Study of Macular Pigments in Glaucoma

Degree in Doctor of Medicine

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Declaration

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Summary

The macula can be involved in the early stage of glaucoma, as evidenced by the presence of paracentral scotoma on visual field tests (Drance 1969) or thinning of the ganglion cell complex (GCC) layer on optical coherence tomography (OCT) scans (Hood et al. 2013). In the human retina, the ganglion cells are most densely located at the macula and about 50% are concentrated within 4.5 mm of the fovea (Curcio & Allen 1990). Macular pigment (MP), which is composed of the hydroxycarotenoids lutein (L), zeaxanthin (Z) and meso-zeaxanthin (meso-Z), is also known to be most densely concentrated around the fovea (Trieschmann et al. 2008). Both L and Z are solely of dietary origin, while meso-Z can be synthesised de novo from retinal L (Bone et al. 1993).

Disability glare is a visual complaint that can be present during the early stage of age-related macular degeneration (AMD) (Scilley et al. 2002) and glaucoma (Nelson et al. 2003, Goldberg et al. 2009). The aetiology of glare disability in glaucoma is poorly understood and currently, little can be offered to the patient to alleviate this debilitating problem. Oral dietary MP supplementation has been shown to augment macular pigment optical density (MPOD) and improve visual function including glare in individuals with AMD (Weigert et al. 2011, Liu et al. 2015) and those without underlying ocular disease (Stringham & Hammond 2008, Loughman et al. 2012). Our earlier work had demonstrated that MP levels may be compromised in glaucoma (Igras et al. 2013). This provided a rationale for the exploration of MP in glaucoma in particular, (a) the study of the relationship between MP and glaucoma-related structural parameters and visual function respectively, and (b) to evaluate the effect of MP supplementation on glaucoma structure and function.

The Macular Pigment & Glaucoma Trial (ISRCTN registry number: 56985060) was a placebo-controlled, double-masked study where subjects with a diagnosis of open angle glaucoma (OAG) were randomly assigned to either receive a daily oral MP supplement (10mg L, 2 mg Z & 10mg meso-Z) (Active group) or placebo for a period of 6 months. MPOD at 0.25°, 0.5° & 1° retinal eccentricity were measured using a customised heterochromatic flicker photometry technique at baseline and

at the end of the trial. Glaucoma-related structural parameters were captured using RTVue Fourier-domain OCT. Standard automated perimetry (HVF) 24-2 & 10-2 patterns, mesopic contrast sensitivity with glare (mCSg), photo-stress recovery time (PRT), self-reported visual symptoms and dietary questionnaire were evaluated.

A total of 88 subjects with OAG were recruited into the study. Of this, 83 completed the trial (Active group, n = 43; Placebo group, n = 40). A cross-sectional analysis of the baseline date showed that MPOD was peaked centrally at 0.25° of retinal eccentricity and declined at more peripheral eccentricities. Mean deviation (MD) of HVF 24-2 and 10-2 significantly correlates with MPOD at 0.25° and 0.5° of retinal eccentricities. Those with foveal-involved GCC loss (n = 52) had significantly lower MPOD at 0.25°, 0.5° & 1° of retinal eccentricities, greater glaucoma severity (lower glaucoma-related OCT parameters, worse HVF 24-2 & 10-2 MDs), worse low spatial *f* mCSg (at 1.5cpd & 3cpd) and a prolonged PRT in comparison to those without foveal involvement. Those who reported glare symptoms had a significantly lower MPOD including those with foveal involvement. Those with foveal field loss displayed lower MPOD, overall worse HVF 24-2 and 10-2 MD, and higher PRT. At follow-up, a significant increase in MPOD was observed at 0.5° of retinal eccentricity for those in the Active group with foveal-involved GCC loss. No effect on glaucoma-related OCT parameters and visual function were observed in the Active group at the end of the trial.

In summary, MPOD was found to be reduced with worsening glaucoma severity especially when the fovea was involved. We showed a significant relationship between MP and HVF 10-2 MD, a measure of severity of central VF loss. Furthermore, the inter-relationships observed between MP and mCSg suggest that lower MPOD in the presence of global central field loss as a potential factor in the development of glare symptoms among glaucoma subjects. MPOD augmentation, albeit small, was observed following 6 months of oral dietary MP supplementation. Future studies with a longer duration of MP supplementation are required to better evaluate MPOD response and to investigate the effect on visual function.

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Abbreviations List

Age-related macular degeneration (AMD)

Body mass index (BMI)

Contrast sensitivity (CS)

Cycles per degree (cpd)

Early Treatment Diabetic Retinopathy Study (ETDRS)

Enhanced Macular Map 5 (EMM5)

Frequency (*f*)

Functional Acuity Contrast Test (FACT)

Ganglion cell complex (GCC)

Heterochromatic Flicker Photometry (HFP)

Logarithm of the Minimum Angle of Resolution (LogMAR)

Lutein (L)

Macular pigment (MP)

Macular pigment optical density (MPOD)

Mean deviation (MD)

Mesopic contrast sensitivity under glare condition (mCSg)

Meso-zeaxanthin (meso-Z)

Open angle glaucoma (OAG)

Optical coherence tomography (OCT)

Optic nerve head (ONH)

Primary open angle glaucoma (OAG)

Photo-stress recovery time (PRT)

Retinal ganglion cell (RGC)

Retinal nerve fiber layer (RNFL)

Reactive oxygen species (ROS)

Ultra-violet (UV)

Visual field (VF)

Zeaxanthin (Z)

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Chapter 1: Introduction

1.1 Background

Retinal neurodegenerative disorders such as glaucoma and age-related macular degeneration (AMD), are leading causes of worldwide blindness. With the rapidly growing and ageing population worldwide, there will be an increase in the number of individuals affected by glaucoma and AMD (The Eye Diseases Prevalence Research Group 2004), which poses a significant socio-economic burden. The prevalence of glaucoma is estimated to increase from 60 million in 2010 to 80 million by 2020 (Quigley & Broman 2006). Current treatment modalities for glaucoma are limited to halting disease progression and do not restore lost visual function. It is well recognised that vision impairment from glaucoma is a major contributing factor to falls (Ivers et al. 1998) and motor vehicle collisions (Haymes et al. 2007).

Glaucoma is characterised by a gradual loss of the retinal ganglion cells (RGC) and their axons (Quigley et al. 1989, Quigley HA et al. 1980, Sommer et al. 1977, Sommer et al. 1984). The RGC axons form the retinal nerve fibre layer (RNFL) and collectively these fibres make up the optic nerve. In the human retina, the ganglion cells are most densely located at the macula and about 50% are concentrated within 4.5 mm of the fovea (Curcio & Allen 1990). In the early stage of glaucoma, the patient is asymptomatic. Up to 25% of the RGC axons can be damaged before recordable field loss is detected on visual field testing. The finding of paracentral scotoma on visual field tests during the early stage of glaucoma suggested that the macula may be involved in this disease process (Drance 1969). Zeimer and colleagues first demonstrated reduced macular thickness in glaucoma using the slit-scanning retinal thickness analyser (Zeimer et al. 1998). Studies have utilised the optical coherence tomography (OCT) scan to demonstrate that there is thinning of the ganglion cell complex (GCC) layer in glaucoma (Tan et al. 2009, Hood et al. 2013).

AMD is a condition that affects the macula, the central area of the retina responsible for central vision. In early AMD, yellowish deposits, known as drusen, and alterations in retinal pigmentation can be seen on ophthalmoscopy. These findings are indicative of retinal pigment epithelium cell dysfunction (Zarbin 1998). It is predicted that cases of early AMD will increase approximately 96% from 9.1 million in 2010 to 17.8 million in 2050 (Rein et al. 2009). Late AMD can be classified as geographic atrophy or choroidal neovascularisation, either of which results in photoreceptor cell loss and visual impairment (Zarbin 1998). Geographic atrophy accounts for 25% of cases of severe central visual loss while choroidal neovascularisation accounts for the remaining 75% of cases (Klein et al. 1997).

1.2 Oxidative stress

Oxidative stress plays a crucial role in ageing (Malinin et al. 2011). Glaucoma and AMD are both diseases associated with the ageing eye. The pathogenesis of either condition is multifactorial but there is strong evidence that oxidative stress plays an important role (Izzotti et al. 2000, Beatty et al. 2000). Oxidative stress results in tissue damage when there is an imbalance between the production of reactive oxygen species (ROS) and the capability to scavenge these toxic reactive intermediates through the action of antioxidants. The eye is a unique organ as it is highly aerobic and is constantly exposed to light (ultra-violet (UV) and short-wavelength light), and environmental chemicals such as cigarette smoking, all of which predisposes to the generation of ROS. Other sources of free radicals include ageing and inflammation (Machlin & Bendich 1987).

Oxidative stress plays a role in the pathogenesis of glaucoma and this has been demonstrated to occur at the level of the trabecular meshwork, retina and optic nerve head (Tezel 2006, Cuenca et al. 2014). Insults such as high intraocular pressure (Moreno et al. 2004), light exposure (Osborne et al. 2006, Huang et al. 2014), mitochondrial dysfunction (Abu-Amero et al. 2006) and decreased ocular blood flow (Flammer et al. 2002) can result in the generation of ROS in the retina of the glaucomatous eye that ultimately lead to RGC death.

In the ageing eye, there is a linear reduction in the trabecular meshwork cells (Alvarado et al. 1981) and this is related to the increasing deleterious effects of

oxidative stress (De La Paz & Epstein 1996). It was previously demonstrated that oxidative damage to DNA is higher in the trabecular meshwork cells of humans with glaucoma than in healthy controls (Izzotti et al. 2003) and this is correlated with intraocular pressure and visual field in those with primary open angle glaucoma (POAG) (Sacca` et al. 2005). Tezel et al has shown an increase in oxidatively modified retinal proteins in a rat model of experimental glaucoma (Tezel et al. 2005). Retinal oxidative stress can cause apoptosis of the RGCs (Moreno et al. 2004). At the optic nerve head where the lamina cribrosa cells are located, our group has shown increased intracellular ROS in glaucoma patients when compared to normal controls (McElnea et al. 2011) further implicating the role of oxidative stress. Pseudoexfoliation glaucoma is a form of glaucoma with a more aggressive clinical course compared to the more prevalent POAG. It has been shown that oxidant levels were higher in the serum and aqueous humour of individuals with pseudoexfoliation glaucoma compared to age-matched controls (Koliakos et al. 2008). Light-induced RGC apoptosis via the disruption of mitochondrial function can also contribute to glaucoma (Osborne 2008).

The retina is particularly susceptible to oxidative stress due to its high oxygen consumption, high proportion of polyunsaturated fatty acids and its exposure to visible light (Tezel 2006). There is a growing body of evidence that cumulative oxidative injury leads to the development of AMD (Beatty et al. 2000) and that antioxidant supplementation such as vitamins A (beta-carotene), C and E, zinc and lutein/zeaxanthin supplementation halt disease progression (Age-Related Eye Disease Study Group 2001, Age-Related Eye Disease Study 2 Research Group 2014). There is evidence that dietary macular pigment (MP) supplementation may confer a protective role in the pathogenesis of AMD (Snodderly 1995, Landrum et al. 1997). Oral dietary MP supplementation has also been shown to augment macular pigment optical density (MPOD) and improve visual function in individuals with AMD (Weigert et al. 2011, Liu et al. 2015) and those without underlying ocular disease (Stringham & Hammond 2008, Loughman et al. 2012).

1.3 Macula

The macula is responsible for our central vision and allows us to perceive high acuity, high-resolution vision and colour. It is located at the centre of the retina and

consists of a yellow pigmentation (macula lutea). This region has a central foveal depression that contains the highest density of RGCs (Curcio & Allen 1990). The fovea is about 1-1.5mm wide and is located at the centre of the macula (Figure 1).

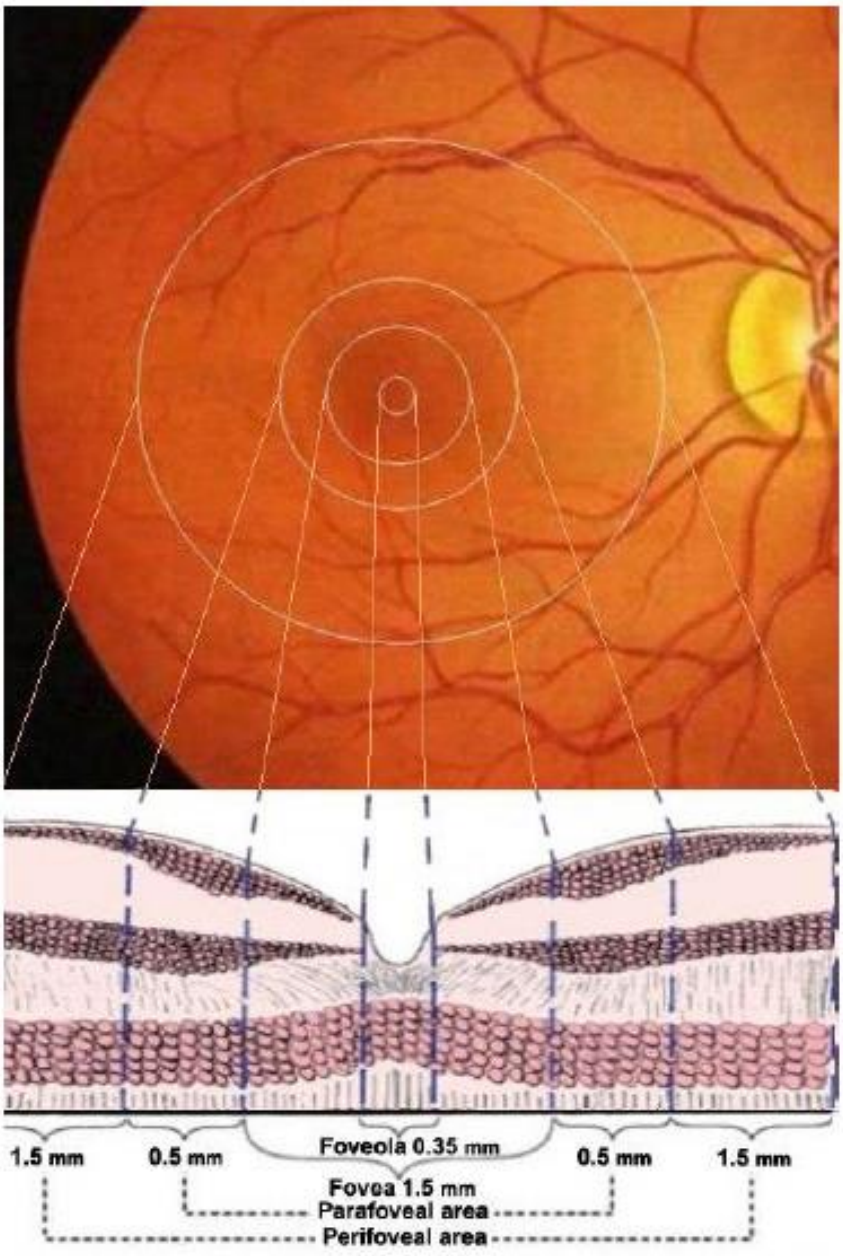


Figure 1. Schematic diagram showing the human retina macula.

1.4 Macular pigment

MP, which is composed of the hydroxycarotenoids lutein (L), zeaxanthin (Z) and meso-zeaxanthin (meso-Z), is most densely concentrated around the fovea

(Trieschmann et al. 2008). MP is predominantly localised in the inner retinal layers of a human macula; fibres of Henle at the fovea and inner nuclear layer at the parafoveal site.

Both L and Z are solely of dietary origin (for example dark green leafy vegetables, coloured fruits and vegetables, eggs), while meso-Z can be synthesised de novo from retinal L (Bone et al. 1993). Numerous studies have shown that the macular carotenoids are effective antioxidants with the ability to scavenge free radicals, quench triplet sensitizers and singlet oxygen, and limit lipid peroxidation (Khachik et al. 1997, Li et al. 2010, Bhosale & Bernstein 2005). Secondly, MPs are believed to play a role in visual performance (Acuity and Visibility hypotheses) (Wooten & Hammond 2002). This is attributed, in part, by MPs' preferential spectral absorption for short-wavelength blue light. Additionally, MPs' capabilities to attenuate longitudinal chromatic aberration and to reduce scattered short-wavelength blue light in the background serve to improve retinal image quality and target contrast respectively.

1.5 Macular pigment optical density

The study of L & Z as potential nutritional therapy is important as their levels can be measured using non-invasive *in vivo* methods unlike most nutrients in tissues. The heterochromatic flicker photometry (HFP) is an example of an *in vivo* method that utilises psychophysical technique to measure the MPOD (Bone & Landrum 2004). It has good test-retest reliability with reproducible MP measurements (Hammond et al. 1997) and demonstrates good agreement with results generated by absorbance spectra using *in vitro* liposome bound L and Z (Bone et al. 1992). A detailed description on HFP is discussed in chapter 2. Other *in vivo* techniques include fundus reflectometry, autofluorescence imaging, resonance Raman spectroscopy. High performance liquid chromatography (Bone et al. 1985) and micro-densitometry (Snodderly et al. 1991) are examples of *ex vivo* techniques for the measurement of MPs.

1.6 Optical Coherence Tomography

OCT is a quick, non-contact, non-invasive diagnostic tool that provides the identification of retinal morphologic details that previously can only be seen

through histopathologic analysis (Figure 2). It was first introduced nearly 3 decades ago (Huang et al. 1991) and it has now become an important imaging device to detect early glaucoma damage before optic disc cupping becomes clinically evident. It also plays a vital role in monitoring for glaucoma progression. In this study, OCT will be used to measure and assess GCC and RNFL thickness at the macula. Further details on the assessment of the macula using the OCT is described in chapter 2.

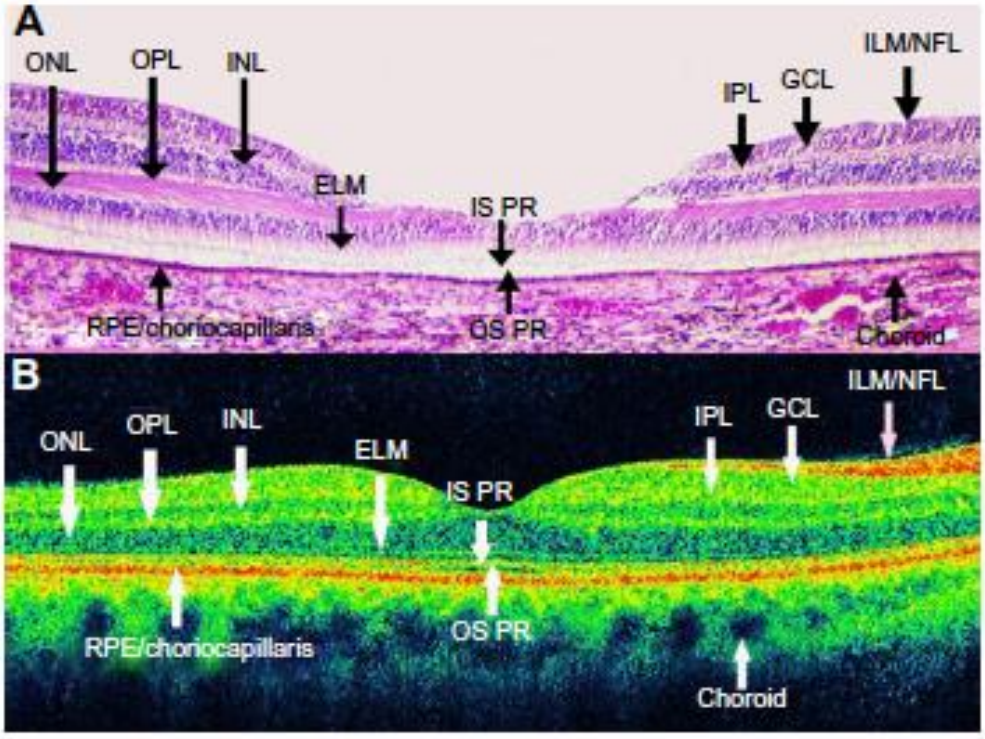


Figure 2. (A) Histological section of a human fovea, (B) OCT of a normal human macula (ILM/NFL, internal limiting membrane, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer of Henle; ONL, outer nuclear later; ELM, external limiting membrane; IS PR, inner segments of photoreceptors (hyoid and ellipsoid segments); OS PR, photoreceptor's outer segments; RPE/choriocapillaris, retinal pigment epithelium and choriocapillaris complex (Modified from Arevalo et al. 2013).

