b>			
_____			Office Code <input type="text"/> <input type="text"/>
_____			Office Code <input type="text"/> <input type="text"/>
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 <input type="checkbox"/>	a thin layer <input type="checkbox"/>	a thick layer <input type="checkbox"/>
g) Did you use any fat or oil for home frying or cooking?		Yes <input type="checkbox"/> No <input type="checkbox"/>	
If yes, please give full details of the one or two types you used most (e.g. Tesco Pure Vegetable Oil).			
<b>If you did not use any fat or oil for home frying or cooking, please go straight on to section 18.</b>			
_____			Office Code <input type="text"/> <input type="text"/>
_____			Office Code <input type="text"/> <input type="text"/>

### 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



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	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

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## 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) _____ Brand name and details _____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
b) _____ Brand name and details _____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
c) _____ Brand name and details _____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7
d) _____ Brand name and details _____	_____	1 2 3 4 5+	R M 1 2 3 4 5 6 7

## 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 E

### Questionnaire 2

This is a food frequency questionnaire designed to assess your dietary intake of two antioxidants, Lutein and Zeaxanthin. You are asked to answer two questions for each food

- 1) **Number of times you eat that food. Choose one of the following**
  - a. per month,
  - b. per week
  - c. *or* per day.

If you never eat that food, tick the box directly under 'Never consumed'

- 2) **Portion size**

Use the pictures from the front page of the first questionnaire to answer this question.

- 1 portion = 1 apple/1 orange etc  
2 tangerines  
1 slice of melon  
1 small glass of fruit juice  
2-3 tablespoons of vegetables

Food Item	Number of Servings				Portion size
	Per month	per week	per day	Never consumed	
<b>Vegetables</b>					
Broccoli					
Brussels sprouts					
Cabbage					
Spinach					
Peas					
Carrots					
Peppers					
Lettuce					
Sweetcorn					
Tomatoes					
Squash					
Minestrone soup					
Tomato Juice					
Tomato puree					
Vegetable juice					
Courgette					
<b>Fruits</b>					
Oranges					
Peach					
Melon					
Grapefruit					
Fruit cocktail(canned)					
Watermelon					
Papaya					
Tangerine					
<b>Other</b>					
Eggs					
Kelloggs Cornflakes					
Turnip					
Kale					
Celery					
Herbs/Spices – cornflour, paprika, basil, parsley					

## **Appendix F**

### **Investigation of the Relationship between Dietary Antioxidants**

#### **-and Eye Health Patient Information Sheet:**

##### **Background**

There is increasing evidence that higher intakes of antioxidants could protect against oxidative and light damage in the eye. Research has shown that a low dietary intake of two antioxidants in particular, Lutein and Zeaxanthin, is associated with an increased risk of a chronic eye disease, Age Related Macular Degeneration. They are present in large quantities in the retina of the eye and are collectively referred to as 'macular pigment'. Without macular pigment or a low density macular pigment your eye is more susceptible to damage.

##### **Invitation to Participate**

You are invited to participate in a research study designed to measure your macular pigment level and complete two dietary questionnaires. This will allow us to identify any relationship(s), which may exist between macular pigment and dietary intake of antioxidants.

Each volunteer will be asked to attend the Dublin Institute of Technology in Kevin St for a once off visit of approximately one hour.

The following will take place during your your 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.
- You will be asked to complete a brief questionnaire to gather information about your diet and other demographic details. This questionnaire covers the following areas: contact details; lifestyle details; personal medical history; weight and height..
- A blood sample will be taken to measure macular pigment levels in your blood.
- We will measure your macular pigment using a specialised vision testing technique.

You will be asked to fill in two dietary questionnaire's which are an important measure of eye health.