1.7 Glare & Visual Performance

Glare is defined as difficulty seeing in the presence of a bright light and this can be

broadly divided into 2 types namely disability glare and discomfort glare (Mainster & Turner 2012). Disability glare impairs vision when light scatters within the eye. This occurs when there is a bright light on a background of low light levels resulting in the loss of image contrast and difficulty seeing dull objects near the source of glare. For example, walking down a dark street with poorly shielded streetlamps or driving at night while viewing oncoming car headlights**.** In contrast, an individual with discomfort glare will have discomfort without visual impairment for example when looking into the snow at high altitudes with sunlight reflecting off it. Patients with early AMD suffer from disability glare compared with elderly individuals with no AMD (Scilley et al 2002). Disability glare is also a common difficulty encountered by glaucoma patients even in those with mild visual loss (Nelson et al. 1999, Nelson el al 2003, Goldberg et al. 2009).

The aetiology of glare disability in glaucoma is poorly understood and currently, little can be offered to the patient to alleviate this debilitating problem. Recent evidence pertaining to the involvement of the macula in glaucoma (Hood et al. 2013), however, has provided a rationale for the exploration of MP in relation to functional (including glare-related) visual loss associated with glaucoma. It has been shown that healthy individuals with higher MPOD experience less disability glare and demonstrate better photo-stress recovery times (PRT) (Stringham et al. 2011, Stringham & Hammond 2007, Hammond et al. 2013). Furthermore, oral dietary MP supplementation has been shown to increase MPOD, and thereby improve glare disability and PRT in healthy individuals (Stringham & Hammond 2008, Loughman et al. 2012). Dietary MP supplementation has also been shown to increase MPOD and thereby elicit an improvement in visual function including visual acuity (VA), contrast sensitivity (CS) and subjective glare recovery in AMD subjects (Liu et al. 2015).

1.8 Oral dietary MP supplementation

Oral dietary MP supplements are currently widely available over the counter and have been shown to have a safe profile (Connolly et al. 2011). Other studies have provided evidence to support that oral dietary MP supplementation is capable of augmenting MPOD (Nolan et al. 2011, Loughman et al. 2012, Beatty et al. 2013, Trieschmann et al. 2007, Richer et al. 2011, Murray et al. 2013, Aleman et al. 2001,

Duncan et al. 2002, Aleman et al. 2007). Moreover, in AMD where oxidative stress plays a major pathogenic role (as in glaucoma), replenishing the depleted MPs is associated with favourable visual outcomes (Beatty et al. 2013, Richer et al. 2011, Murray et al. 2013, Liu et al. 2015).

Individuals with higher MPOD have less disability glare and shorter photo-stress recovery time (PRT) (Stringham & Hammond 2007). It has also been shown that when healthy subjects were supplemented with 12 mg daily L and Z for 6 months, MPOD significantly increases and disability glare and PRT improve (Scilley et al. 2002). Loughman et al studied the relationship between MPOD and visual performance such as visual acuity (VA), mesopic and photopic contrast sensitivity (CS), glare sensitivity and PRT in 142 young healthy subjects. In contrast to the other studies, it was found that both VA and CS were positively associated with MPOD but glare sensitivity and PRT did not demonstrate this relationship (Loughman et al. 2010). This may be related to a different glare source (tungsten lamp) that was used in this particular study whereby less than 10% of the emitted light was in the spectral absorption for MP and hence explain less beneficial effects on glare disability and PRT. Recently, Stringham et al published further data to support the correlation of MPOD and better visual performance in glare using a system whereby factors such as iris pigmentation and pupil size were also studied. It was shown that individuals with smaller pupil size during glare presentation have higher visual discomfort (Stringham et al. 2011).

A number of clinical trial involving supplementation with high doses of L for extended periods of time have failed to demonstrate any toxicity in ocular and non-ocular tissues for example 20mg of L daily for 6 months (Stringham & Hammond 2007) and 30mg of L daily for 140 days (Stringham & Hammond 2008). The only documented adverse reaction that has been reported as a result of L supplementation in humans has been carotenodermia, which is a harmless and reversible cutaneous hyperpigmentation (Loughman et al. 2010, Stringham et al. 2011). Recently, a clinical trial, carried out at the Waterford Institute of Technology, treated individuals with 5.9mg of L, 10.6mg of meso-Z and 1.2mg of Z for 6 months and demonstrated the safety of oral consumption of macular carotenoids (Connolly et al. 2011). Blood clinical pathology (renal and liver

function, lipid and haematologic profiles, and markers of inflammation) was tested before and after supplementation and was found to be normal except for total cholesterol and LDL, which had a baseline value outside normal reference range (Connolly et al. 2011).

1.9 Study Rationale

At the conception of this study, there was no published data on the relationship between MP and glaucoma. In a pilot study carried out by our research group, it was shown for the first time that MPOD was lower in glaucoma patients compared to normal age-matched controls (Igras et al. 2013). This study evaluated MPOD levels in 40 glaucoma patients to 54 age-matched normal controls using heterochromatic flicker photometry at 0.50 of retinal eccentricity. Glaucoma severity was determined by standard automated perimetry Humphrey field analyser Swedish Interactive Threshold Algorithm (SITA) 24-2 test using the mean deviation (MD) classification; mild (<-6 dB), moderate (>-6 to -12dB) and severe (>-12dB). The pilot study did not find any association between glaucoma severity and MPOD. This finding is likely to be reflected by the fact that it had a small sample size and Humphrey visual field (HVF) 24-2 test was used instead of a central or macular visual field test (HVF 10-2) to correlate with the anatomical location of MP at the macula.

The possible role of oxidative stress in glaucoma, coupled with emerging evidence that (a) the structural integrity of the macula is affected early in glaucoma, and (b) MP levels may be compromised in glaucoma (Igras et al. 2013), were sufficient to prompt the investigation into the relationship between MP and structural aspects of glaucoma in the macular region. Given that MP is largely concentrated to the central 1° of retinal eccentricity of the macula, we were keen to explore the finding of foveal-involved glaucoma using the OCT imaging.

Based on the evidence that lower MPOD may be associated with disability glare (Stringham et al. 2011, Stringham & Hammond 2007, Hammond et al. 2013), this study was designed to evaluate whether MP may relate to functional performance in the glaucomatous eye.

Given the established evidence linking higher MPOD to better ocular health and visual function, and the viable mechanisms by which MPOD may be adversely affected in glaucoma, it is paramount to investigate whether replenishing MPs in the glaucomatous eye is of benefit. The outcome of MP replacement in glaucoma has not been studied before. The Macular Pigment and Glaucoma Trial was designed to answer these research questions.

1.10 Study objectives

- To evaluate the relationship between MPOD and structural parameters of the macula and optic nerve head in glaucomatous eyes.
- To study the relationship between MPOD and glare disability in OAG.
- To investigate the effect of oral dietary MP supplementation in glaucoma.

Chapter 2: Methodology

This section will be outlined under the following sub-headings:

1. Study Design
2. Assessments
3. Statistical Analysis

2.1 Study Design

A study protocol outlining the study design and methodology was presented into the Research Ethics Committee (REC) of the Mater Misericordiae University Hospital, Dublin, and the Dublin Institute of Technology for approval. Following the approval from the REC, the study was registered with the ISRCTN (Registry number: 56985060) prior to recruitment of the first study subject.

This was a placebo-controlled, double-masked study whereby all glaucoma subjects were randomised into the treatment arm (oral dietary MP supplementation – MacuShield®, Macuvision Europe Ltd, Solihull, UK) or placebo arm. Each daily dose of MacuShield® contains 10mg L, 10mg meso-Z and 2mg Z. The duration of intervention was for 6 months. Figure 1 outlines the schematic diagram of Study Design and Procedures.

2.1.1 Recruitment of study subjects

Subjects were recruited from the eye clinic of the participating centres. Information leaflet (Appendix A) was given and written informed consent (Appendix B) obtained from all suitable study subjects.

2.1.2. Subject inclusion criteria

- Diagnosis of open angle glaucoma (OAG)
- Age 18 years and above
- Capacity to give informed consent and adhere to study protocol

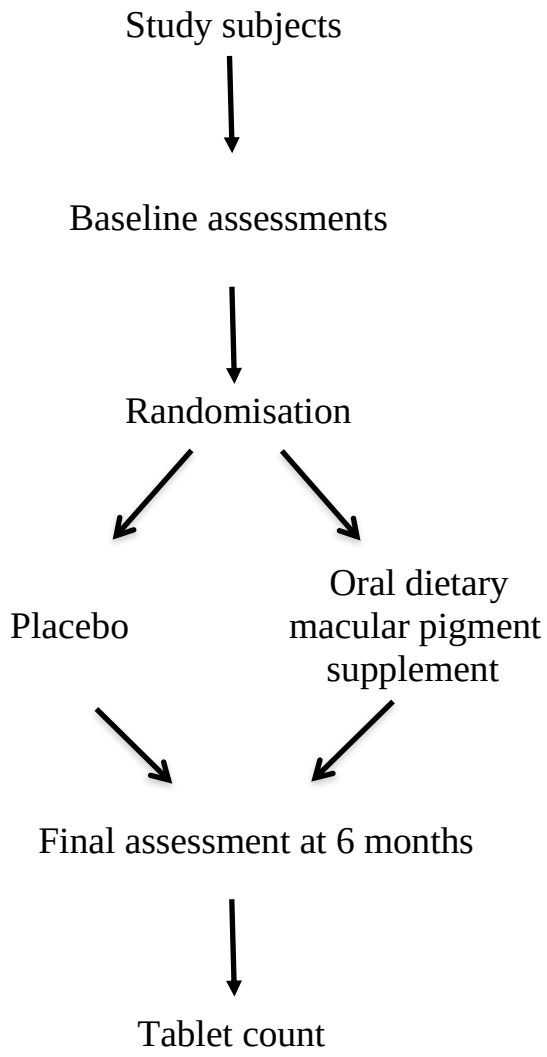


Figure 1. Schematic diagram of Study Design and Procedures.

OAG was defined as the presence of glaucomatous optic disc cupping with associated visual field loss in an eye with a gonioscopically open anterior chamber drainage angle. The different OAG subtypes that were considered for inclusion were (a) primary open-angle glaucoma (POAG) (intraocular pressure > 21 mmHg), (b) normal tension glaucoma (intraocular pressure ≤ 21 mmHg), (c) pseudoexfoliation glaucoma (fibrillar deposits in the anterior segment of the eye), and (d) pigment dispersion glaucoma (peripheral iris trans-illumination and pigment deposition throughout the anterior segment structures of the eye). All subjects had characteristic and reproducible glaucomatous field defects on standard

automated perimetry (Humphrey Visual Field Analyser 24-2 Swedish Interactive Threshold Algorithm Fast program).

2.1.3 Subject exclusion criteria

- Underlying ocular pathology such as AMD, diabetic retinopathy or moderate to significant cataract (using Lens Opacity Classification System III grading)
- Early Treatment Diabetic Retinopathy Study (ETDRS) Logarithm of the Minimum Angle of Resolution (LogMAR) visual acuity > 0.30
- Previous ocular surgery other than for cataract extraction or glaucoma drainage procedure
- Co-existing blue-light filter intraocular lens implant
- History of diabetes mellitus
- Oral dietary MP supplementation within the past 6 months

2.1.4 Data Collection & Study eye

Demographic information including age, gender, type of glaucoma, years diagnosed with glaucoma, history of smoking (current smoker, ex-smoker and never smoked), and body mass index (kg/m^2) and lens status (phakic vs pseudophakic) were recorded for each subject. All subjects also underwent a comprehensive slit-lamp bio-microscopy examination. Only one eye from each research participant will be used for this study. If both eyes met the inclusion criteria, the study eye was determined by random assignment using the software program, Research Randomizer (Version 4.0) (Urbaniak et al. 2013). Study subjects were reviewed at baseline and finally at 6 months. At each visit, vision-related assessments were carried out.

2.1.5 Baseline assessments:

- ❖ Best corrected visual acuity (ETDRS LogMAR Chart)
- ❖ Slit-lamp bio-microscopy examination
- ❖ Macular pigment optical density (MPOD) (using Macular Densitometer)

- Glaucoma-related structure parameters such as ganglion cell complex thickness, macular peripapillary RNFL thickness and central foveal thickness (using SD-OCT, RTVue-100)
- Standard automated perimetry, Humphrey 24-2 and 10-2 tests
- Glare sensitivity and contrast sensitivity (using Optec 6500 device)
- Photo-stress recovery time, PRT (using MDD-2 Macular Adaptometer)
- Dietary questionnaire, LZQ^{TM} (Appendix C)
- Glaucoma Activity Limitation (GAL-9) questionnaire (Appendix D)
- TyPE Spec questionnaire – to assess glare symptoms (Appendix E)

2.1.6 Randomisation and Blinding

All study subjects were each given a unique identification code for example GMP01, GMP02, GMP03, etc. After the initial baseline assessments, study subjects were randomly assigned to receive either placebo or oral dietary MP supplementation. A coding system was applied in the randomisation software to generate the batch number/treatment that the study subjects were to receive. This randomisation was double-blinded, which means neither the participants nor the investigators know the identity of the capsules given. At the end of the clinical trial, the investigators were informed the identity of the study product by the sponsor.

2.1.7 Investigational Product

The trial preparation, which consists of either MacuShield® (active ingredients include 10mg L, 10mg meso-Z and 2mg Z) or placebo, were manufactured by MacuVision Europe Ltd, a company based in Solihull, United Kingdom. The placebo did not contain any active ingredients but sunflower oil only. The placebo looked identical to MacuShield® in its preparation size, colour, smell and taste. MacuShield® is derived from naturally occurring dietary carotenoids and has been shown to be safe for human consumption. Oral dietary MP supplementation will not affect the patients' glaucoma management. The trial preparation was produced according to Good Medical Practice as laid down in the European directives.

Both the placebo and MacuShield® capsules were supplied to us in loose packaging in 2 separate boxes labelled with different batch numbers. At this stage of the trial, only the sponsor was aware of the identity of each batch numbers. In this study, dosage regimen consists of oral consumption of 1 capsule on a daily

basis for 6 months. All study subjects were given a supplement pack (placebo or active intervention) at their baseline visit. Each standard 135ml tablet container can hold 90 capsules (3-month supply). Therefore, each study subject received 2 tablet containers. There were 14 capsules in excess of the 6 months intervention in each supplement pack to ensure that each subject had enough supply until their final follow-up visit. Each study subject was instructed to take one capsule daily with food (co-consumption with fat to encourage carotenoid uptake into the serum) for a period of 6 months. Each container was labelled to include information such as batch number, expiry date and directions for taking the capsule. Other information such as study subject's details and date of commencement were added to the tablet container at the time of enrolment into the clinical trial. Products had a shelf-life of 24 months and were stored in a cool, dry place and away from direct light.

2.1.8 Monitoring of Adverse Effects & Compliance

Study subjects were contacted either by a phone call or a text message during the course of the study to identify any possible adverse effects of the dietary MP supplementation and to ensure compliance. They were reminded on a frequent basis to take their capsules. Study subjects' general practitioners were informed by letter of the enrolment of their patients into the study.

2.1.9 Discontinuation criteria

Study subjects were encouraged to adhere to the study protocol. However, where a study subject decides to discontinue from the study prematurely, he/she will be required to return any unconsumed capsules. If the reason for discontinuation is not due to an adverse event or adverse reaction, we will not be informing the participant the nature of the treatment until the end of the research study so that the study will not be jeopardised. MacuShield® has been widely studied and used in other clinical trials with proven safety record.

2.1.10 Breaking codes

Each study subject was given a randomisation code such as GMP01 for the first subject, GMP02 for the second subject and so on. When a study subject withdraws prematurely from the study, whether voluntarily or due to inter-current illness, their identification code was not reassigned to another study subject.

2.1.11 Quality Control and Quality Assurance

The Study Protocol contains detailed and clear instructions for carrying out the study procedures. The site for the study was equipped with all the necessary instruments. A qualified ophthalmologist (WFS) verified the eligibility of the subjects at the screening visit before randomisation in the study. For the testing of MPOD, glare sensitivity, contrast sensitivity (CS), PRT and visual field testing, the lighting of the room where these tests were carried out was controlled and consistent for all study subjects.

2.1.12 Data Handling and Record Keeping

All study subjects were given an identification code to maintain anonymity. Only the chief investigator, investigator and authorised healthcare professional had access to the study data. At the end of the study, all data was kept and locked away at the Institute of Ophthalmology, No.60, Eccles Street for 5 years.

2.1.13 Financing and Insurance

The Howard Foundation was the sponsor for this study and indemnity cover was in place.

2.2 Assessments

The assessments for each study subject were recorded in the Case Report Form (Appendix F).

2.2.1 Measurement of Visual Acuity

The ETDRS LogMAR test chart used was computer-generated (Version 1.1; KyberVision, Quebec, Canada) and visual acuity was tested at a viewing distance of 4m, using a Sloan ETDRS letterset, while wearing current distance spectacle prescription where required.

2.2.2 Slit-lamp bio-microscopy

All study subjects underwent slit-lamp bio-microscopy assessment to confirm the suitability of inclusion into the study. The Lens Opacity Classification System III (LOCS III) (Chylack et al, 1993) grades cataracts by type (cortical, C1-5 and posterior subcapsular, P1-5) and nuclear appearance (nuclear colour, NC1-6 and nuclear opalescence, NO1-6). The LOCS III standards were used to categorise eyes according to lens appearance as either "mild cataract" (grades C1-2, P1-2, NC1-2 and/or NO1-2) or "no cataract" (C0, P0, NC0 and/or NO0). Exclusion criteria comprised of any moderate-to-significant cataract (LOCS III grade C3-5, P3-5, NC3-5 or NO3-5), any ocular disease such as AMD, glaucoma drainage procedure, presence of a blue-filter intraocular lens.

2.2.3 Measurement of MPOD

Heterochromatic Flicker Photometry (HFP) is a psychophysical technique for measuring MPOD that has been validated against the absorption spectrum of MP in vitro (Bone et al. 1992). It has also been shown to provide reliable data when compared to other methods of measuring MPOD such as fundus reflectometry and fluorophotometry (Wooten et al. 1999; Howells et al. 2011). As the HFP technique is not dependent on or affected by regional variation in sensitivity at the central and peripheral retinal loci (either normal or disease induced variations) (Wooten et al. 1999), the HFP technique remains appropriate for MPOD measurement among glaucoma subjects. In this study, MPOD was measured using the Macular

Densitometer (Macular Metrics, Rehoboth, Massachusetts, USA), a device originally described by Wooten and colleagues (Wooten et al. 1999).

Subjects viewed a stimulus consisting of a square wave alternating blue (460 nm) and green (550 nm) flickering LED light source. Subjects were required to make iso-luminance matches, which were perceived as the point of cessation of flicker (null flicker). All subjects were shown a training video prior to being tested with the densitometer, and they were afforded the opportunity to practice the technique. A customised HFP approach was used (cHFP), a refined technique that allowed the investigator to predetermine the optimal flicker frequency for each subject, hence enabling a more discrete end point for the test and minimizing the variance between readings (Stringham et al. 2008). In this study, the spatial profile of MP at 0.25°, 0.5° and 1° of retinal eccentricity was measured under conditions of dimmed light at a viewing distance of 18.5 inches (47 cm) with distance optical correction where required. Five readings were obtained to produce a mean MPOD value at each retinal eccentricity, which was deemed reliable and acceptable for inclusion in the study when the standard deviation of measures was 0.05 or less for each test point.

2.2.4 Fourier-domain Optical Coherence Tomography

All study subjects were scanned using the RTVue FD-OCT system, which has a scan speed of 26,000 A-scans/second and 5 μm axial resolution. The protocols used included (a) the RNFL 3.45 (retinal nerve fibre layer thickness at 3.45 mm diameter around optic disc) scan, (b) the ONH (optic nerve head) scan, (c) the EMM5 (Enhanced Macular Map 5) scan, and (d) the GCC (ganglion cell complex) scan.

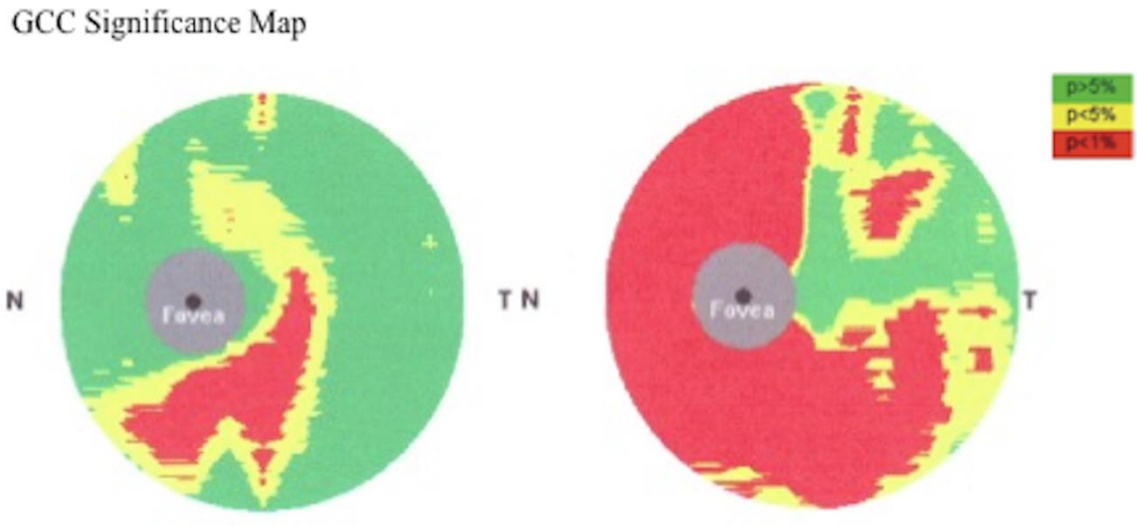
The RNFL 3.45 protocol acquired four 3.45 mm diameter circular scans centred on the optic disc and provided the average RNFL thickness at the temporal, superior, nasal and inferior quadrants (peripapillary RNFL). The ONH scan pattern consisted of 12 radial line scans of 3.4 mm length and 13 concentric rings (1.3 – 4.9 mm diameter) centred on the optic disc, providing a detailed optic disc/rim/cup analysis. The EMM5 scan pattern captured 29,438 data points across the macula over 0.90 seconds and provided information such as the full retinal and inner retinal thickness at the fovea, parafovea and perifovea regions which are 1 mm (~ 3.3°), 3 mm and 5 mm in diameter respectively.

MP is localised within the foveal and parafoveal regions and therefore these regions were a priority focus in this study. The EMM5 scan image was excluded from analysis in the presence of a poor-quality image (signal strength < 40), segmentation error, off-centre fixation and/or out of range image (Ho et al. 2009). The GCC scan pattern specifically provided a comprehensive ganglion cell assessment by measuring the thickness of the nerve fibre layer (ganglion cell axons), ganglion cell layer (ganglion cell body) and inner plexiform layer (ganglion cell dendrites) at the macula. The macular region was scanned over 0.58 seconds and consists of 1 horizontal line and 15 vertical lines at 0.5 mm intervals. The scan was centred 1 mm temporal to the fovea to produce a 7 mm square region. The RTVue software cropped the outer 0.5 mm region to provide a 6 mm GCC thickness map that was used for the calculation of the GCC Significance Map. This 6 mm x 6 mm map spans an area which is equivalent to the central 20° on a visual field map. If the signal strength index was poor at < 40 or if the scan was misaligned, the images were not used.

2.2.4.1 GCC Subgroups

In the GCC color-coded Significance Map, the central, grey foveal area (1.5 mm / 5° diameter) covers the region where the GCC thickness is too thin to be evaluated. In a normal eye, the MP is most densely located within the diameter of this grey area. Beyond approximately 7° eccentricity, retinal MP becomes optically undetectable (Bone et al. 1998). Glaucoma subjects were divided into 2 subgroups based on their GCC Significance Map to better represent the GCC thickness in relation to the anatomical location of the MP. All diagnostic parameters in the GCC Significance Map were colour coded to indicate whether there was any significant GCC thickness reduction; $P < 1\%$ was coded as red, $P < 5\%$ was yellow and $P \geq 5\%$ was green. If the perimeter of the grey foveal area was green in colour, it was classified as 'Fovea-Not-Involved' (Figure 2A). If the red scale encroached up to the grey area, it was classified as 'Fovea-Involved' (Figure 2B). A general linear model analysis was used to determine the effect of foveal involvement ('Fovea-Not-involved' vs 'Fovea-Involved' subgroups) and age, years diagnosed with

glaucoma, smoking status and body mass index (BMI) on the dependent variable, MPOD.



A. B.

Figure 2. GCC Significance Maps. (A) 'Fovea-Not-involved' subgroup, (B) 'Fovea-Involved' subgroup

2.2.5 Standard Automated Perimetry

The Swedish Interactive Threshold Algorithm Standard 24-2 and 10-2 programmes available in the Humphrey Visual Field Analyser (HVFA II, Carl Zeiss Meditec, Jena, Germany) were used to assess differential light sensitivity as a measure of global glaucomatous visual field (VF) loss and residual visual function in the central retina (within 10° of fixation) respectively. Mean deviation (MD) was used to define the severity of glaucoma (extent of functional loss) and was included in data analysis. Unreliable visual field (VF) plots, as determined by a fixation loss of > 33% and/or false positive/negative rates > 20%, were excluded from analysis.

In the pilot study carried out by our research group (Igras et al. 2013), a SITA fast 24-2 programme was used and we have chosen the same test for better comparison of the outcomes between the 2 studies. However, we have chosen a SITA standard 10-2 programme to capture a central visual field loss.

2.2.5.1 VF Subgroups

MP is most concentrated within the central 1° of the fovea. Although the 24-2 test pattern is useful in providing a global scale of glaucomatous VF loss, it uses a 6° x 6° grid which only has 4 points within the central 4.2° radius of fixation. In contrast, the 10-2 test pattern uses a 1° x 1° grid, and therefore is more precise in delineating foveal VF loss, if any. The central 4 points in the 10-2 test pattern corresponds to within 1.4° of the fovea centre.

In an attempt to better understand foveal visual loss in relation to the anatomical location of MP, we categorised the 10-2 test pattern into (a) No foveal visual loss, and (b) Foveal visual loss (Figure 3). We applied the Hodapp-Parrish-Anderson criteria, a system that is generally used for the 24-2 test pattern, to the 10-2 pattern deviation plot in this instance to allow us to objectively determine the involvement of foveal visual loss. If there were a cluster of 3 or more non-edge points in a location typical for glaucoma, all of which were depressed on the pattern deviation plot at $P < 5\%$ level and 1 of which is depressed at $P < 1\%$ level, and that this involves any of the central 4 points, it was categorised as 'Foveal visual loss'. For the study of MP and central visual function, we will focus on the MD of the 10-2 test for the purpose of statistical analysis.

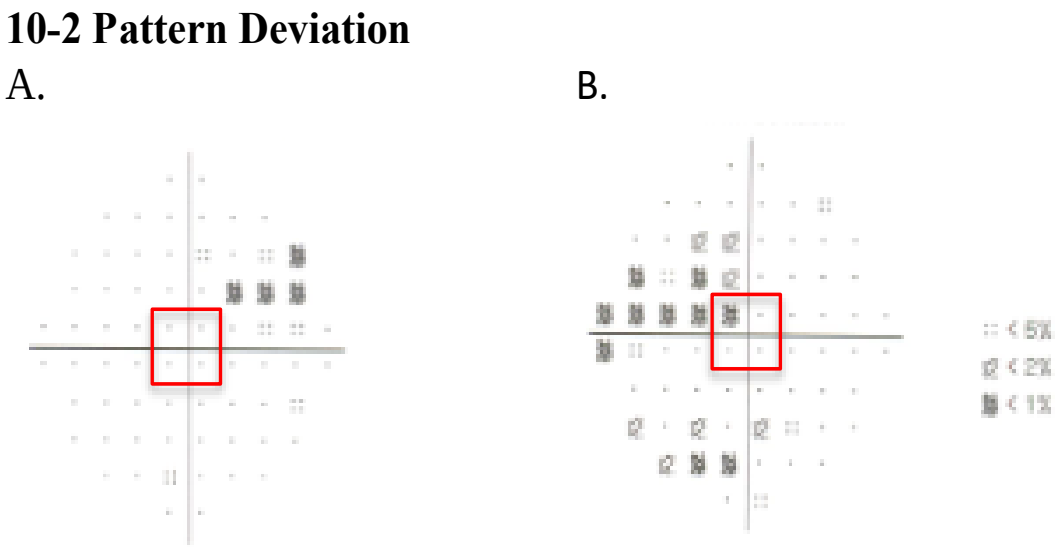


Figure 3. The red box outlines the central 4 test points (-1°, 1°) that are within 1.4° of the foveal centre of the Humphrey visual field 10-2 pattern deviation. (A) No foveal visual loss. (B) Foveal visual loss.

2.2.6 Contrast Sensitivity with Glare

The Optec® 6500 device (Stereo Optical Co., Inc., Chicago, IL) was used to objectively measure mesopic (3.0 cd/m²) contrast sensitivity (CS) under glare conditions (mCSg) in all subjects. All testing was carried out on natural size pupils under a constant ambient light. The Optec® 6500 is based on the Functional Acuity Contrast Test (FACT) (Appendix G), which consists of a series of sine-wave grating charts and is designed to test sensitivity across five spatial frequencies (*f)* (1.5, 3, 6, 12 and 18 cycles per degree (cpd)) and 9 levels of contrast in 0.15 log CS decrements. The in-built glare source consists of 12 white LEDs that are arranged circumferentially to the grating charts. The glare source was pre-set to provide a medium intensity luminance of 42 Lux (Loughman et al. 2010; Nolan et al. 2010). With distance glasses, if necessary, each subject was required to identify the orientation of the grating (right, up or left) without guessing, starting from the lowest spatial *f* and the highest contrast. The last correct response for each spatial *f* was plotted on a CS curve. When the subject was not able to see the highest contrast setting at any spatial *f,* the result was documented as half the lowest CS value for that spatial *f*.

2.2.7 Photo-stress Recovery

PRT was measured using the portable, handheld MDD-2 Macular Adaptometer™ (Health Research Sciences, LLC, FL) device, which has previously been used in other studies (Newsome et al. 2009; Loughman et al. 2014). It consists of a xenon flash source capable of producing a 200 μsec duration flash that is subsequently filtered to block infrared and ultraviolet light before reaching the viewing eye. Each subject was instructed to hold the device up close to the study eye and to look through the 12-mm diameter-viewing aperture, which contained a +8 Diopter-focusing lens. At the viewing aperture, the xenon flash reaches a peak irradiance of 4.5 W/cm^2 and the stimulus is of 0.41-radian angular subtense. At the start of the test, it was ascertained that each subject was able to recognise a LED display number. The test was abandoned if it was not identifiable to the subject.

Afterwards, a push-button was pressed to produce the xenon flash. The subject was required to call-out a new numerical stimulus (single digit) that appeared simultaneous to the flash. This prompted the examiner to press a button to end the test. The time taken for the subject to correctly identify the stimulus was recorded as the PRT. If the response was incorrect, the test was repeated approximately 20 minutes later. An upper PRT limit of 60 sec was incorporated into the device, which automatically re-sets for a new test thereafter. In the event that the subject did not respond within 60 sec, the test was abandoned.

2.2.8 Glare Symptoms

In the evaluation of subjective glare symptoms, the subjects were asked the following question: "Do you suffer from glare?" Examples of different scenarios (glare from a poorly shielded streetlamp in a dark street; glare from an oncoming car headlight whilst driving at night; glare from low-lying sun in the winter) were used to provide context and assist them in answering this question better.

2.2.9 Dietary Intake of Lutein and Zeaxanthin

A self-administered, semi-quantitative food frequency questionnaire known as the Lutein Zeaxanthin Questionnaire (LZQ), was used to assess the dietary intake of lutein and zeaxanthin (Carotenoid & Health Laboratory, Jean Mayer USDA Human Nutrition Centre on Aging, Tufts University, USA) (LZQ 2009) as has been used in other MPOD studies (Scanlon et al. 2015). This information allowed us to control for any MPOD disparity that may arise from different dietary habits among the glaucoma subjects.

2.2.10 Self-reported Visual Function

All study subjects filled out 2 vision-related quality of life questionnaires. The Cataract TyPE Spec questionnaire was used to assess visual function and outcomes following cataract extraction and has been found to be reliable and valid (Lawrence et al. 1999). However, due to limitations associated with the use of a classic test theory, Gothwal and colleagues applied Rasch analysis to generate a modified 11-item Cataract TyPE Spec questionnaire (subscale: glare, near vision, daytime driving, distance vision) (Gothwal et al. 2009). The latter showed good validity in measuring visual function in particular the overall scale and in 2 subscales: near

vision and glare (Gothwal et al. 2009). Though this questionnaire was developed with the intention to assess visual function in cataract surgery, given the good reliability and validity of the Rasch-scaled scores, we used it to evaluate self-reported visual function in our subjects with glaucoma. In the glare subscale, subjects were asked if they were hindered, limited or disabled by glare during their usual activities, reading shiny paper (such as a magazine), driving towards the sun or oncoming headlights and when walking outside on a sunny day. For the purpose of statistical analysis, we focused on the overall and glare scores.

The GAL-9 questionnaire has been validated using the Rasch model (Khadka et al. 2011) and therefore used in this study to assess glaucoma-specific visual functioning among our subjects. The GAL-9 consists of 9 items instead of 15 items from the GQL-15 (Nelson et al. 2003) and has been shown to have more superior psychometric properties.

2.3 Statistical Analysis

We performed sample size calculation based on a priori assumption of $\alpha = 0.05$ (type I error) and $\beta = 0.20$ (type II error). A study of 86 subjects has the statistical power of 80% (β-1) to detect a 6% difference in MPOD between subjects receiving the oral dietary MP supplementation (active group) and those receiving placebo when the standard deviation is determined to be 0.1. With the assumption of a maximum dropout rate of 10%, at least 47 subjects in the active group and 47 subjects in the placebo group were required to complete the trial.

Results were analysed using the statistical software package SPSS (Version 22.0; IBM Corp, New York, USA). The Kolmogorov-Smirnov test (if $n > 50$) or Shapiro-Wilk W test (if $n \leq 50$) was used to assess normality of all variables prior to statistical analysis. All data are presented as mean ± SD for normally distributed data, and as median (range) for non-normal distributions. Parametric tests (Pearson correlation coefficient, independent t-test) or non-parametric tests (Spearman Rho test, Mann-Whitney U-test) were carried out where appropriate. Most of the quantitative variables that were investigated exhibited a normal distribution except for age, duration of glaucoma diagnosis, body mass index, peripapillary RNFL thickness, superior macular RNFL thickness, cup-disc area ratio and optic disc rim area. Various statistical analyses including an independent t-test, Mann-Whitney U-test, ANOVA with post hoc tests, Pearson or Spearman correlation coefficients were computed where appropriate.

2.3.1 Analysis of glaucoma-structure parameters

GCC thickness subgroup analyses ('Fovea-Not-Involved' and 'Fovea-Involved') were performed to explore relationships, if any, with other glaucoma-related OCT parameters and MPOD. A conservative 1% level of significance was adopted throughout the analysis to offset the risk of a Type I error given the multiple comparisons and correlations conducted, even though the majority of statistical tests were unrelated. GCC thickness subgroup analyses ("Fovea-Not-Involved" and "Fovea-Involved") were performed to explore relationships, if any, with other glaucoma-related OCT parameters and MPOD.

2.3.2 Analysis of MPOD and visual function parameters

As the presence of a cataract can influence mCSg results, we controlled for this factor in our analysis. Linear regression analysis was also performed; R^2 value signifies the percentage of variability in the dependent variable that can be explained by the model with the independent variable (MPOD). When comparing categoric data, a Chi-square test was used. A 5% statistical significance level of was adopted throughout the analysis.

2.3.3 Analysis of outcome post-intervention (Oral dietary MP supplement vs Placebo)

Comparisons of variables between the active and placebo groups were conducted using statistical analyses such as independent *t*-tests, paired *t*-tests, Wilcoxon Signed Ranks tests, Chi-square tests and Mann-Whitney tests, as appropriate. Additionally, baseline MPOD was divided into tertile subgroups for further analysis. MPOD response was assessed by performing a paired *t*-test on the means of MPOD at baseline ($MPOD_{baseline}$) and at follow-up ($MPOD_{follow\text{-}up}$) for each intervention group. MPOD response was also calculated as the change in MPOD (ΔMPOD), which was calculated by subtracting $MPOD_{follow\text{-}up}$ from $MPOD_{baseline}$. A negative or zero ΔMPOD after active intervention was regarded as a lack of response at that particular retinal eccentricity. A non-responder was defined as an individual with a complete lack of MPOD response at all retinal eccentricities. Baseline MPOD was also divided into tertile subgroups for further analysis.

Chapter 3: Results

The Macular Pigment and Glaucoma Trial was a prospective, placebo-controlled, double-masked randomised study (ISRCTN registry number: 56985060). A total of 88 subjects with a diagnosis of OAG were recruited into this study.

3.1 Demographics

The demographics for all 88 glaucoma subjects are summarised in Table 1. The right eye was selected for inclusion in 51 subjects (58%), and the left eye for the remaining 37 subjects (42%).

Table 1. Subject Characteristics (N = 88)

Characteristic	Result
Age, Median (Range) (y)	67 (36 - 84)
Sex, n (%)	
Male	48 (54.5)
Female	40 (45.5)
Study eye LogMAR, Mean ± SD	0.03 ± 0.10
Type of glaucoma, n (%)	
POAG	45 (51.1)
NTG	31 (35.2)
PXG	9 (10.2)
PDG	3 (3.4)
Duration of glaucoma, Median (Range) (y)	6 (0.5 - 32)
Smoking habits, n (%)	
Never smoked	42 (47.7)
Ex-smoker	36 (40.9)
Current smoker	10 (11.4)
Body mass index, Median (Range) (kg/m2)	25.5 (18.5 - 42)
Lutein intake, Median (Range) (mg/dL)	0.7 (0 – 13.2)
Zeaxanthin intake, Median (Range) (mg/dL)	0.1 (0 – 1.2)

IQR = Interquartile range; SD = standard deviation; POAG = primary open-angle glaucoma; NTG = normal tension glaucoma; PXG = pseudoexfoliation glaucoma; PDG = pigment dispersion glaucoma

3.2 Baseline MPOD

Valid MPOD data was obtained for 69 subjects at 0.25° retinal eccentricity (mean ± SD = 0.23 ± 0.14), 81 subjects at 0.5° eccentricity (0.19 ± 0.12) and 59 subjects at 1° eccentricity (0.12 ± 0.09). Only 53 subjects had complete MPOD data at all 3 retinal eccentricities. Of these subjects, 46 displayed a typical MP spatial profile with a central peak at 0.25° and relative decline at more peripheral eccentricities, while the remaining 7 subjects exhibited atypical MP spatial profiles which peaked at 0.5° of retinal eccentricity.

The results will be presented in 3 sections:

3.3 Analysis of MPOD and glaucoma-structure parameters

3.4 Analysis of MPOD and visual function parameters

3.5 Trial outcome (Oral dietary MP supplement vs Placebo)

3.3 Analysis of MPOD and glaucoma-structure parameters

A conservative 1% level of significance was adopted throughout the analysis of MPOD and glaucoma-structure parameters to offset the risk of a Type I error given the multiple comparisons and correlations conducted, even though the majority of statistical tests were unrelated. There was no statistically significant correlation observed between MPOD and age (r = -0.21, P = 0.08 at 0.25° eccentricity; r = -0.23, P = 0.04 at 0.5° eccentricity; r = -0.17, P = 0.19 at 1° eccentricity), or length of time since diagnosed with glaucoma (r = -0.24, P = 0.05 at 0.25° eccentricity; r = -0.09, P = 0.44 at 0.5° eccentricity; r = -0.11, P = 0.40 at 1° eccentricity), although there was a trend towards significance (P = 0.01 – 0.05) in both instances at some eccentricities.

Similarly, no statistically significant correlation was observed between MPOD and body mass index at any eccentricity (r = -0.13, P = 0.28 at 0.25° eccentricity; r = 0.01, P = 0.93 at 0.5° eccentricity; r = -0.08, P = 0.57 at 1° eccentricity). Independent t-test analysis showed no gender-based difference in MPOD (P > 0.01 for all). One-way ANOVA revealed no statistically significant effect of smoking habits (current smoker, ex-smoker and never smoked) on MPOD. While there was

no statistically significant correlation between MPOD and OCT structural parameters at the strict 0.01 threshold, borderline significant (P = 0.01 – 0.05) correlations were observed between MPOD and a number of topographical measures including inferior peripapillary RNFL thickness, inferior GCC thickness, inner retinal thickness at the fovea, cup-disc area ratio and optic disc rim area (see Table 2).