**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 of the quality of your diet.

**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.

## Appendix G

### Comparison of 2 Heterochromatic Flicker Photometers and Evaluation of SightRisk Questionnaire as a Clinical Tool

Subject Number: \_\_\_\_\_

Patient Information Sheet:

#### **Background**

Age Related Macular Degeneration (AMD) is a **progressive disease of the retina in which the light-sensing cells in the central area of vision (the macula) stop working and eventually die**. There is increasing evidence that higher concentrations of macular pigment in the eye protect against oxidative damage, which is the suspected cause of AMD. Research has show that this pigment is only available to the body via dietary intake. The macular pigment accumulates in the retina and acts as an anti oxidant. Low antioxidant levels, in particular Lutein and Zeaxanthin are associated with increased risk of developing AMD.

Measuring the macular pigment density in the eye should eventually provide a more complete understanding of its functional role in the retina and the relationship between macular pigment levels and the development of AMD. A psychophysical method using heterochromatic flicker photometry (HFP) is one of the few non-invasive methods available to measure this pigment in the eye.

#### **Invitation to Participate**

You are invited to participate in a research study designed to measure your macular pigment level and complete a dietary questionnaire. This will allow us to identify any relationship(s), which may exist between macular pigment and dietary intake of antioxidants.

Each volunteer will be asked to attend the Dublin Institute of Technology in Kevin St for a once off visit of approximately one hour. The following will take place during your 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.
- You will be asked to complete a brief questionnaire. This covers the following areas: contact details; lifestyle details; personal medical history; weight and height.
- We will measure your macular pigment using 2 instruments.
  - a) The Macular Metrics Densitometer TM and
  - b) The MPOd (Tinsley Instruments)

We will also measure your best-aided visual acuity (BVA) and correlate this value with macular pigment optical density (MPOD).

### 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 of the quality of your diet.

### 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.

### Consent Form

Date: \_\_\_\_\_

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.
4. I agree to take part in the above study and hereby give my consent to have any necessary measurements taken and my data analysed.

\_\_\_\_\_



Name of Volunteer

Date

Signature of Volunteer

\_\_\_\_\_  
Name of Witness

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Witness

# COMPASS

## Collaborative Optical Macular Pigment Assessment Study



### CRF 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 1)

<b>DESCRIPTION</b>	<b>EST. TIME MINS</b>
<b>A.</b> Information leaflet discussion and informed consent	5 mins
<b>B.</b> Collection of blood for serum carotenoid analysis	5 mins
<b>C.</b> Demographic, medical history, lifestyle and vision case history questionnaires	20 mins
<b>D.</b> Visual acuity, high and low contrast visual acuity, and refraction	15 mins
<b>E.</b> Colour vision tests: colour anomoloscope; 100 hue test	20 mins
<b>F.</b> Glare tests (Optec + BAT)	10 mins
<b>G.</b> Visual performance questionnaire	10 mins
<b>H.</b> Contrast sensitivity (Metropsis)	15 mins
<b>BREAK</b>	<b>~30 mins</b>
<b>I.</b> Macular pigment optical density spatial profile measurement	35 mins
<b>J.</b> Dietary questionnaire	30 mins
<b>K.</b> Photostress recovery test	10 mins
<b>L.</b> Short wavelength perimetry	15 mins
<b>M.</b> Fundus and iris photographs	5 mins

**A. Informed consent**

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: \_\_\_\_\_

**B. 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°C?  
yes                      no                                           

If yes,

Time of centrifugation: \_\_\_\_\_

Name of person obtaining blood: \_\_\_\_\_

Signature of person obtaining blood: \_\_\_\_\_

**C. Demographic, medical history, lifestyle and vision case history questionnaires**

Forename: \_\_\_\_\_ Surname: \_\_\_\_\_

Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Contact No(s): \_\_\_\_\_

Email: \_\_\_\_\_

Date of birth: \_\_\_\_\_ Age: \_\_\_\_\_(years)  
(DD/MM/YYYY)

**Please circle number corresponding to correct answer. All questions must be answered unless otherwise specified.**

1. Sex

- Male ..... 1
- Female ..... 2

If female, stage of Menopause (please circle):

- Pre ..... 1
- During ..... 2
- Post ..... 3

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. 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 6.1

c) How long has it been since you last smoked?

- |                     |                          |
|---------------------|--------------------------|
| Less than 1 day     | <input type="checkbox"/> |
| Less than 7 days    | <input type="checkbox"/> |
| Less than 1 month   | <input type="checkbox"/> |
| Less than 3 months  | <input type="checkbox"/> |
| Less than 6 months  | <input type="checkbox"/> |
| 6 months to a year  | <input type="checkbox"/> |
| Greater than 1 year | <input type="checkbox"/> |

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)? \_\_\_\_\_

6.1 Are you commonly exposed to second-hand smoke at home or in the work place? yes  no

7. 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 every day..... 5
- I drink twice a day or more..... 6

b) What is your average alcohol consumption on a weekly basis?

- 1 unit a week..... 1
- 2-5 units a week..... 2
- 6-10 units a week..... 3
- > 10 units a week..... 4

8. 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.)

---

---

9. 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? (e.g. age-related macular degeneration, glaucoma etc.)	1	2

If a family member, what is their relation to you, and what eye disease do/did they have?

---

### 10. 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**

If you exercise, how long would each session usually last?  **minutes**

### 11. Body Mass Index (BMI)

Please record the subject's weight and height in the spaces provided

Weight.....  **Kg**

Height.....  **M**

BMI.....  **Kg/M<sup>2</sup>**





Do you use a computer?

Yes	No
<b>1</b>	<b>2</b>

If yes,

Do you ever suffer eyestrain associated with using the visual display unit (VDU)?

Yes	No
<b>1</b>	<b>2</b>

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
<b>1</b>	<b>2</b>

Do you ever suffer headaches?

Yes	No
<b>1</b>	<b>2</b>

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)**

---

---

**D. High contrast visual acuity and refractive error**

**1: High Contrast (HC) Visual Acuity (LogMAR)**

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 spx)..... R  L

**2: Refractive Error**

Please record the subject's refractive error for both eyes:

R \_\_\_\_\_ Best Corrected HC VA

L \_\_\_\_\_ Best Corrected HC VA

**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 (eye with best corrected HC VA)

*Note: The study eye is the eye with best corrected visual acuity. If corrected visual acuity is the same in both eyes the dominant eye is thereafter used as the study eye.*