Table 2. Relationship between MPOD and OCT parameters

	MPOD 0.25°		MPOD 0.5°		MPOD 1°	
	R*	*P*	R*	*P*	R*	*P*
Peripapillary RNFL thickness						
Average	0.15†	0.23	0.16†	0.18	0.07†	0.60
Superior	0.13†	0.31	0.12†	0.30	0.02†	0.89
Inferior	0.21†	0.09	0.23†	0.05	0.15†	0.28
Macular RNFL thickness						
Average	0.11	0.36	0.18	0.12	0.12	0.36
Superior	0.05†	0.68	0.11†	0.35	0.03†	0.83
Inferior	0.18	0.14	0.21	0.07	0.16	0.22
GCC thickness						
Average	0.15	0.24	0.16	0.15	0.22	0.09
Superior	0.06	0.63	0.11	0.33	0.13	0.33
Inferior	0.21	0.09	0.18	0.11	0.27	0.04
Foveal thickness						
Full retina	0.09	0.52	0.18	0.15	0.18	0.22
Inner retina	0.19	0.17	0.29	0.02	0.23	0.11
Parafoveal thickness						
Full retina	0.03	0.85	0.09	0.46	0.07	0.66
Inner retina	0.16	0.24	0.20	0.11	0.14	0.35
Cup-disc-area ratio	-0.27†	0.03	-0.25†	0.03	-0.28†	0.04
Optic disc rim area	0.28†	0.02	0.22†	0.06	0.22†	0.10

MPOD = macular pigment optical density; OCT = optical coherence tomography; RNFL = retinal nerve fibre layer; GCC = ganglion cell complex; *P* = significance (2-tailed)

*Pearson coefficient correlation (unless indicated otherwise)

†Spearman coefficient correlation

3.3.1 OCT parameters and GCC subgroups

Of the 88 GCC scans completed, 3 demonstrated poor signal strength and were therefore eliminated from the analysis. In total, there were 33 subjects (38.8%) in the 'Fovea-Not-Involved' subgroup and 52 subjects (61.2%) in the 'Fovea-Involved' subgroup.

Figure 1 shows the MPOD spatial profile for all glaucoma subjects, and demonstrates the clear difference in MPOD between the 'Fovea-Not-Involved' and 'Fovea-Involved' subgroups. Table 3 shows the different types of glaucoma within the GCC subgroups and other characteristics. The 2 most common types of glaucoma within the 'Fovea-Involved' group were POAG (n = 22) and normal tension glaucoma (NTG) (n = 23). Between these subgroups, no significant differences in MPOD or any OCT parameters were observed ($P > 0.01$ for all).

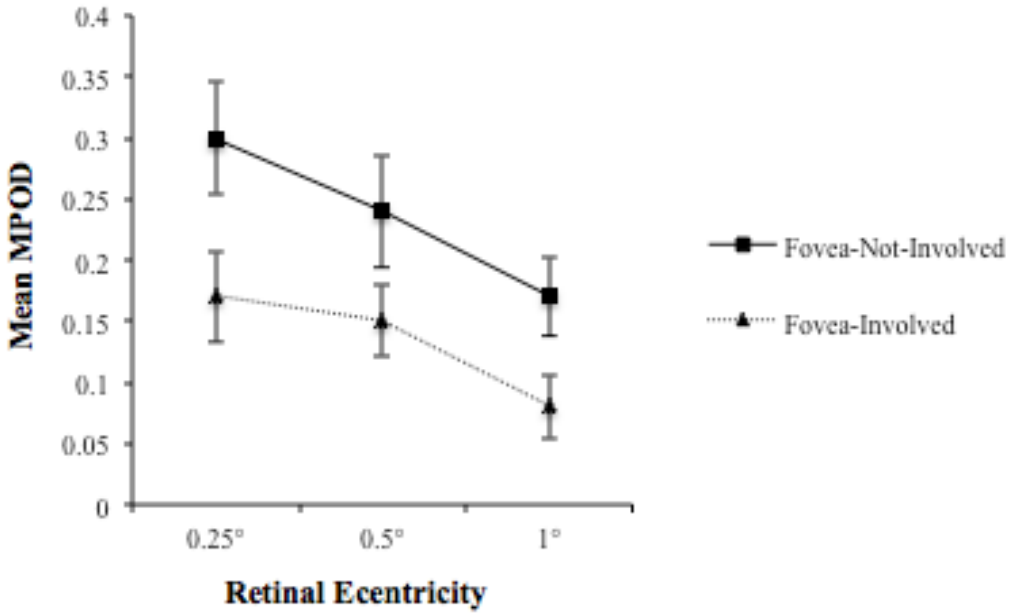


Figure 1. Mean macular pigment optical density (MPOD) spatial profile for glaucoma subjects.

Table 3. Types of Glaucoma and Characteristics

	Fovea-Not-Involved (N = 33)			Fovea-Involved (N = 52)		
Types of Glaucoma	n	HVF 24-2 MD (dB)*	Average GCC (µm), Mean	n	HVF 24-2 MD (dB)*	Average GCC (µm), Mean
POAG	22 (50%)	-5.72†	77.31	22 (50%)	-10.41†	70.11
NTG	7 (23%)	-5.30	83.85	23 (77%)	-8.79	71.47
PXG	2 (22%)	-9.68	75.90	7 (78%)	-16.24	68.66
PDG	2 (100%)	-4.50	74.83	0	N/A	N/A

POAG = primary open-angle glaucoma; NTG = normal tension glaucoma; PXG = pseudoexfoliation glaucoma; PDG = pigment dispersion glaucoma; HVF = Humphrey visual field; MD = mean deviation; GCC = ganglion cell complex; N/A = not applicable

*Mean (unless indicated otherwise)

†Median

Within GCC subgroups no statistically significant correlation was observed between MPOD (at 0.25°, 0.5° and 1° retinal eccentricities respectively) and age, length of time since diagnosed with glaucoma and body mass index ($P > 0.01$ for all). There was no significant gender-based difference in MPOD at all retinal eccentrities within the GCC subgroups (Independent t-test, $P > 0.01$ for all). Furthermore, one-way ANOVA did not reveal any significant effect of smoking habits on MPOD in either subgroup ($P > 0.01$ for each).

A statistically significant difference in MPOD between the 'Fovea-Not-Involved' and 'Fovea-Involved' GCC subgroups was observed at all retinal eccentricities ($P < 0.001$ for all - Table 4). Furthermore, the 'Fovea-Involved' group exhibited more severe glaucoma as determined by thinner RNFL and GCC, larger cup-disc area ratio and a smaller optic disc rim area ($P < 0.01$ for each). MPOD did not, however, correlate significantly with OCT structural parameters for either the 'Fovea-Not-Involved' or 'Fovea-Involved' GCC subgroup ($P > 0.01$ for all). A general linear model analysis confirmed a significant effect of foveal involvement ('Fovea-Not-involved' vs 'Fovea-Involved' subgroups) on MPOD at all retinal eccentricities ($P \leq 0.001$), but no effect of age, years diagnosed with glaucoma, smoking status and body mass index on MPOD ($P = 0.12$ to 0.72 at 0.25° retinal eccentricity, $P = 0.34$ to 0.99 at 0.5° retinal eccentricity, $P = 0.49$ to 0.88 at 1° retinal eccentricity).

Table 4. Comparison between GCC Subgroups

	Fovea-Not-Involved	Fovea-Involved	*P*
MPOD, Mean ± SD (degree)			
0.25	0.30 ± 0.12	0.17 ± 0.11	**<0.001***
0.50	0.24 ± 0.12	0.15 ± 0.10	**<0.001***
1.00	0.17 ± 0.07	0.08 ± 0.08	**<0.001***
RNFL thickness, Mean ± SD (μm)			
Peripapillary RNFL	77.58 ± 9.80	69.33 ± 8.34	**<0.001***
Macular RNFL	79.66 ± 12.55	69.70 ± 10.65	**<0.001***
GCC thickness, Mean ± SD (μm)	78.46 ± 7.71	70.38 ± 9.34	**<0.001***
Foveal thickness, Mean ± SD (μm)			
Full fovea	270.00 ± 19.55	253.02 ± 23.08	**0.002***
Inner fovea	86.54 ± 11.93	76.76 ± 14.84	**0.005***
Outer fovea	183.46 ± 11.19	176.26 ± 11.91	0.013*
Parafoveal thickness, Mean ± SD (μm)			
Full parafovea	306.03 ± 20.06	285.45 ± 18.99	**<0.001***
Inner parafovea	119.06 ± 12.25	106.20 ± 9.42	**<0.001***
Cup-disc area ratio, Median (Range)	0.76 (0.36 to 0.97)	0.88 (0.55 to 0.98)	**<0.001†**
Rim area, Median (Range) (mm2)	0.43 (0.06 to 0.86)	0.22 (0.05 to 0.71)	**<0.001†**
HVF 24-2 MD, Median (Range) (dB)	-5.46 (-1.45 to -26.46)	-10.54 (-0.09 to -31.06)	0.018†

GCC = ganglion cell complex; MPOD = macular pigment optical density; SD = standard deviation; RNFL = retinal nerve fibre layer; HVF = Humphrey visual field; MD = mean deviation; *P* = Significance (2- tailed)

*Independent T test

†Mann-Whitney U test

Median lutein intake in the 'Fovea-Not-Involved' group was 0.7 mg/dL (range 0 – 13.2), while that in the 'Fovea-Involved' group was 0.8 mg/dL (range 0 – 13.1). These respective intakes were not statistically significantly different (Mann-Whitney U-test, $P = 0.95$). Similarly, we did not find any significant difference in zeaxanthin intake between the 'Fovea-Not-Involved' group (median = 0.1 mg/dL, range 0 – 0.5) and the 'Fovea-Involved' group (median = 0.1 mg/ dL, range 0 – 1.2) (Mann-Whitney U-test, $P = 0.44$).

3.4 Analysis of MPOD and visual function parameters

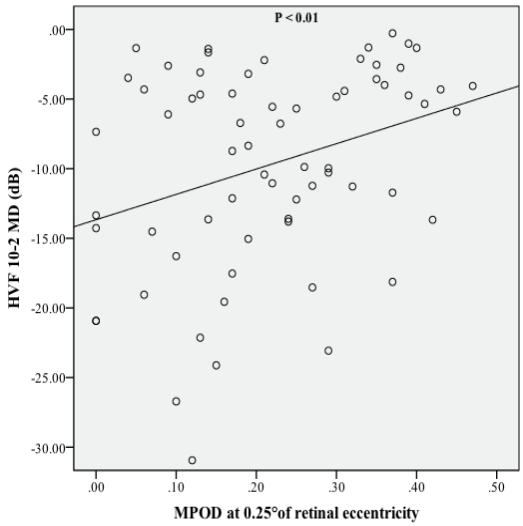
A 5% statistical significance level of was adopted throughout the analysis. No significant relationship was observed between MPOD and potential confounders for any relationship that might exist between MPOD, visual function and other demographic parameters, including gender, time diagnosed with glaucoma, type of glaucoma, body mass index, lens status or smoking status ($P > 0.05$), except for age, which demonstrated a statistically significant relationship with MPOD at 0.5° of retinal eccentricity only (r = -0.23, $P = 0.04$). Subsequent analyses involving MPOD at 0.5° of retinal eccentricity were corrected for age.

3.4.1 Visual Field Loss

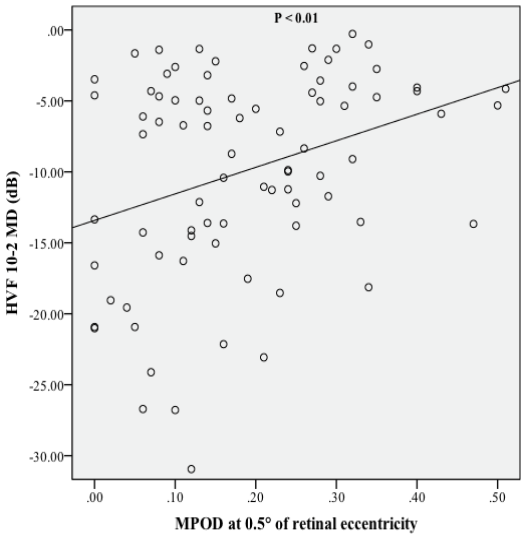
The median for HVF 24-2 and HVF 10-2 MD was -7.85 dB each with a range of -0.09 dB to -32 dB and -0.28 dB to -30.9 dB respectively. HVF 24-2 MD was significantly correlated with MPOD (r = 0.33, $P < 0.01$ at 0.25°; r = 0.33, $P < 0.01$ at 0.5°; r = 0.31, $P = 0.02$ at 1°). Using the 10-2 pattern deviation plots, we found 51.5% (n = 45) of subjects without foveal visual loss in contrast to 48.9% (n = 43) with foveal visual loss. However, none of the latter displayed the findings of depression of all 4 central points on the 10-2 pattern deviation plots suggesting the possibility of reliable subject fixation of the macular densitometer stimulus targets to within 1.4° of the foveal centre. MPOD at all retinal eccentricities were positively and statistically significantly correlated to the total amount of central visual field loss as measured using the HVF 10-2 MD (r = 0.33, $P < 0.01$ at 0.25°; r = 0.33, $P < 0.01$ at 0.5°; r = 0.32, $P = 0.01$ at 1°) (Figure 2). Linear regression

analyses between MPOD (0.25°, 0.5° and 1° of retinal eccentricity respectively) and HVF 10-2 MD showed a R^2 ranging from 10% to 12%.

A.



B.



C.

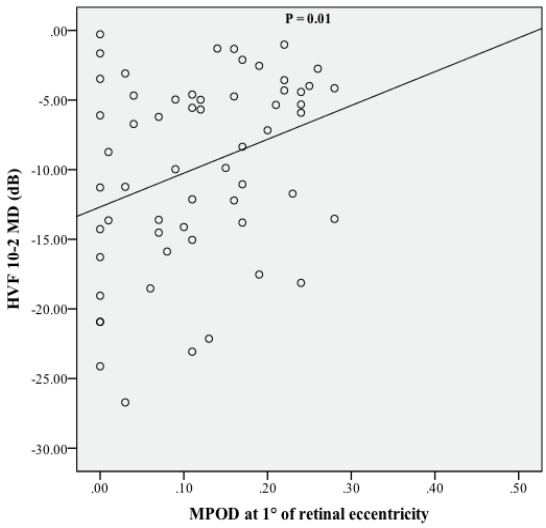
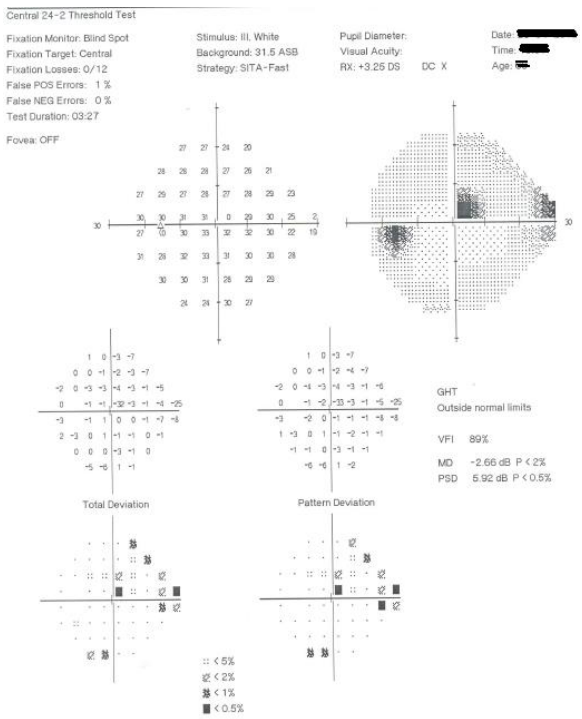


Figure 2. Scatter plots (A-C) show the correlation between MPOD and HVF 10-2 MD.
HVF, Humphrey visual field; MD, mean deviation; MPOD, macular pigment optical density.

3.4.2 VF Subgroups

Figures 3-5 show examples of HVF 24-2 and 10-2 patterns of study subjects. They illustrate the findings of no foveal field loss (Figures 3B & 4B) and foveal field loss (Figure 5B) respectively.

A.



B.

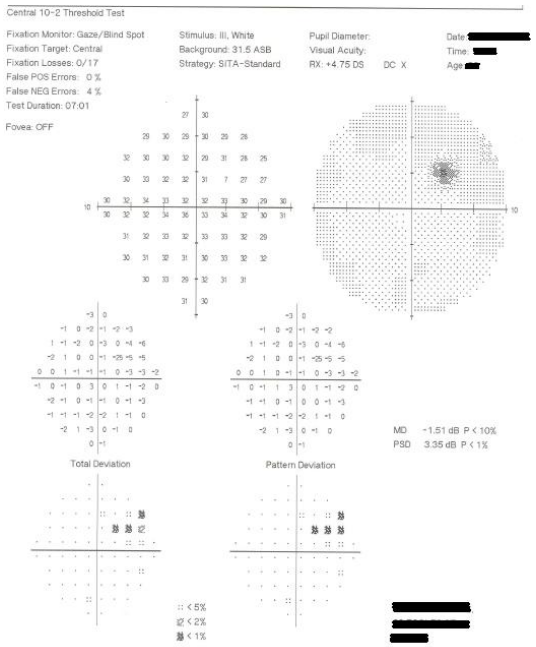
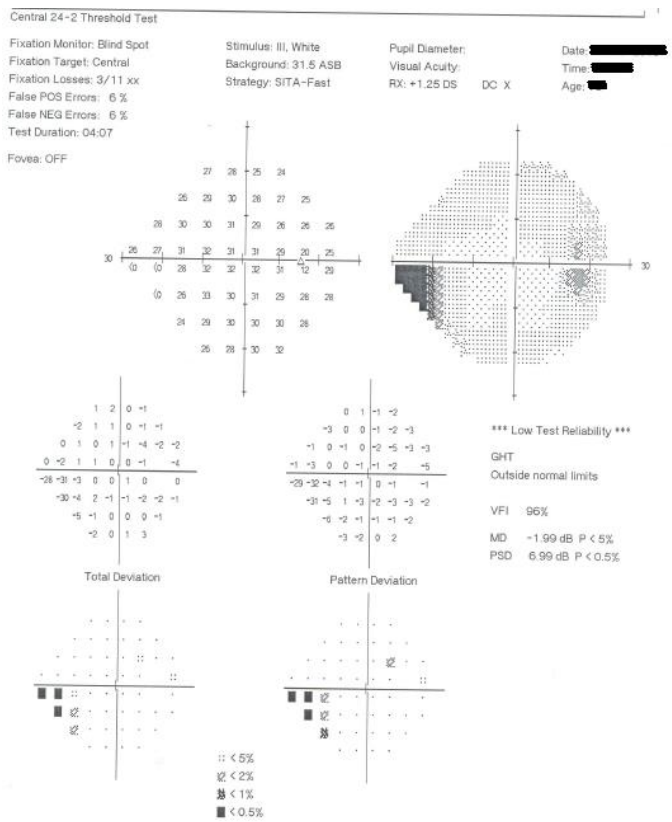


Figure 3. Visual fields of a subject (GMP010) with a diagnosis of normal tension glaucoma. (A) HVF 24-2, (B) HVF 10-2

A.



B.