R  L

**E. Colour Vision**

**FM 100 Hue Test 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

*Note: Insert polar co-ordinate error chart.*

## Oculus HMC Anomaloscope Results

Moreland Mix

Range  Mid-point   $AQ_{min}$    $AQ_{max}$

*Note: Insert Graph here*

**F. Glare / Photosensitivity**

**Functional Acuity Contrast Test (FACT)**

Total Correct Patches

Night Time No Glare

Night With Glare

Day Without Glare

Day With Glare

Attach Graph

**Brightness Acuity Test**

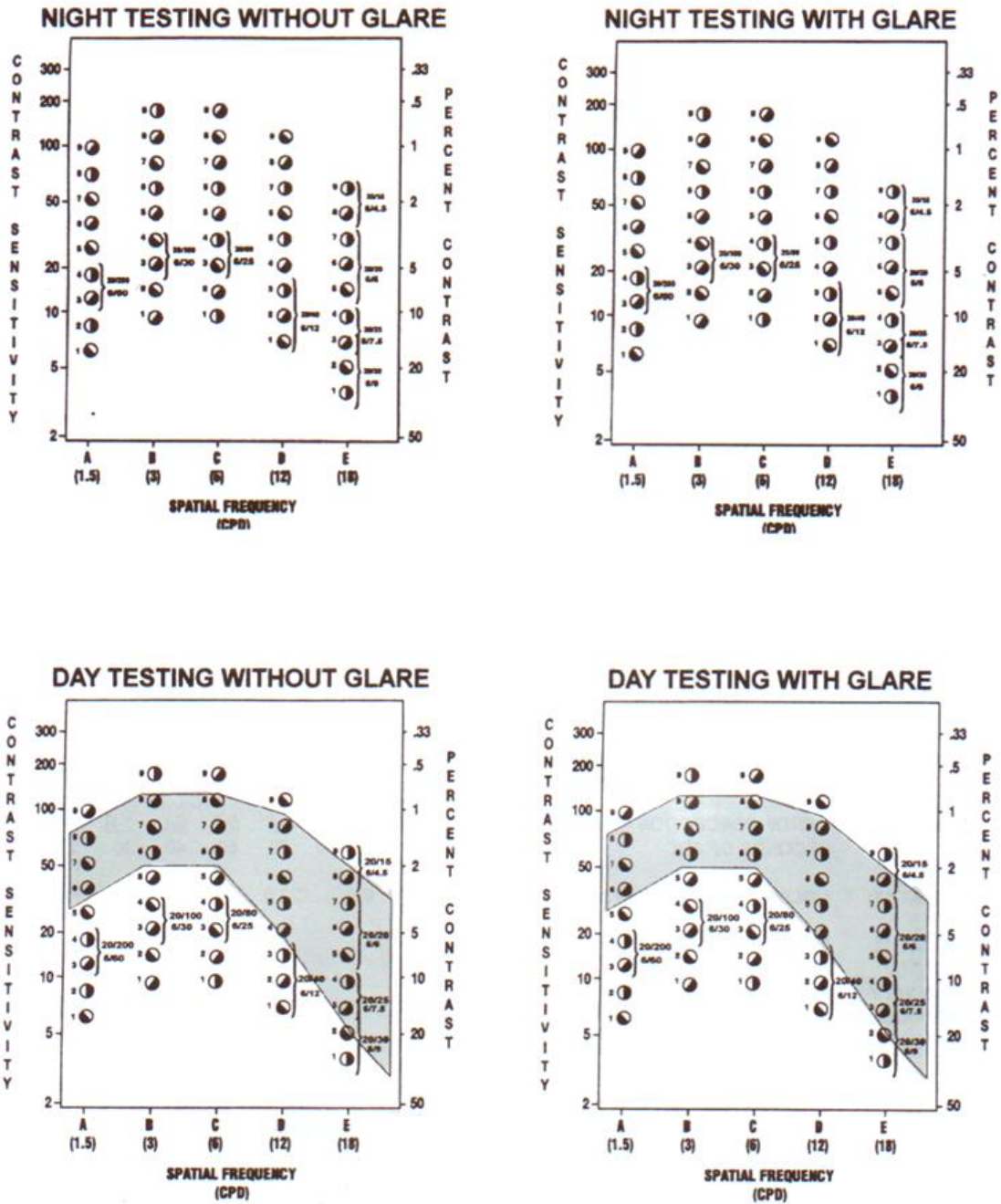
**Contrast Sensitivity**

Aided VA

Low Glare VA (12 ft lamberts)

Medium Glare VA (100 ft lamberts)

High Glare VA (400 ft lamberts)



### G. Visual performance questionnaire

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**

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

**Quantitative 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 .....	<b>2</b>
Significantly worse than others .....	<b>1</b>

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) .....	<b>10</b>
Nine.....	<b>9</b>
Eight .....	<b>8</b>
Seven.....	<b>7</b>
Six.....	<b>6</b>
Five .....	<b>5</b>
Four.....	<b>4</b>
Three.....	<b>3</b>
Two .....	<b>2</b>
One.....	<b>1</b>
Zero (worst).....	<b>0</b>

Glare Disability

All of the following questions assume you are using your best corrected vision (with glasses or contact lenses if necessary).

4: I have problems with lights around me causing glare when I'm trying to see something:

Never.....	<b>5</b>
Rarely.....	<b>4</b>
Sometimes.....	<b>3</b>
Often.....	<b>2</b>
Always.....	<b>1</b>
N/A.....	<b>0</b>

5: I have trouble driving when there are headlights from oncoming cars in my field of view:

Never.....	<b>5</b>
Rarely.....	<b>4</b>
Sometimes.....	<b>3</b>
Often.....	<b>2</b>

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

**Quantitative 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

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).