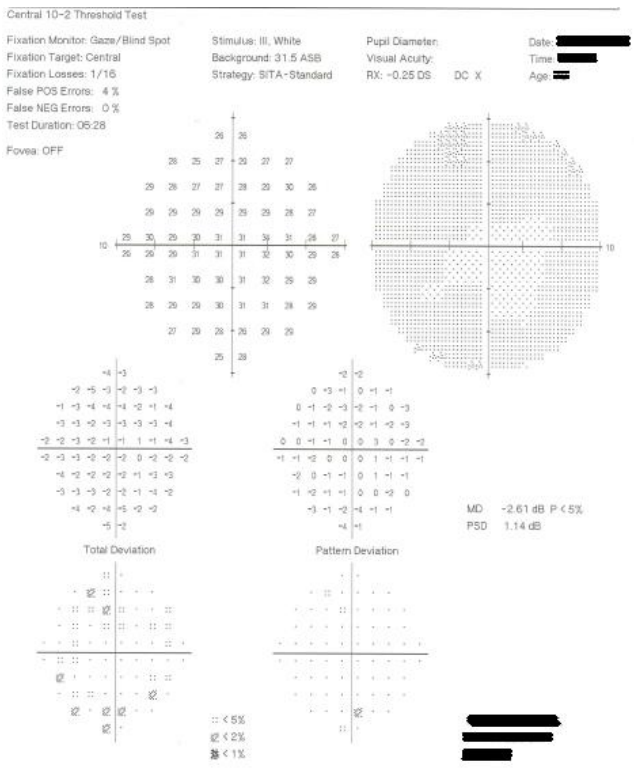
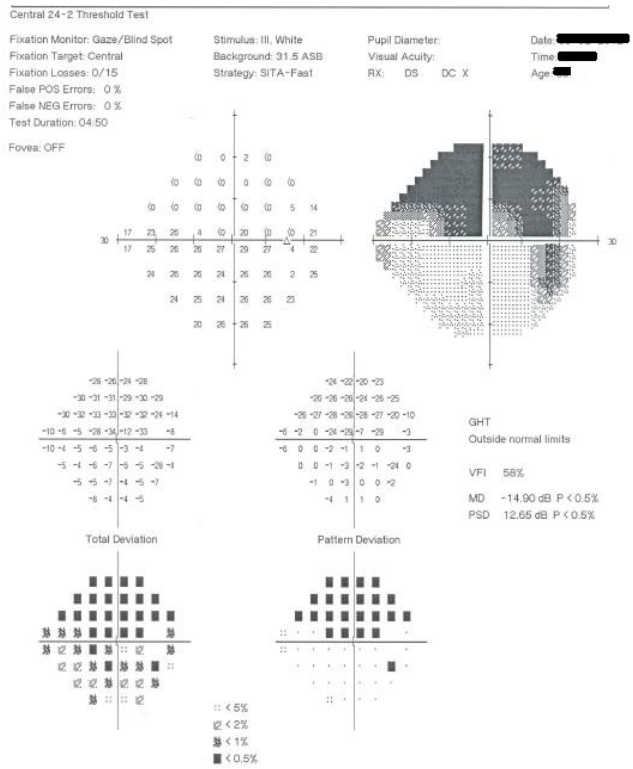


Figure 4. Visual fields of a subject (GMP015) with a diagnosis of primary open angle glaucoma.
(A) HVF 24-2, (B) HVF 10-2

A.



B.

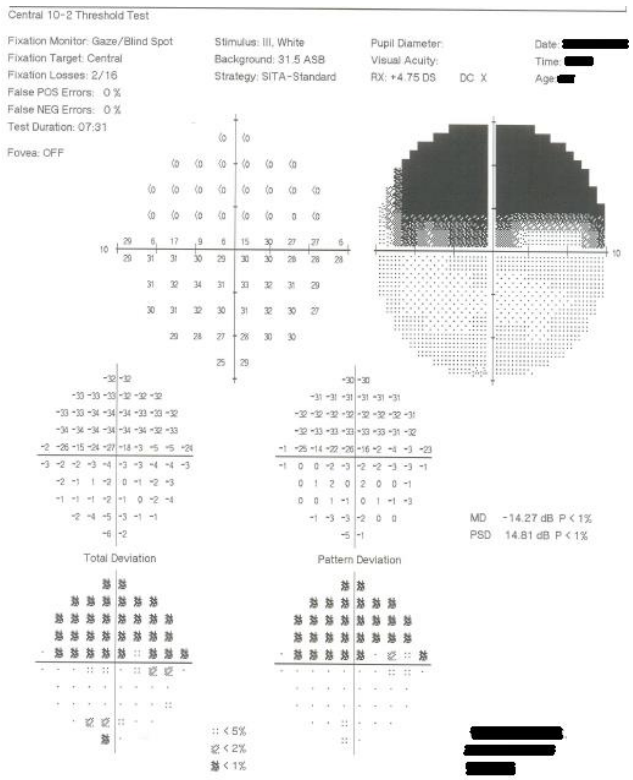


Figure 5. Visual fields of a subject (GMP017) with a diagnosis of primary open angle glaucoma.
(A) HVF 24-2, (B) HVF 10-2

Table 5 shows the different types of glaucoma within the VF subgroups. Table 6 shows the results between VF subgroups and visual function. Those with foveal field loss had significantly lower MPOD at 0.25° ($P < 0.01$) and 0.5° retinal eccentricities ($P = 0.02$) in comparison to those without foveal field loss. Our results also showed that those with foveal field loss had significantly worse HVF 24-2 MD, HVF 10-2 MD and PRT respectively.

Table 5. Types of Glaucoma and VF Subgroups

	No Foveal Field loss (N = 45)			Foveal Field loss (N = 43)		
Types of Glaucoma	n	HVF 24-2 MD (dB)*	HVF 10-2 MD (dB)*	n	HVF 24-2 MD (dB)*	HVF 10-2 MD (dB)*
POAG	28 (62.2%)	-6.48†	-4.71†	17 (39.5%)	-13.11	-15.42
NTG	9 (20.0%)	-4.05	-3.53	22 (51.2%)	-9.52	-13.04
PXG	5 (11.1%)	-12.54	-6.29	4 (9.3%)	-17.58	-21.26
PDG	3 (6.7%)	-5.93	-6.85	0	N/A	N/A

HVF = Humphrey visual field; MD = mean deviation; N/A = not applicable; NTG = normal tension glaucoma; PDG = pigment dispersion glaucoma; POAG = primary open-angle glaucoma; PXG = pseudoexfoliative glaucoma; VF = visual field

*Mean (unless indicated otherwise)

†Median

Table 6. Comparison between VF Subgroups

	No Foveal Field Loss	Foveal Field Loss	P Value
MPOD, Mean ± SD (degree)			
0.25	0.27 ± 0.13	0.18 ± 0.14	0.004*
0.50	0.22 ± 0.13	0.15 ± 0.12	0.019*
1.00	0.13 ± 0.09	0.10 ± 0.09	0.233*
LogMAR VA, Mean ± SD	0.03 ± 0.11	0.03 ± 0.10	0.960*
HVF 24-2 MD, Median (range) (dB)	-5.50 (-0.33 to -31.06)	-11.18 (-0.09 to -32)	0.004†
HVF 10-2 MD, Median (range) (dB)	-4.68 (-0.28 to -24.12)	-14.27 (-3.48 to -30.94)	<0.001†
Photo-stress recovery time, Median (range) (s)	15 (8 to 58)	21 (9 to 59)	0.029†
Mesopic CS with glare, Median (range)			
1.5 cpd	9 (3.5 to 50)	9 (3.5 to 36)	0.341†
3 cpd	15 (5 to 114)	15 (5 to 40)	0.541†
6 cpd	6 (6 to 85)	6 (6 to 45)	0.587†
12 cpd	4 (4 to 15)	4 (4 to 11)	0.638†
18 cpd	2 (2 to 4)	2 (2)	1†
Average overall spatial *f*	7.8 (4.1 to 53.6)	8.2 (4.1 to 19.6)	0.684†
Average low spatial *f*	11 (4.8 to 83.0)	11 (4.8 to 30.7)	0.653†
Average high spatial *f*	3 (3 to 9.5)	3 (3 to 6.5)	0.638†

GCC = ganglion cell complex; MPOD = macular pigment optical density; SD = standard deviation; RNFL retinal nerve fiber layer; HVF = Humphrey visual field; MD = mean deviation; *P* = Significance (2- tailed)

*Independent T test

†Mann-Whitney U test

3.4.3 Glare & Self-reported Visual Function

With controlling for the presence of mild cataract, MPOD was found to be statistically significantly correlated with mCSg for low spatial *f*s only, at both 0.25° (3 cpd: r = 0.25, *P* = 0.04) and 0.5° (3 cpd: r = 0.23, *P* = 0.04) of retinal eccentricity. No significant correlation was found between MPOD at 1° and mCSg. Linear regression analyses showed a low R^2 value between MPOD and mCSg for low spatial *f*s (range 3% to 7%). Mann-Whitney U-test was performed to evaluate any possible effect of cataract on glare and no significant differences in mCSg were found at any *f* between those with and those without mild cataract (*P*-values range 0.75 – 1.0).

In response to the glare symptoms question, 61% (n = 54) of subjects stated that they suffer from glare symptoms, with a median duration of 4.5 years (range 0.5 – 30). There was no statistical difference between self-reported glare symptoms and glaucoma subtypes. Those who suffered from glare symptoms had a significantly lower MPOD at all eccentricities relative to those without glare symptoms (Figure 6), but no difference in terms of age, gender, length of glaucoma diagnosis, body mass index, lens status or dietary intake of lutein and zeaxanthin (Table 7).

The duration of self-reported glare symptoms correlated significantly with the overall score for the Cataract TyPE Spec questionnaire and its glare subscale (Spearman Rho, r = 0.53, *P* < 0.001 each) and with GAL-9 questionnaire (r = 0.31, *P* = 0.003) respectively. However, the Cataract TyPE Spec questionnaire and its glare subscale, and the GAL-9 questionnaire did not correlate with MPOD (*P* > 0.01). We found borderline significance between the Cataract TyPE Spec glare subscale and mCSg at 1.5 cpd (r = -0.24, *P* = 0.02) and 3 cpd r = -0.22, *P* = 0.04) respectively. We examined the relationship between glaucoma-related functional parameter (HVF 24-2 and 10-2 MD respectively) and glaucoma-specific self-reported visual functioning (GAL-9 questionnaire) and found those with worse glaucoma have poor vision-related quality of life (*P* < 0.01 each).

3.4.4 Photo-stress Recovery

Valid PRT data was available for 73 subjects with a median of 19 seconds (range 8 – 59). The remaining subjects were either unable to see the display number in the Macular Adaptometer^{TM} (n = 4) or to respond within the 60 sec limit following the xenon flash (n = 11). There was no correlation between MPOD and PRT ($P > 0.05$ for all).

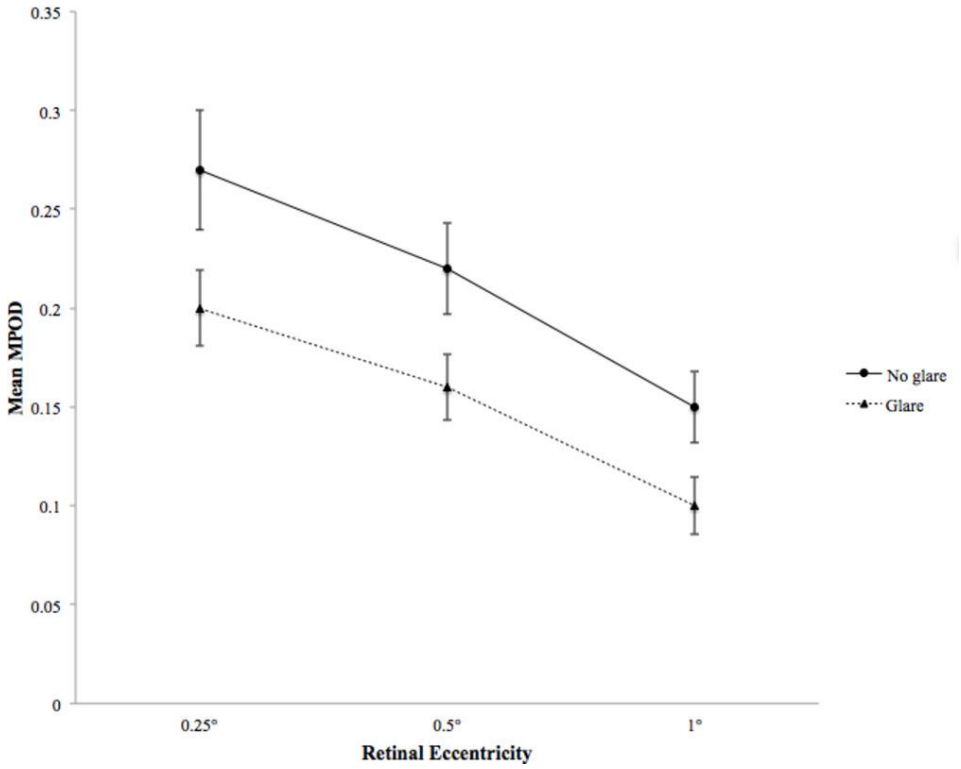


Figure 6. Graph demonstrates lower MPOD levels in those with symptoms of glare compared with those who were symptom free.
MPOD, macular pigment optical density.

Table 7. Self-reported Glare Symptoms and their Characteristics

	No Glare	Glare	*P*
MPOD			
0.25°, Mean ± SD	0.27 ± 0.15	0.20 ± 0.13	0.05*
0.5°, Mean ± SD	0.22 ± 0.13	0.16 ± 0.12	0.04*
1°, Median (range)	0.15 (0 to 0.28)	0.09 (0 to 0.25)	0.05†
Age, Mean ± SD	64.5 ± 9.6	66 ± 10.4	0.51*
Gender			
Male, n (%)	16 (47.1%)	32 (59.3%)	
Female, n (%)	18 (52.9%)	22 (40.7%)	0.28‡
Length of glaucoma diagnosis, Median (range) (y)	6 (0.5 to 15)	6.5 (0.5 to 32)	0.60†
Body mass index, Median (range) (kg/m2)	26.3 (18.7 to 39)	25.3 (18.5 to 42)	0.80†
Lens status			
Phakic, n (%)	30 (40%)	45 (60%)	
Pseudophakic, n (%)	4 (30.8%)	9 (69.2%)	0.56‡
Dietary intake, Median (range) (mg/dL)			
Lutein	0.85 (0 to 13.2)	0.7 (0 to 5.2)	0.52†
Zeaxanthin	0.1 (0 to 1.1)	0.1 (0 to 1.2)	0.15†
HVF 10-2 MD, Median (range) (dB)	-7.85 (-0.28 to -24.12)	-7.75 (-1.02 to -30.94)	0.44†

*Independent T test

†Mann-Whitney U test

‡Chi-square test

MPOD = macular pigment optical density; SD = standard deviation; y = years; kg/m2 = kilogram per square metre; mg/dL = milligrams per decilitre; HVF = Humphrey visual field; MD = mean deviation; dB = decibels; *P* = Significance (2- tailed)

3.4.5 GCC Subgroups

No confounding differences were found between the GCC subgroups for gender, BMI, length of time diagnosed with glaucoma, dietary intake or smoking habits except age ('Fovea-Not-Involved' vs 'Fovea-Involved', 62.6 ± 10.4 years vs 67.9 ± 9.0, $P = 0.02$), which was accounted for in subsequent analyses. A general linear analysis confirmed that age did not have a significant effect on parameters such as low spatial *f* mCSg, PRT, HVF 10-2 MD and self-reported glare symptoms ($P > 0.05$ for all).

Low spatial *f* mCSg was significantly worse in the 'Fovea-Involved' subgroup compared to those without foveal-involvement (1.5 cpd: Z = -2.26, $P = 0.02$; 3 cpd: Z = -2.36, $P = 0.02$) (Table 8). Additionally, the 'Fovea-Involved' subgroup had a prolonged PRT in comparison to the 'Fovea-Not-Involved' subgroup (Z = -2.41, $P = 0.02$). In the 'Fovea-Involved' subgroup, low spatial *f* mCSg was positively and statistically significantly correlated with MPOD at 0.25° (1.5 cpd: r = 0.37, $P = 0.02$; 3 cpd: r = 0.43, $P = 0.01$) and 0.5° (1.5 cpd: r = 0.29, $P = 0.05$; 3 cpd: r = 0.41, $P = 0.01$) of retinal eccentricity, whilst no significant correlation was observed at 1° and higher mCSg spatial *f*s. Of those with significant correlations, R^2 was found to be between 8% and 20%. No significant correlation was observed between MPOD and mCSg in the 'Fovea-Not-Involved' subgroup ($P > 0.05$ for all). Furthermore, MPOD was significantly lower at all retinal eccentricities in those with foveal-involvement who reported glare symptoms compared to those without glare symptoms (0.25°: $P = 0.05$, t = -1.99; 0.5°: $P < 0.01$, t = -2.92; 1°: $P = 0.01$, t = -2.63) whilst no significant difference was observed in the 'Fovea-Not-Involved' subgroup ($P > 0.05$ for all). No significant correlation was observed between MPOD and PRT within either GCC subgroup ($P > 0.05$). Residual visual function, as determined by HVF 10-2 MD, was significantly worse (Z = -4.42, $P < 0.001$) in the 'Fovea-Involved' subgroup (median, -12.17dB (range -0.28 to -26.78)) in comparison to the 'Fovea-Not-Involved' subgroup (median, -4.42 (range -1.02 to -20.62)).

Table 8. Comparison of visual function between GCC subgroups

	Fovea-Not-Involved	Fovea-Involved	*P*
mCSg, Median (range) (cpd)			
1.5	13 (3.5 to 50)	9 (3.5 to 36)	0.023
3	20 (5 to 114)	10 (5 to 40)	0.018
6	6 (6 to 85)	6 (6 to 45)	0.212
12	4 (4 to 15)	4 (4 to 8)	0.127
18	2 (2 to 4)	2	0.388
PRT, Median (range) (s)	15 (8 to 59)	20 (9 to 58)	0.015
HVF 10-2 MD, Median (range) (dB)	-4.42 (-1.02 to -20.62)	-12.17 (-0.28 to -26.78)	<0.001

GCC = ganglion cell complex; mCSg = mesopic contrast sensitivity under glare condition; cpd = cycles per degree; PRT = photo-stress recovery time; s = seconds; HVF = Humphrey visual field; MD = mean deviation; dB = decibels

3.5 Trial Outcome

3.5.1 MPOD Response - All Subjects

Of the 88 subjects recruited into the trial, 83 subjects completed it, with 1 dropout in the active group (n = 43) and 4 in the placebo group (n = 40) giving a dropout rate of 5.7%. Table 9 shows subjects' demographic and characteristics at baseline. Overall, 5 subjects returned more than 14 tablets at the end of the trial (active group, n = 1 (23 tablets); placebo group, n = 4 (90, 70, 60 and 20 tablets respectively)). It is important to note that not all subjects were able to perform the experiment using the Macular Densitometer to yield reliable MPOD readings at all retinal eccentricities. For those in the active group, valid $MPOD_{baseline}$ and $MPOD_{follow\text{-}up}$ data were available for 74.4% (32/43) of subjects at 0.25° of retinal eccentricity, 83.7% (36/43) at 0.5° and 51.2% (22/43) at 1° respectively. In the placebo group, valid $MPOD_{baseline}$ and $MPOD_{follow\text{-}up}$ data were available for 57.5% (23/40) of subjects at 0.25° of retinal eccentricity, 80% (32/40) at 0.5° and 62.5% (25/40) at 1° respectively.

Table 10 shows the comparison between the means for $MPOD_{baseline}$ and $MPOD_{follow\text{-}up}$ for subjects in the active and placebo groups respectively. Factors such as age, gender, year(s) diagnosed with glaucoma, smoking habit (pack years) and body mass index were not significantly different between those in the active and placebo groups. Following intervention, we found a statistically significant increase in MPOD at 0.25° of retinal eccentricity in the active group ($t = -2.18$, $P = 0.04$) while no change was seen in the placebo group ($t = -1.17$, $P = 0.26$). There was no significant difference in the dietary MP intake at baseline and final visits of those receiving the active intervention (lutein intake, $Z = -0.92$, $P = 0.36$; zeaxanthin intake, $Z = -1.93$, $P = 0.07$).

$MPOD_{baseline}$ measurements of those in the active group were divided into tertile subgroups; ΔMPODs were compared to those with the lowest and highest $MPOD_{baseline}$ measurements respectively. We observed a statistically significant higher ΔMPOD at 0.5° of retinal eccentricity in those with the lowest $MPOD_{baseline}$ in comparison to those with the highest $MPOD_{baseline}$ ($Z = -2.41$, $P = 0.02$) whilst

no significant difference was seen at 0.25° and 1° of retinal eccentricity. We found a significant linear correlation between ΔMPOD and cigarette pack year history at all retinal eccentricities (at 0.25°: r = 0.27, $P = 0.04$; at 0.5°: r = 0.25, $P = 0.04$; at 0.1°: r = 0.29, $P = 0.05$). There was no correlation between ΔMPOD and age, body mass index or length of glaucoma diagnosis at all retinal eccentricities ($P > 0.05$). We did not find any significant difference between ΔMPOD and glaucoma diagnosis (POAG and NTG subgroups) at any retinal eccentricity ($P > 0.05$ for all).

Table 9. Subject characteristics at baseline

Characteristic	Active group N = 43	Placebo group N = 40	*P*
Age, Mean ± SD (y)	65.7 ± 10.8	64.6 ± 9.4	0.62
Sex, n (%)			
Male	23 (53.5)	21 (52.5)	
Female	20 (46.5)	19 (47.5)	1
Duration of glaucoma, Median (Range) (y)	6 (1 – 22)	7 (0.5 – 32)	0.89
Smoking habits, n (%)			
Never smoked	19 (44.2)	20 (50)	
Ex-smoker	4 (9.3)	6 (15)	
Current smoker	20 (46.5)	14 (35)	0.55
Study eye LogMAR, Median (Range)	0 (-0.16 to 0.24)	0.06 (-0.2 to 0.24)	0.01
Body mass index, Median (Range)	25.4 (18.5 – 39.0)	25.5 (19.8 – 42.0)	0.86

SD = standard deviation; y = year; LogMAR = logarithm of the minimum angle of resolution; *P* = Significance (2-tailed)

Table 10. Comparison between baseline and follow-up MPOD for each intervention group

MPOD	Active group		n	*P*	Placebo group		n	*P*
(Mean ± SD)	Baseline	Follow-up			Baseline	Follow-up		
0.25°	0.27 ± 0.14	0.31 ± 0.12	32	0.04	0.22 ± 0.11	0.24 ± 0.11	23	0.26
0.5°	0.23 ± 0.12	0.25 ± 0.10	36	0.07	0.19 ± 0.12	0.21 ± 0.13	32	0.08
1°	0.14 ± 0.08	0.15 ± 0.09	22	0.70	0.13 ± 0.09	0.13 ± 0.10	25	0.90

MPOD = macular pigment optical density; SD = standard deviation; *P* = 2-tailed significance

3.5.2 MPOD Response – Responders and non-responders

There was no significant difference in MPOD response between those with POAG and NTG at all retinal eccentricities (Chi-square test, $P > 0.05$). Overall, 46.5% (20/43) of subjects in the active group had a complete dataset of MPOD spatial profile at baseline and final visits. Of these, 40% (8/20) showed positive ΔMPOD responses at all retinal eccentricities (full-responders), 35% (7/20) were partial responders (4 subjects with positive ΔMPOD at 1 retinal locus and 3 subjects at 2 retinal loci respectively) and 25% (5/20) did not display any MPOD response at any retinal eccentricity (complete non-responders).

There was no significant difference in MPOD response (including those with no ΔMPOD) between the active and placebo groups at all retinal eccentricities (at 0.25°, $P = 0.09$; at 0.5°, $P = 0.32$; at 0.1°, $P = 0.31$). In the active group, a positive ΔMPOD was observed in 71.9% (23/32) of subjects at 0.25° of retinal eccentricity, 58.3% (21/36) at 0.5° and 54.5% (12/22) at 1° respectively. Subjects that had a positive ΔMPOD did not show any significant change in oral dietary lutein or zeaxanthin intake from baseline to follow-up ($P > 0.05$ at all retinal eccentricities). An analysis comparing subjects in the active group who displayed a positive ΔMPOD to those in the placebo group showed a statistically significant MPOD response at all retinal eccentricities (at 0.25°, $Z = -3.45$, $P < 0.001$; at 0.5°, $Z = -4.19$, $P < 0.001$; at 1°, $Z = -2.84$, $P = 0.004$) (Figure 7).

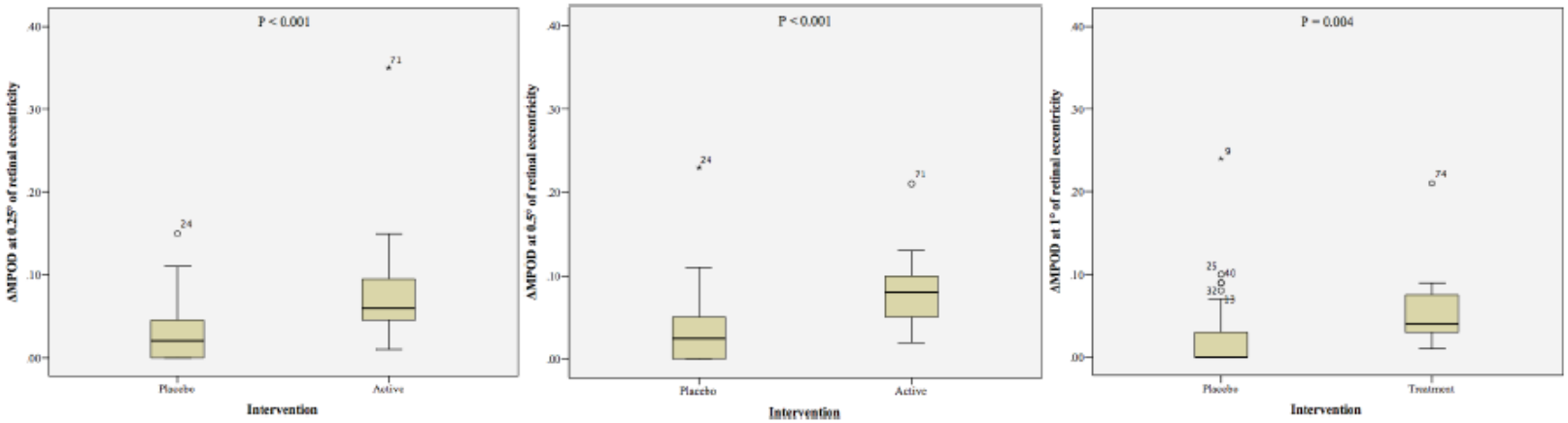


Figure 7. Box and whiskers plots compare the ΔMPOD between those the placebo group and the responders in the active group at individual retinal eccentricities.

In the placebo group, there was a cohort of subjects with a positive ΔMPOD. At 0.25° of retinal eccentricity, 56.5% (13/23) of subjects had a positive ΔMPOD (median = 0.04, range 0.02 to 0.15). This could be explained by the significantly higher oral dietary lutein intake from baseline to final follow-up (Z = -1.97, *P* = 0.05); no difference was observed in zeaxanthin intake (Z = -2, *P* = 0.13). A positive ΔMPOD was observed for 62.5% (20/32) of subjects at 0.5° of retinal eccentricity and 32% (8/25) at 1° respectively, in the absence of any significant influence from either dietary lutein or zeaxanthin (*P* > 0.05 for all). A comparison of positive ΔMPOD between the placebo and active groups showed a significantly higher MPOD response in the active group at 0.5° of retinal eccentricity (Z = -2.02, *P* = 0.04) but not at 0.25° (Z = -1.36, *P* = 0.18) or 1° (Z = -1.48, *P* = 0.15) respectively.

In the active group, lower baseline MPOD level was found in those with a positive ΔMPOD in comparison with no ΔMPOD at 0.25° of retinal eccentricity (0.24 ± 0.13 vs 0.35 ± 0.13, *P* = 0.03) and at 0.5° (0.18 ± 0.08 vs 0.29 ± 0.13, *P* < 0.01) whilst no difference was observed at 1° (0.14 ± 0.08 vs 0.14 ± 0.09, *P* = 0.96) (Figure 8). However, no correlation was observed between ΔMPOD and baseline MPOD or baseline dietary intake at all retinal eccentricities (*P* > 0.05 for all) in those with a positive ΔMPOD. There was a preponderance for male gender in those with a positive ΔMPOD at 0.25° of retinal eccentricity (Fisher's exact test, *P* = 0.05) but this finding was not seen at 0.5° or 1° of retinal eccentricity respectively. There was no significant difference in subject characteristics (age, year(s) diagnosed with glaucoma, smoking habit, body mass index or foveal involvement) between those with a positive ΔMPOD and no ΔMPOD at all retinal eccentricities.

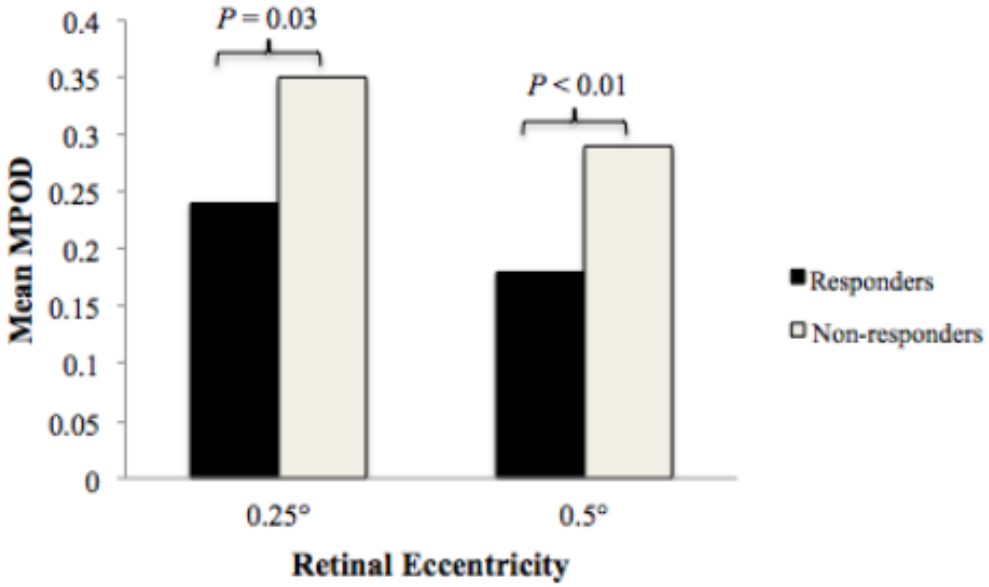


Figure 8. Graph illustrates a statistically significant lower baseline MPOD in the responders group compared to the non-responders.

3.5.3 MPOD Response - GCC Subgroups

Of the 83 subjects who completed the trial, 80 had good quality GCC scans that allowed us to sub-analyse data according to foveal involvement. In the active group, there were 16 subjects with "Fovea-Not-Involved" glaucoma and 25 with "Fovea-Involved" glaucoma. Table 11 shows the MPOD response between the GCC subgroups of those in the active group. A statistically significant increment in MPOD at 0.5° of retinal eccentricity was found in those with "Fovea-Involved" glaucoma (t = -2.74, P = 0.01). Although a linear trend towards increasing MPOD was observed at 0.25°, the result was not statistically significant (t = -1.87, P = 0.08). There was no difference between the means for $MPOD_{baseline}$ and $MPOD_{follow\text{-}up}$ at all retinal eccentricities for subjects with "Fovea-Not-Involved" glaucoma receiving the active intervention. We found no significant difference in ΔMPOD between the "Fovea-Involved" and "Fovea-Not-Involved" subgroups (P > 0.05). Only 9 subjects with "Fovea-Not-Involved" glaucoma and 10 subjects with "Fovea-Involved" glaucoma had complete dataset of MPOD spatial profiles at

baseline and follow-up. There were 5 full-responders, 1 partial responder and 3 non-responders in the “Fovea-Not-Involved” subgroup. In the “Fovea-Involved” subgroup, we observed 3 full-responders, 5 partial responders and 2 non-responders. In the placebo group, there were 15 subjects with “Fovea-Not-Involved” glaucoma and 24 with “Fovea-Involved” glaucoma. No significant difference in mean $MPOD_{\text{baseline vs follow-up}}$ was observed in the placebo group ($P > 0.05$ for all).

Table 11. MPOD response based on GCC Subgroups in the Active group

MPOD (Mean ± SD)	Fovea-Not-Involved		n	*P*	Fovea-Involved		n	*P*
	Baseline	Follow-up			Baseline	Follow-up		
0.25°	0.28 ± 0.14	0.31 ± 0.12	15	0.36	0.25 ± 0.11	0.29 ± 0.09	16	0.08
0.5°	0.23 ± 0.13	0.23 ± 0.11	15	0.68	0.22 ± 0.09	0.26 ± 0.08	20	0.01
1°	0.16 ± 0.09	0.17 ± 0.07	9	0.60	0.12 ± 0.07	0.13 ± 0.11	12	0.71

GCC = ganglion cell complex; MPOD = macular pigment optical density; SD = standard deviation; *P* = 2-tailed significance

3.5.4 MPOD Response - VF Subgroups

We found no significant difference in ΔMPOD between the "No Foveal Field Loss" and "Foveal Field Loss" subgroups ($P > 0.05$).

3.5.5 Effects on Glaucoma Structure and Function

No significant improvement was observed in glaucoma structure as measured by SD-OCT, or glaucoma function such as mCSg, PRT or VF 10-2, following oral dietary MP supplementation ($P > 0.05$ for all) for both the active and placebo groups at 6 months follow-up. There was no significant correlation between ΔMPOD and glaucoma structure and function parameters respectively. Subgroup analyses by GCC subgroups (Table 12) and by MPOD response (responder vs non-responder) respectively, were also performed to compare intervention outcomes but no significant improvement in glaucoma structure or functional parameters were evident. Instead, we observed a statistically significant worsening of some parameters (full foveal and parafovea thickness, mCSg at 1.5cpd and 3cpd respectively) in the Fovea-Involved subgroup at the final follow-up period representing natural disease progression.

Table 12. Outcomes following oral dietary macular pigment supplementation between GCC subgroups

	Fovea-Not-Involved		n	*P*	Fovea-Involved		n	*P*
	Baseline	Follow-up			Baseline	Follow-up		
RNFL thickness, Median (range) (µm)								
Peripapillary RNFL	71 (62 to 88)	70 (64 to 83)	16	0.21	68 (57 to 100)	68 (52 to 98)	24	0.50
Macular RNFL	72.2 (63.1 to 88.5)	71.5 (61.2 to 90.9)	15	0.45	72.5 (54.8 to 98.7)	71.5 (52.3 to 100.4)	25	0.15
GCC thickness, Mean ± SD (µm)	75.6 ± 6.8	77.5 ± 6.8	15	0.20	70.6 ± 9.4	69.0 ± 8.8	25	0.07
Foveal thickness, Mean ± SD (µm)								
Full fovea	268.5 ± 19.5	267.1 ± 20.5	14	0.42	257.8 ± 23.7	254.3 ± 24.1	19	0.05
Inner fovea	86.6 ± 11.6	87.1 ± 12.3	14	0.72	81.8 ± 16.4	79.7 ± 14.1	19	0.27
Parafoveal thickness, Mean ± SD (µm)								
Full parafovea	304.2 ± 14.7	302.2 ± 16.5	14	0.10	287.6 ± 18.6	284.5 ± 18.0	19	<0.01
Inner parafovea	119.1 ± 9.1	117.6 ± 10.7	14	0.24	107.3 ± 9.4	107.9 ± 9.5	19	0.51
Cup-disc area ratio, Median (range)	0.78 (0.58 to 0.96)	0.83 (0.52 to 0.98)	16	0.10	0.88 (0.65 to 0.97)	0.90 (0.64 to 0.97)	25	0.80
Rim area, Median (range) (mm2)	0.37 (0.14 to 0.75)	0.29 (0.05 to 0.84)	16	0.11	0.21 (0.06 to 0.62)	0.22 (0.06 to 0.62)	25	0.78
mCSg, median (range) (cpd)								
1.5	13 (3.5 to 50)	13 (3.5 to 36)	16	0.92	9 (3.5 to 36)	3.5 (3.5 to 25)	25	0.03
3	24.5 (5 to 57)	24.5 (5 to 57)	16	0.13	15 (5 to 40)	10 (5 to 40)	25	0.01
6	9 (6 to 64)	6 (6 to 33)	16	0.80	6 (6 to 45)	6 (6 to 33)	25	0.14
12	4 (4 to 11)	4 (4 to 15)	16	1.00	4 (4 to 8)	4 (4 to 8)	25	0.25
18	2	2 (2 to 4)	16	1.00	2	2	25	1.00
PRT, median (range) (s)	12.5 (8 to 59)	12.5 (8 to 36)	12	0.62	20 (10 to 53)	18 (10 to 42)	12	0.18
HVF 10-2 MD, median (range) (dB)	-4.23 (-1 to -20.6)	-4.4 (-0.1 to -19)	16	0.87	-11.17 (-0.3 to -26.8)	-11.13 (-1.8 to -28.3)	24	0.23

cpd = cycles per degree; dB = decibels; GCC = ganglion cell complex; HVF = Humphrey visual field; mCSg = mesopic contrast sensitivity under glare conditions; MD = mean deviation; PRT = photo-stress recovery time; RNFL = retinal nerve fiber layer; SD = standard deviation; *P* = Significance (2- tailed)

Chapter 4: Discussion

The discussion will be presented under 3 sections:

1. MP and glaucoma-structure parameters
2. MP and visual function parameters
3. Macular Pigment and Glaucoma Trial outcome

4.1 MP and glaucoma-structure parameters

MP has previously been shown to be lower in glaucoma compared to healthy controls (Igras et al. 2013, Ji et al. 2016). This study extends those findings and suggests that a continuum exists, whereby, not only is MPOD lower in the presence of glaucoma but is further compromised in more severe cases of the condition, and, in particular, where foveal ganglion cells are involved. We also found borderline significant correlations between MPOD in the overall group and OCT-derived topography measures, which may further imply a relationship between MP and glaucoma-related structural parameters. We did not include the use of a control group in this trial as this had been studied by our research group (Igras et al. 2013).

The San Diego Macular Pigment study did not find any difference in MP level between glaucoma eyes and healthy controls (Daga et al. 2018) and this can be explained by the difference in study design, subject demographics (age, diet, BMI) and glaucoma severity. In agreement with this study, we did not find any relationship between MP levels and RNFL thickness. However, when we further categorised glaucomatous disease severity using the GCC subgroup analysis, we found that those with fovea-involved glaucoma had statistically significantly lower MPOD at 0.25°, 0.5° and 1° of retinal eccentricities in comparison to those without foveal involvement ($P < 0.001$ for all). Interestingly, MPOD at 0.25°, 0.5° and 1° of retinal eccentricities in the ‘‘Fovea-Not-Involved’ group were comparable to that reported in the normal population (see Table 4). We believe that the analysis of MPOD in the presence of glaucoma should be done in a manner that respects the localisation of (a) MPs at the central macula (fovea), and (b) glaucoma damage. In the early stages of glaucoma disease (with the exception of normal tension glaucoma), structural damage is often not located at the macula. Hence, the use of

a global RNFL thickness parameter may not accurately delineate the relationship between MP and glaucoma.

In our study, only half of the POAG patients had foveal involvement compared to a large proportion of those with NTG (77%). This finding was not surprising to us given that paracentral scotoma is a common finding in the early course of NTG. PXG glaucoma is generally recognized as a more progressive glaucoma subtype. This may explain why the majority (78%) of patients in the PXG group had foveal-involved glaucoma and displayed worst glaucoma parameters (HVF 24-2 MD and average GCC thickness respectively) compared to other glaucoma subtype.

Beatty and colleagues have previously demonstrated the finding of an age-related decline in MPOD in individuals with healthy eyes, while an earlier study by Bone et al has shown the contrary (Beatty et al. 2001; Bone et al. 1988). Given that glaucoma is a progressive retinal degenerative disease that tends to affect the ageing individual, one may speculate that increasing age may, in part, be contributing to the decline in MPOD with advancing disease. Although subjects in the "Fovea-Involved" subgroup were older than that in the "Fovea-Not-Involved" subgroup (mean age, 67.9 years vs 62.6 years respectively), this difference was only borderline significant (Independent t-test, $P = 0.02$). Our data, however, are in keeping with the work by Bone and colleagues (Bone et al. 1988), and do not support the existence of a relationship between MPOD and age. Our earlier study (Igras et al. 2013) had shown MPOD to be significantly lower in glaucomatous eyes compared to age-matched controls. With our current data, it would seem that age is not a confounding factor, but the presence of foveal GCC loss is a major determinant of MPOD levels.

Adipose tissue acts as a major storage organ for carotenoids and therefore may compete with the retina for their uptake. It has been shown that an inverse relationship exists between MPOD and BMI in those with healthy eyes (Hammond et al. 2002). In our study, we did not find any correlation between MPOD and BMI.