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

**Quantitative 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

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).

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

**Quantitative 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

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).

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

**Quantitative 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

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) .....	<b>10</b>
Nine.....	<b>9</b>
Eight .....	<b>8</b>
Seven.....	<b>7</b>
Six.....	<b>6</b>
Five .....	<b>5</b>
Four.....	<b>4</b>
Three.....	<b>3</b>
Two .....	<b>2</b>
One.....	<b>1</b>
Zero (worst).....	<b>0</b>

### **Scoring the VFNQ-30**

The purpose of the VFNQ-30 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 Quantitative (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.

## Subject Score

### Colour Discrimination

(a) Functional Analysis

(b) Quantitative/Comparative Analysis

(c) Subject Satisfaction Score

### Glare Disability

(a) Functional Analysis

(b) Quantitative/Comparative Analysis

(c) Subject Satisfaction Score

### Acuity / Spatial Vision

(a) Functional Analysis

(b) Quantitative/Comparative Analysis

(c) Subject Satisfaction Score

### Light / Dark Adaptation

(a) Functional Analysis

(b) Quantitative/Comparative Analysis

(c) Subject Satisfaction Score

### Daily Visual Tasks

(a) Functional Analysis

(b) Quantitative/Comparative Analysis

(c) Subject Satisfaction Score

## **H. Contrast Sensitivity Function**

Photopic CSF

Attach Contrast Sensitivity versus Spatial Frequency (CSF) Plot Below

Attach Data Sheet with minimum contrasts defined for all spatial frequencies

Mesopic CSF

Attach Contrast Sensitivity versus Spatial Frequency (CSF) Plot Below

Attach Data Sheet with minimum contrasts defined for all spatial frequencies

### I. Macular Pigment Optical Density Spatial Profile

Record the Critical Flicker Frequency (CFF) values and calculate the Optimal Flicker Frequency (OFF) values as per COMPASS densitometer 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°	CFF-8	
0.5°	CFF-7	
1°	CFF-7	
1.75°	CFF-7	
3°	CFF-9	
7°	CFF-14	

Predicted CFF		Radiance		
Predicted CFF Actual CFF	0.25 deg			MPOD =
Predicted CFF Actual CFF	0.5 deg			MPOD =
Predicted CFF Actual CFF	1 deg			MPOD =
Predicted CFF Actual CFF	1.75 deg			MPOD =
Predicted CFF Actual CFF	3 deg			MPOD =
Predicted CFF Actual CFF	7 deg			MPOD =

*Note: Please attach graph.*

**J. Diet Questionnaire**

*Note: Please attach complete dietary questionnaire.*

**K. Photostress Recovery**

Pupil Size

Bleaching Exposure Time = 30 sec  
Bleaching Exposure Distance = 15 cm

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	Foveal Sensitivity dB	Time (secs)
Baseline (Pre-Bleaching)	<input type="text"/>	
1 (Post Bleaching)	<input type="text"/>	<input type="text"/>
2	<input type="text"/>	<input type="text"/>
3	<input type="text"/>	<input type="text"/>
4	<input type="text"/>	<input type="text"/>
5	<input type="text"/>	<input type="text"/>
6	<input type="text"/>	<input type="text"/>
7	<input type="text"/>	<input type="text"/>
8	<input type="text"/>	<input type="text"/>
9	<input type="text"/>	<input type="text"/>
10	<input type="text"/>	<input type="text"/>

**Baseline dB**

**Percentage sensitivity reduction post-bleaching**

**Return to Baseline Sensitivity**

**L. Short Wavelength Automated Perimetry**

Reliability Indices                      Pass                       Fail



Attach reliable field plot printout below:

Mean Central Sensitivity (db)

Foveal threshold

1 degree

2 degree

3 degree

4 degree

5 degree

Overall

*Note: Please insert Perimetry printout*

**M. 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? \_\_\_\_\_

Comments

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Signature of person obtaining images: \_\_\_\_\_