In human eyes, the most central part of the fovea (also known as the foveal pit) measures 200μm in diameter and is a region where only the cone photoreceptors

can be found (Curcio & Allen 1990). From here, the inner retinal structures are displaced radially to form the foveal slope and eventually at the parafovea (thickest portion of the retina), the ganglion cells are packed into 6 layers thick. MP can be localised to the fibres of Henle and plexiform layers at the fovea and the inner and outer plexiform layers at the parafovea (Trieschmann et al. 2008). This is of relevance as the 3-innermost retinal layers preferentially affected in glaucoma are (a) nerve fibre layer (ganglion cell axon), (b) ganglion cell layer (ganglion cell body) and (c) inner plexiform layer (ganglion cell dendrites) (Tan et al. 2008). Collectively, these 3 retinal layers constitute the ganglion cell complex (GCC) as measured by the RTVue FD-OCT.

Due to limitations of the RTVue FD-OCT to reliably evaluate GCC thickness at the fovea, we described a simple method to allow us to distinguish the presence/absence of foveal involvement in glaucoma subjects (see Methods section). MPOD was significantly lower in subjects who exhibited evidence of GCC thinning which encroached on the foveal zone ('Fovea-Involved' GCC) relative to those without foveal GCC involvement. Given that the macular layers where MP is housed appear to be affected by glaucoma, this may explain, at least in part, the observation herein that MPOD is lower in individuals with more severe glaucoma, and in particular, those with GCC loss at the fovea. However, our data in this study is not sufficient enough to make any conclusions on causal inferences.

In our earlier work (Igras et al. 2013), it had been shown for the first time that MPOD was significantly lower in glaucomatous eyes compared to controls. Also utilising the HFP technique, Igras and colleagues (Igras et al. 2013) showed that mean MPOD ± SD was 0.39 ± 0.24 in a similar cohort of normal controls, in comparison to 0.19 ± 0.12 at 0.5° of retinal eccentricity in this study. The current study was not designed to repeat that investigation, but rather to extend the study in an effort to better understand the relationship between MPOD and glaucoma-related structural parameters. As such, the lack of a normal control group is not a substantial limitation to the current study.

Igras and colleagues (Igras et al. 2013) had also previously reported that MPOD did not correlate with glaucoma severity (HVF 24-2 MD) whereas this present

study had demonstrated a borderline significant (P = 0.01 – 0.05) correlation between MPOD and glaucoma-related structural parameters as measured by RTVue FD-OCT in the overall group. Furthermore, we also found that those with foveal-involved glaucoma had more severe disease (OCT parameters, $P < 0.01$; HVF 24-2 MD, $P = 0.02$) and demonstrated lower MPOD ($P < 0.001$) compared to those without foveal-involvement. This disparity between the 2 studies likely reflects limitations in our earlier study, which had a smaller sample size (and therefore lacked statistical power), and where, importantly, a central visual field test (HVF 10-2) and OCT scans to capture foveal loss were not employed. The majority of the subjects in this present study had foveal-involved glaucoma, whilst this information was not available in our earlier study for analysis. Therefore, the differences in results between the studies do not suggest a contradiction, but rather emphasizes the differences in the study methodology.

We also observed that a small subset of our glaucoma subjects (7 out of 53 subjects (13%) with complete MPOD data at all eccentricities) exhibited an atypical MP spatial profile, which peaked at 0.50 degrees of retinal eccentricity. Six out of these 7 subjects had foveal-involved glaucoma. In a study of healthy subjects, Kirby and colleagues found that atypical MP spatial profile was related to the foveal slope such that the steeper the foveal depression, the steeper the MP distribution (Kirby et al. 2009). The significance of an atypical MP spatial profile in glaucoma is currently unknown and therefore further studies are warranted to better understand it.

In the current study, both the full parafoveal ($P < 0.001$) and foveal thickness (P = 0.002) were significantly lower in the 'Fovea-Involved' group, which exhibited greater glaucoma severity. In a study of POAG patients with isolated superior or inferior hemifield defects, it was shown that the retinal thickness at the parafovea and fovea linearly correlated with glaucoma severity (Inuzuka et al. 2013). Furthermore, they also showed that the parafoveal and foveal thickness were significantly reduced in the corresponding hemifield defects when compared to the normal side. Another study demonstrated the finding of a thin parafoveal ring on time-domain OCT imaging in subjects with more advanced glaucoma but not for full foveal thickness (Kanis et al. 2010). This discrepancy in findings may be

related to the use of different OCT imaging modalities across the studies, for example, time-domain OCT has been shown to exhibit a higher percentage of clinically significant inaccurate central foveal thickness compared to FD-OCT (Ho et al. 2009).

A statistically significantly reduced outer foveal thickness was observed in the 'Fovea-Involved' group ($P = 0.01$). This finding suggests that cone photoreceptors may be affected in foveal-involved glaucoma. A histological study on human eyes with a diagnosis of chronic glaucoma has previously demonstrated cone photoreceptor swelling and loss (Nork et al. 2000). Furthermore, fundus reflectometry and OCT imaging techniques have provided evidence of the loss of foveal cone outer segment integrity in subjects with POAG with advanced central visual field defects (Kanis et al. 2010), while loss of cone density in glaucomatous eyes has been shown to correspond with retinal locations of greater visual sensitivity loss (Choi et al. 2011). Although this study does not provide definitive evidence of cone photoreceptor loss, the observed involvement of those structures where MP is housed, including significantly thinner parafovea and inner fovea, and a borderline significantly thinner outer fovea, may provide some explanation as to our finding of lower MPOD in foveal-involved glaucoma.

The macula is susceptible to oxidative injury as it is highly aerobic, constantly exposed to light (UVA and short-wavelength blue light are particularly hazardous), and environmental chemicals such as cigarette smoking (Beatty et al. 2000). Furthermore, the presence of high levels of polyunsaturated fatty acids predisposes to the generation of reactive oxygen species. RGCs are highly dependent on mitochondria for energy and are particularly vulnerable to oxidative stress (Chen & Chan 2006). The finding of lipofuscin accumulation in glaucomatous eyes emphasises the importance of oxidative stress in glaucoma pathogenesis (McElnes et al. 2014). Ghanem and colleagues studied the levels of malondialdehyde, an antioxidant enzyme, in aqueous humour samples of POAG patients (n = 30) and found that it was significantly correlated with visual field loss ($P < 0.001$) indicating that increased oxidative stress is associated with more glaucoma damage (Ghanem et al. 2010). L (Li and Lo 2010) and Z (Nakajima et al. 2009) have been shown to confer a protective effect against oxidative stress-induced cell damage in

in vitro studies using the RGC-5 cell line. Likewise, L has been shown to increase the survival of the RGC by decreasing oxidative stress in a model of acute retinal ischemia/reperfusion in mice (Li et al. 2009). We postulate that an environment of high oxidative stress such as glaucoma can cause MP to be depleted, and thereby potentially explain the finding of lower MPOD in glaucomatous eyes exhibiting foveal involvement and more severe damage.

4.1.1 Study limitations

We were not able to obtain complete MPOD data at all 3 retinal eccentricities in some glaucoma subjects as they found the HFP task somewhat difficult to perform and time-consuming with consequent fatigue. As the HFP technique is a psychophysical test, it is observer-dependent and may explain the difficulty in carrying out the task. Furthermore, the presence of glaucoma may have contributed to additional challenges. Despite that, HFP is still considered a reliable and practical method to measure MPOD (Wooten et al. 1999). However, it may be worthwhile incorporating a less onerous and time-consuming imaging test such as fundus autofluorescence in future studies (Howells et al. 2011, Waldstein et al. 2012).

Currently, an imaging system that can accurately measure the GCC thickness at the fovea is lacking. The Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA) uses the Ganglion Cell Analysis protocol to measure 2 retinal layers at the macula, namely the ganglion cell and inner plexiform layers, unlike the RTVue FD-OCT that measures 3 layers including the nerve fibre (Grewal and Tanna 2013). In the context of studying MP, Cirrus HD-OCT may have a slight advantage over RTVue FD-OCT as its macular area of analysis is centred on the fovea compared to 1 mm temporal to the fovea in the latter. Nevertheless, both OCT devices are not capable of reliably measuring the ganglion cell layer at the fovea. Our method of determining whether GCC loss affects the foveal region by referring to the GCC Significance Map of the RTVue FD-OCT remained effective in providing us with sufficient data for analysis in this study.

Given the limitations of this study, we can only postulate as to the likely explanation for the finding that MPOD is lower in foveal-involved glaucoma.

Firstly, as discussed earlier, it may be possible that when the foveal and parafoveal structures are affected in glaucoma, loss of the MP housing initiates MPOD depletion. Secondly, low levels of MPOD, either due to poor dietary intake or impaired carotenoid absorption/metabolism/transportation, may predispose RGC to oxidative damage due to the lack of its protective antioxidant effect, leading to eventual foveal damage. Thirdly, given that the macula is a highly aerobic tissue and that glaucoma creates an environment of chronic oxidative stress, MP may be constantly used up to scavenge free radicals leading to MPOD depletion. This may be especially evident in those with fovea-involved glaucoma, where the RGC are under immense oxidative stress and MP storage depletes faster than it can be replenished. Augmentation of MPOD by dietary modification and/or supplementation, may, therefore, be desirable in glaucoma patients, particularly those with evidence of foveal involvement. The potential protective role of oral MP supplementation against foveal damage in glaucoma, however, remains to be elucidated.

4.1.2 Conclusion

This was the first study to investigate the relationship between MP and macula and optic nerve head topography in glaucoma at the time our initial findings were published. Our study compliments previous findings that glaucoma is associated with lower MPOD levels (Igras et al. 2013, Ji et al. 2016), and extends the relationship such that MPOD, it appears, is lower in more severe cases of glaucoma exhibiting foveal involvement. Further research is merited to better define the causative roles of oxidative stress, impaired ocular blood flow and other factors that might influence MP levels in glaucoma. In addition, it will be important to evaluate whether oral dietary MP supplementation in glaucoma patients can (a) increase MPOD, and (b) affect glaucoma-related structural parameters.

4.2 MP and visual function parameters

This study demonstrates for the first time that a relationship exists between MPOD and disability glare in the glaucomatous eye. Low MP levels appear to be associated with poorer mCSg at low spatial *f* and with increased symptoms of disability glare. More interestingly, it appears that this relationship between MPOD, mCSg and glare symptoms in individuals with glaucoma is mediated by foveal involvement.

Glare-affected visual performance under mesopic conditions linearly correlates with the optical density of MP among glaucoma subjects, particularly at lower spatial *f*. Our finding that higher MP is associated with improved glare-related visual performance is consistent with other reports (Stringham & Hammond 2008, Stringham et al. 2011, Hammond et al. 2013), although some studies have failed to replicate such findings for mesopic CS (Loughman et al. 2012, Loughman et al. 2014). The inconsistency in the results among studies may be explained by the variation in population demographics, disease status and study methodology. Mesopic conditions were prioritised herein due to previous observations that impaired dark adaptation and disability glare can be found in glaucoma (Nelson et al. 2003, Velten et al. 2001, Glen and Crabb 2015), coupled with evidence that glare-affected CS in the home environment is compromised due to poorer lighting (Bhorade et al. 2013). Although the study of MP in the glaucomatous eye is novel, other studies of MP in the non-glaucomatous eye have indicated that higher MPOD and oral dietary MP supplementation can be beneficial towards improving CS including CS under the influence of glare (Stringham et al. 2011, Stringham & Hammond 2007, Stringham & Hammond 2008, Hammond et al. 2013, Loughman et al. 2012).

Depressed levels of CS in the glaucomatous eye can be explained in part, by the disturbance at the macula (Sun et al. 2008). Our finding that mCSg at low spatial *f* significantly correlates with MPOD only in the 'Fovea-Involved' subgroup is not surprising as the fovea represents the most central anatomic location where MP density is highest. GCC thickness is linearly correlated with MPOD (Ji et al. 2016); lower central MP in those with foveal-involved glaucoma may, therefore, be

accountable, at least in part, for glare disability in these eyes. However, given the weak association between MPOD and mCSg at low spatial *f*s, there are other factors, in particular retinal changes (retinal ganglion cells and photoreceptors) in the glaucomatous eye that require consideration. Studies have demonstrated an association between decreased CS in the glaucomatous eye and retinal ganglion cell dysfunction (Venture & Porciatti 2005) or death (Kerrigan-Baumrind et al. 2000). In addition, MP is localised to the photoreceptor and retinal ganglion cell layers (Trieschmann et al. 2008). Therefore, the loss of retinal ganglion cells (Venture & Porciatti 2005, Kerrigan-Baumrind et al. 2000) and photoreceptors (Nork et al. 2000) as encountered in glaucoma, can contribute to lower MPOD. We would like to highlight that the retinal factors affecting CS and storage of MP cannot be excluded in our experiments. Our finding of a relationship between MPOD and mCSg in the glaucomatous eye is novel and interesting, and future studies are required to interpret this further.

Those who reported the experience of symptomatic glare exhibited lower MPOD levels. This provides additional evidence that glare disability is potentially linked to residual MP levels in glaucoma. Our overall findings suggest that lower levels of MPOD in the glaucomatous eye might represent a contributory factor to the effects of reduced mCSg and symptomatic glare disability. Furthermore, we observed that the relationships between MPOD and both mCSg and glare symptoms respectively are isolated to those with foveal GCC loss. This suggests that further emphasis should be given to GCC analysis in glaucoma as foveal involvement relates to lower MPOD and poorer residual visual function. There is substantial evidence to support the role of MP in improving visual performance in healthy eyes (Wooten & Hammond 2002, Stringham & Hammond 2007, Stringham & Hammond 2008, Stringham et al. 2011, Hammond et al. 2013, Loughman et al. 2010, Loughman et al. 2012) and in those with AMD (Liu et al. 2015). Oral dietary MP supplementation can augment MPOD and thereby effect an improvement in visual function such as disability glare, PRT and CS under glare conditions (Stringham & Hammond 2008, Loughman et al. 2012, Liu et al. 2015). Further research is needed to investigate the therapeutic potential of oral dietary MP supplementation for improving glare disability especially in those with foveal-involved glaucoma.

Those with pseudoexfoliation glaucoma (PXF) or pigment dispersion glaucoma (PDG) can have structural changes in the anterior segments of the eye in particular iris defects which can contribute to glare symptoms. In this trial, we did not find any significance between self-reported glare symptoms and glaucoma subtypes. However, for future studies of MPOD and glaucoma function, it would be beneficial to only include glaucoma subtypes such as POAG and NTG.

There is strong evidence in the literature to suggest good structure-function relationship between macular RGC thinning and macular VF loss as measured by HVF 10-2 (Hood et al. 2013). In addition, it is also shown that the macular OCT/VF relationships have localised arcuate characteristics in the centre of the macula (Lee et al. 2017).

We included HVF 10-2 as an ancillary test to capture macular function for this purpose. Our finding that HVF 10-2 MD was significantly worse in the foveal GCC loss subgroup in comparison to those without foveal involvement reinforces previous findings where MPOD positively correlated with GCC thickness and where those with foveal involvement displayed worse MPOD (Ji et al .2016). Although this study does not define the relationship between field loss, MPOD and glare-related visual performance, the correlation between central visual field loss and MPOD represents a novel finding in the field of glaucoma and worthy of further research.

In keeping with our results, published work by Igras and co-workers (Igras et al. 2013) and that from Ji and colleagues (Ji et al. 2016) showed that MP levels are lower in glaucoma, but the San Diego Macular Pigment study did not find this relationship (Daga et al. 2018). The glaucoma subjects in the San Diego Macular Pigment study had less glaucomatous damage in contrast to that in our study (mean HVF 24-2 MD, -6.4 dBs vs –7.85 dBs). Besides utilising HVF 24-2, our study also included HVF 10-2 as the latter is more precise in mapping out central visual field loss; this is important when studying the relationship between MP and visual function. In our study, we found that those with “Foveal Field Loss” had lower MPOD at 0.25° ($P < 0.01$) and 0.5° ($P = 0.02$) of retinal eccentricities in comparison to those with “No Foveal Field Loss” (see Table 6). In addition, those

with “Foveal Field Loss” had significantly worse glaucoma disease than those with “No Foveal Field Loss”: (Median HVF 24-2 MD, -11.2 dB vs -5.5 dB, $P < 0.001$; Median HVF 10-2 MD, -14.3 dB vs -4.68 dB, $P < 0.001$) (see Table 6). Interestingly, MPOD at 0.25° and 0.5° of retinal eccentricities in the “No Foveal Field Loss” group were comparable to that reported in the normal population. This highlights the potential of missing crucial information if subgroup analyses of foveal field loss were not performed in this study.

The mechanisms underlying an abnormal PRT among individuals with glaucoma remains unclear. Our findings indicate that a relationship between MPOD and photo-stress recovery is confined to those with foveal-involved glaucoma, who demonstrated a prolonged PRT. In a study comparing healthy controls and those with diabetes mellitus (with and without diabetic retinopathy subgroups), there was no difference in PRT between groups (Loughman et al. 2014). However, the study did not include those with diabetic maculopathy. There are conflicting results in the literature surrounding the relationship between MPOD and PRT. In a study of normal healthy controls, Stringham and colleagues (Stringham et al. 2011) and Hammond and colleagues (Hammond et al. 2013) each showed that MPOD correlated with photo-stress recovery in normal healthy controls whilst Loughman and co-workers reported otherwise (Loughman et al. 2010). The variability of findings in the published literature may be explained by the differences in ocular pathology that were studied, and the methods used to evaluate photo-stress recovery and MPOD respectively.

There are limitations to this study. The HFP task can be challenging for some individuals and this may affect the acquisition of MPOD measurements. We have applied stringent criteria and excluded readings that had large variances between them. This may have resulted in a high exclusion rate in the MPOD data that were available for statistical analysis. In the future, the use of fundus autofluorescence to measure MPOD will help eliminate this problem (Putnam 2017). Another concern regarding the use of HFP in measuring MPOD in the glaucomatous eye is the ability of the subject to fixate and report the absence of flicker in the stimulus targets. In this study, 48.9% of the subjects demonstrated some element of foveal visual field loss. This was determined by referring to the central 4 points

(corresponding to 1.4° of the foveal centre) of the 10-2 pattern deviation plots. As no subjects had evidence of light sensitivity depression in all central points, and as all subjects demonstrated acceptable fixation stability throughout the test, inability to reliably maintain central fixation of the stimulus targets was not likely to be an issue. Despite this, we do acknowledge that we cannot exclude the possibility that patients' glaucomatous foveal scotoma may have impacted their fixation capacity.

4.2.1 Study limitations

The lack of a healthy control group could be considered a drawback in this study. However, our main aim was to investigate the relationship between MPOD and glaucoma-related functional parameters, in particular glare disability, rather than the comparison of visual function between glaucoma and controls. The latter has previously been widely studied in the literature and it is known that functional measures such as CS with and without glare and PRT are affected in those with glaucoma, and therefore was not the primary focus in this study. The cross-sectional nature of the study also limits the interpretation of the relationships that have been shown to exist but does serve to guide future research.

'Fovea-Involved' glaucoma subjects were older in comparison to those without foveal-involvement, although the age difference was small and not clinically meaningful. Co-existing ocular pathologies such as cataract and macular changes are more common with increasing age and may affect visual functions such as mCSg and PRT respectively. We meticulously excluded individuals with moderate-to-significant cataract using the LOCS III grading and therefore the possibility of a cataract as a confounding factor on mCSg was minimised. One possible limitation in this study is the absence of specific grading of the type and severity of mild cataract in this study. We showed, however, that cataract (those without cataract vs those with mild cataract) had no effect on mCSg in our study subjects. Furthermore, subsequent correlation analyses between MPOD and mCSg were also controlled for the presence of mild cataract, so this potential limitation is relatively minor. Any individuals with co-existing AMD were not recruited into this study. We also excluded those with an underlying diagnosis of diabetes mellitus as the finding of concurrent diabetic retinopathy has been linked with reduced MPOD (Scanlon et al. 2015). Any possible residual age-related

confounding effects were controlled for in our statistical analysis, thereby negating the potential issue.

Another limitation of this study is the inability to disentangle the effects of disability and discomfort glare. Although the Optec® 6500 device provides a consistent glare environment for our experiments, it is not discernible whether the measurements recorded are solely disability glare alone. Discomfort glare may be a contributing factor to participant experience of glare. Our finding that those with self-reported glare symptoms have lower MPOD relative to those without symptoms is a potentially important finding. Interpretation of this finding, however, needs to be tempered in relation to the nature of the question posed to participants. The question was qualitative in nature, and not associated with a Likert or other scale to categorise responses. Furthermore, the response was not probed to elucidate additional detail as to the nature of the symptoms where present. Despite this limitation, our findings appear robust, and therefore warrant consideration for future work.

4.2.2 Conclusion

This study extends previous findings that MPOD is lower in glaucoma (Igras et al. 2013) and associated with structural ganglion cell complex losses at the fovea. It demonstrates that lower MPOD levels among glaucoma subjects is associated with (a) more severe glaucomatous VF loss, (b) poorer CS for low spatial *f* stimuli under glare conditions and (c) self-reported symptoms of glare. More importantly, glaucoma related psychophysical tests appear to be related to foveal GCC loss.

It is therefore possible to conclude that MP may have a role in the visual functional status of individuals with glaucoma, although a causal link has yet to be fully established. It is important to note that MP is only found in the central macula and therefore will not entirely explain the global symptom of disability glare but may be a contributing factor. The study of MP in glaucoma is of importance as the therapeutic potential to increase MPOD with an oral dietary MP supplement to improve disability glare is likely to appeal to glaucoma patients exhibiting such symptoms should it work. To explore this concept further, the Macular Pigment and Glaucoma Trial (ISRCTN registry number: 56985060) has been designed to

evaluate the MPOD response to an oral dietary MP supplement and any effects on glare in glaucoma.

4.3 Macular Pigment and Glaucoma Trial outcome

Baseline results of our research study showed that the presence of disease at the fovea negatively impacts on MP level. This trial shows for the first time that MPOD in glaucomatous eyes can be augmented by a short-term oral dietary MP supplementation. Furthermore, a positive MPOD response is most evident in those with the lowest baseline MPOD. The duration of oral dietary MP supplementation was short in this trial and we did not find any significant effect on glaucoma structure and function. However, the therapeutic potential of oral dietary MP supplements in glaucomatous eyes remains to be validated given the positive MPOD response observed in this trial.

MPOD augmentation following oral MP supplementation has previously been demonstrated in studies involving healthy controls (Nolan et al. 2011, Loughman et al. 2012, Trieschmann et al. 2007), those with AMD (Beatty et al. 2013, Richer et al. 2011, Murray et al. 2013, Liu et al. 2015) and other retinal degenerative disorders respectively (Aleman et al. 2001, Duncan et al. 2002, Aleman et al. 2007). This study shows that MPOD augmentation is possible in the presence of a glaucomatous disease. Direct comparison of MPOD response following oral MP supplementation is challenging due to the variability in subject demographics, ocular health status, methodology of MPOD measurement, formulation of oral MP supplements and the duration of supplementation between studies (Nolan et al. 2011, Loughman et al. 2012, Beatty et al. 2013, Trieschmann et al. 2007, Richer et al. 2011, Murray et al. 2013, Aleman et al. 2001, Duncan et al. 2002, Aleman et al. 2007). In our study, we found significant MPOD response at 0.25° of retinal eccentricity but not at 0.5° and 1° respectively, and the magnitude of MPOD response was low relative to other studies (Loughman et al. 2012, Beatty et al. 2013, Trieschmann et al. 2007, Murray et al. 2013). We did not find any significant factor in our study's active group that may influence MPOD response. However,

the duration of supplementation was short at 6 months and this may explain the low MPOD response rate. The COMPASS trial (Nolan et al. 2011) found that MPOD response was only significant at 12-months instead of 6-months of oral MP supplementation in young healthy individuals. Although the CLEAR study (Murray et al. 2013) demonstrated a significant increase in MPOD response following 4 months (7.9% change from baseline) of oral MP supplementation, a large magnitude of MPOD change was only recorded at 8 months (23.7%) and 12 months (39%) respectively.

Apart from a short duration of oral dietary MP supplementation, the baseline MPOD level was low and MPOD response rate was slow at all retinal eccentricities in this study. Factors due to glaucoma disease processes at the macula such as oxidative stress (Tezel 2006, Cuenca et al. 2014) and circulatory issues (Tezel 2006, Cuenca et al. 2014, Flammer et al. 2002) may account, to some extent, the results observed. MPOD response following oral dietary MP supplementation has been widely explored in healthy individuals (Nolan et al. 2011, Loughman et al. 2012, Connolly et al. 2011), AMD (Beatty et al. 2013, Trieschnann et al. 2007, Richer et al. 2011, Murray et al. 2013, Liu et al. 2015) and other forms of retinal degeneration (Aleman et al. 2001, Duncan et al. 2002, Aleman et al. 2007) but remains novel in glaucoma. Macular pigments are recognised as effective antioxidants (Khachik et al. 1997, Li et al. 2010, Bhosale & Bernstein 2005) in quenching up free radicals and are considered to be fundamental at the highly oxidative macula of a glaucomatous eye. This process may exhaust any MP stores and retard the repletion process following oral dietary MP supplementation as reflected by the low MPOD response rate observed in our study. Circulatory issues (Tezel 2006, Cuenca et al. 2014, Flammer et al. 2002) due to vascular dysregulation and artherosclerosis may compromise ocular blood flow (micro- and macro-circulation) and further contribute to oxidative stress at the macula. The transport of carotenoids to the retina following an oral intake is dependent on blood flow (Parker 1996) and therefore any poor micro-circulation and macro-circulation issues in glaucoma can further explain the low MPOD response rate.

We found that those with a low baseline MPOD were more likely to show augmentation in MPOD following oral MP supplementation. This finding is in

keeping with other studies (Trieschmann et al. 2007, Murray et al. 2013). It is plausible that when the retinal carotenoid saturation is low, macular MP is more readily replenished when supplemented with an oral dietary MP supplement. Among those receiving oral dietary MP supplementation, we found that baseline MPOD at 0.5° of retinal eccentricity was comparable between the GCC subgroups but yet only those in the "Fovea-Involved" subgroup demonstrated a statistically significant MP augmentation at final follow-up. Oxidative stress has been strongly implicated in the pathogenesis of glaucoma (Tezel 2006, Moreno et al. 2004, Huang et al. 2014) and in ageing (Pinazo-Duran et al. 2014). Carotenoids are important for normal macular function and this is believed to arise from their roles as powerful antioxidants and the filtration of short-wavelength blue light to attenuate photochemical damage and retinal image degradation respectively (Wooten & Hammond 2002). It is possible that in individuals of older age and worse glaucoma severity as observed in the "Fovea-Involved" subgroup, there is a higher level of ROS that may in turn result in the higher retinal carotenoids uptake.

Gut absorption and the transport of carotenoids to the retina for deposition (Erdman et al. 1993) are amongst other factors to consider when studying MPOD response following oral MP dietary supplementation. None of the individuals in this study was known to suffer from any gastrointestinal disorders that may influence the absorption and transport of carotenoids. Xanthophyll-binding proteins such as retinal tubulin, play a role in the retinal uptake of carotenoids and carry saturation properties (Bernstein et al. 1997). These proteins are located at the Henle's fibre layer of the macula. In those with foveal-involved glaucoma, one would expect lower availability of retinal tubulin for carotenoid stabilisation. However, we speculate that under such circumstance, there may be a higher binding affinity and saturation rate of tubulin for carotenoids contributing in part, to a significant augmentation of MP in the "Fovea-Involved" subgroup. As the duration of oral MP supplementation is short in this study, it is unknown whether those in the "Fovea-Not-Involved" subgroup would also demonstrate a significant augmentation of MP should the supplementation be longer.

However, there are some individuals who did not demonstrate any MP augmentation following oral MP supplementation. This phenomenon has

previously been recognised in other studies (Trieschmann et al. 2007, Richer et al. 2011, Murray et al. 2013, Aleman et al. 2001, Duncan et al. 2002, Aleman et al. 2007) and is known as retinal non-response. Some studies found that serum carotenoid levels rise before any change in MPOD is detected and the effect does not necessarily correlate and is variable among individuals (Trieschmann et al. 2007, Richer et al. 2011, Murray et al. 2013). We did not study serum carotenoid levels but accounted for any dietary MP intake with the use of a validated questionnaire (LZQ 2009).

Retinal non-responders can either be evaluated by analysing MPOD response at each retinal eccentricity or by evaluating MPOD response for each individual. We found a higher non-responder rate in our study relative to others, at 0.25° (28% vs 17% in the COMPASS study) and 0.5° (42% vs 20% in the COMPASS study and 21% in the LUNA study respectively) of retinal eccentricities (Nolan et al. 2011, Trieschmann et al. 2007). However, it is important to note that a large proportion of the subjects in this study had foveal involvement while the subjects in the COMPASS study had healthy maculae and those in the LUNA study had non-central involving AMD. To identify the percentage of individuals who were complete non-responders in this study, we analysed those on the active treatment who displayed no ΔMPOD at all retinal eccentricities. Few individuals had acceptable MPOD data at all retinal eccentricities for this sub-analysis, hence the sample size is small. We found that the proportion of complete non-responders in our study to be 25%. This figure is markedly lower in comparison to the rate of non-responders at each retinal eccentricity. It is plausible that the MPOD response at each retinal eccentricity might improve if there was a longer duration of oral MP supplementation.

The hypothesised outcome following oral dietary MP supplementation is for an augmentation in MPOD to result in either preservation or improvement in visual function over the course of the treatment (Nolan et al. 2011, Loughman et al. 2012, Beatty et al. 2013, Richer et al. 2011, Murray et al. 2013). The CARMA study demonstrated a significant improvement in best-corrected VA only after 24 months of oral MP supplementation in those with early AMD (Beatty et al. 2013). The CLEAR study was a double-blinded, placebo-controlled study that showed lutein

supplementation in those with early AMD over a period of one year resulted in a significant MPOD response in comparison to placebo (Murray et al. 2013). However, at the end of oral lutein supplementation, only a subgroup of individuals who exhibited worse baseline VA showed a significant improvement in VA. Interestingly, those receiving placebo demonstrated a significant deterioration in VA indicating the protective effect of lutein. In our study, there was no significant change in visual function of those in the active and placebo groups over the course of 6 months.

Despite the observation of some MPOD augmentation in those receiving the active treatment in our study, we did not observe any effect on visual function. This may be due the modest MPOD response secondary to a short duration of oral dietary MP supplementation. In keeping with our findings, other studies that involve a 6-month oral dietary MP supplementation in those with inherited retinal disorders also did not show any improvement in visual function despite a successful augmentation in MPOD (Aleman et al. 2001. Duncan et al. 2002, Aleman et al. 2007).

4.3.1 Study Limitations

A major limitation in this study is the use of the HFP technique (Macular Densitometer) to capture MPOD. A proportion of individuals were not able to perform the experiment, and this impacted on data acquisition at all the retinal eccentricities. The strict exclusion criteria that we employed in this study further diminished the sample size. In the future, consideration should be given to an imaging technique that minimises patient fatigue such as fundal autofluorescence in the measurement of MPOD. We also acknowledge that the small sample size in this study is a contributory factor to the loss of power and the possibility of some of the non-conclusive findings in this study.

Another weakness of the study was that only 2 visits per subject were available for analysis; at baseline and at final follow-up. One may argue that the rate of change of MPOD may not be entirely accurate and subject to experimental error. However, a strict MPOD standard deviation of at least 0.05 or better was used while any data that did not comply was excluded from analysis. Given the stringent exclusion

criteria of the MPOD data combined with a relatively small sample size in this study and subject difficulty of performing the task, it undoubtedly negatively impacts on the final sample size. Despite that, this study showed novel findings and may serve as a platform for future studies to explore the therapeutic potential of oral dietary MP supplementation in glaucoma. Notably, the duration of supplementation of 6 months is not sufficient to draw any conclusion on the maximal potential of MPOD response. In this respect, it may seem reasonable for future studies to supplement individuals with glaucoma for a longer duration.

4.3.2 Conclusion

Although we did not identify any significant benefit of MP supplementation in this randomised controlled trial, this study is the first to show that oral dietary MP supplementation may have the potential to increase MPOD in the glaucomatous eye, particularly in those with lower baseline MPOD. In addition, those with foveal involvement appear to have a more favourable MPOD response in comparison to those without foveal involvement. There may be a potential role of MP replacement therapy in glaucoma but would require a supplementation study over a longer duration. The therapeutic opportunity of oral dietary MP supplementation in influencing visual function needs to be substantiated in further studies.

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Presentations & Publications

Presentations

1. The association between macular pigment optical density and glaucoma-related structural and functional parameters.
   Siah WF, Loughman J, O'Brien C.
   Irish College of Ophthalmologists Annual Conference – May 2014

2. The Relationship Between Macular Pigment & Glaucoma-related Structural Parameters: A Baseline Evaluation of the Macular Pigment & Glaucoma Trial.
   Siah WF, Loughman J, O'Brien C.
   ARVO annual meeting – May 2014

3. The Relationship Between Macular Pigment & Visual Function Among Glaucoma Subjects: A Baseline Evaluation of the Macular Pigment & Glaucoma Trial.
   Siah WF, O'Brien C, Loughman J.
   ARVO annual meeting – May 2014

4. The association between macular pigment optical density and glaucoma-related structural and functional parameters.
   Siah WF, Loughman J, O'Brien C.
   European Glaucoma Society Conference – June 2014

5. Macular Pigment Optical Density Response Following Oral Dietary Macular Pigment Supplementation in Glaucoma.
   Siah WF, Loughman J, O'Brien C.
   ARVO annual meeting – May 2015

Publications

1. Macular pigment is associated with glare-affected visual function and central visual field loss in glaucoma.
   Siah WF, O'Brien C, Loughman J.
   British Journal of Ophthalmology. 2018;102(7):929-935.

2. Reply. (Lower Macular Pigment Optical Density in Foveal-Involved Glaucoma)
   Siah WF, Loughman J, O'Brien C.
   Ophthalmology. 2016;123(7):e43-4.

3. Lower Macular Pigment Optical Density in Foveal-Involved Glaucoma
   Siah WF, Loughman J, O'Brien C.
   Ophthalmology. 2015;122(10):2029-37.

Appendix A

PATIENT INFORMATION LEAFLET

RESEARCH TITLE: Study of Macular Pigments in Glaucoma

This is a research study looking at the effects of oral dietary macular pigment (MP) supplementation in an individual with glaucoma. MP is a naturally occurring pigment located at the back of the eye. MP plays an important role to maintain good visual function including glare sensitivity. We have found that patients with glaucoma have reduced MP levels in their eye, which is a new finding. Studies have shown that oral dietary MP supplement can increase MP profiles to a normal level. Here, we would like to study whether dietary MP supplementation will reduce glare symptoms, which is a common complaint among glaucoma patients. We would also like to understand whether dietary MP supplementation will have any impact on glaucoma and the individual's quality of life.

Please read carefully.

WHAT IS THE PURPOSE OF THE STUDY?

Background

- Glaucoma is a slowly progressive disease that causes irreversible blindness.
- Glare is defined as difficulty seeing in the presence of a bright light.
- Glaucoma patients often suffer from glare symptoms and the cause of this is poorly understood.
- Macular pigment (MP) is an important and naturally occurring pigment at the macula, which is located at the back of the eye.
- MP is made up of 3 dietary compounds, Lutein (L), Zeaxanthin (Z) and meso-Zeaxanthin (meso-Z).
- It is believed that these plant compounds help protect the eye by absorbing UV and harmful blue light and neutralising free radicals (antioxidant).
- MP is vital in maintaining eye health and has been shown to play a role in reducing glare symptoms.
- Studies have shown that people with higher MP levels have less glare symptoms.
- Clinical trials have shown that the level of MP in an individual can be increased after taking supplements containing L, Z and meso-Z.
- Our research group has shown that glaucoma patients have lower MP levels.

Research Aims

1. Determine whether oral supplementation of L, Z and meso-Z (MacuShield®) can rebuild their pigment profile to normal levels
2. Determine whether glare symptoms can be improved by oral MP supplementation
3. Determine the relationship between MP and visual function in glaucoma patients

WHY HAVE I BEEN CHOSEN?

All glaucoma patients under the care of Professor Colm O'Brien are invited to participate in this research study. We aim to recruit 120 glaucoma patients and 60 healthy controls.

WHO IS ORGANISING THE RESEARCH STUDY?

This research study will be conducted by:

Principal Investigator: Professor Colm O'Brien (Consultant Ophthalmic Surgeon)

Investigator: Dr. James Loughman (Director, Macular Pigment Research Group, Dublin Institute of Technology Vision Research Laboratory)

Authorised Healthcare Professional: Dr. We Fong Siah (Specialist Registrar in Ophthalmology)

WHAT WILL HAPPEN TO ME IF I TAKE PART?

At the start of the study, you will participate in a series of vision-related tests, complete questionnaires, and be supplemented with either a dietary MP supplement capsule (MacuShield®) or placebo. You will take 1 capsule daily, preferably with a meal. The duration of the study is 6 months. Tests will be carried out at baseline (time of recruitment) and repeated at 6 months. Some participants may be invited back for additional testing at 3 months as well. Therefore, each participant will visit the clinic on 2 to 3 occassions. Each visit may vary from 60 minutes to 90 minutes long.

The enrolment into this study will be on a voluntary basis and you will not be paid. The vision-related tests will not cause any distress to you. No eye drops will be instilled, and you will still be able to drive.

This is a randomised, double-blind study. It means that participants will be put into groups and compared. The reason for this is to prevent bias in the study. The computer will randomly assign each participant into the groups. A double-blind trial means that neither you nor your doctor will know which treatment group you are in. This will only be revealed at the end of the study. The chance of you getting the dietary MP supplement capsule (MacuShield®) or placebo is 50%. The placebo will be a dummy tablet that contains no active ingredient but looks exactly like MacuShield®. The placebo will contain sunflower

oil only. You will only be informed the nature of your treatment (either MacuShield® or placebo) at the end of the study. The identity of the treatment will not be revealed before the study ends as this will jeopardise the research.

WHAT ARE MY RESPONSIBILITITES?

You'll be tested at baseline (time of recruitment into the study). This involves participating in tests that will measure visual performance (visual acuity, contrast sensitivity, glare sensitivity, photo-stress recovery time, visual field test) and structural measurements (MP optical density, macular thickness). Research participants will also be required to fill out food questionnaire and glaucoma-related quality of life questionnaire.

Some participants may be asked to return at 3 months as well. The reason for this is because it takes up to 3 months to rebuild the MP levels and testing at this stage may give us more information. Finally, you'll be required to return for a final visit at 6 months. All participants will have to be compliant with the treatment so that the data we get can be interpreted accurately. During the course of the study, Dr. Siah will contact you either by a phone call or a phone text message to remind you to take your capsules and to attend your clinic visits. At the 3-month and 6-month clinic visits, you are required to bring all remaining capsules with you.

WHAT IS MACUSHIELD®?

It is a unique eye supplement made from naturally occurring dietary carotenoids. It contains Lutein 10mg, Zeaxanthin 2mg and Meso-Zeaxanthin 10mg. Research has shown that if you take Meso-Zeaxanthin, the levels of MP will significantly increase especially at the centre of your retina where the vision is the sharpest. It is currently routinely used to reduce the risk of progression of age-related macular degeneration.

ARE THERE OTHER WAYS OF TREATING MY CONDITION?

The use of this treatment will not affect your glaucoma management. This treatment is an additional supplement and is not intended to substitute your current glaucoma medication(s). The cause of glare symptoms among glaucoma patients is poorly understood and this research study may provide us with useful information.

ARE THERE ANY DISADVANTAGES IN TAKING PART IN THIS RESEARCH STUDY? WHAT ARE THE POSSIBLE RISKS OF TAKING PART?

MacuShield® is a natural product that is widely available over the counter. There are no long-term side effects to MacuShield® and has been tested safe for human consumption on

a large-scale clinical trial in Ireland. The only documented and uncommon short-term side effect of Lutein supplementation in humans has been carotenodermia, which is a harmless and reversible skin hyperpigmentation. However, if you do develop any symptoms that you are concerned about, you should contact Dr. We Fong Siah at 087-6164411 or consult your GP.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

You may or may not receive any direct benefit from taking part in the research study. However, information obtained during the course of the research study may help us understand better your condition. We hope that the treatment that you get may reduce your glare symptoms. However, this cannot be guaranteed. The information we get from this research study may help us to treat future patients with glaucoma better.

IS MY DOCTOR BEING PAID FOR INCLUDING ME IN THE RESEARCH STUDY?

No

WILL PATIENT EXPENSES BE MET?

The enrolment into this study will be on a voluntary basis and you will not be paid.

WHAT HAPPENS WHEN THE STUDY ENDS?

You will be informed whether you were treated with MacuShield® or placebo.

ARE THERE ANY RESTRICTIONS ON WHAT I MIGHT EAT OR DO?

No. Your diet will not be restricted as a result of the study.

WHAT IF SOMETHING GOES WRONG?

MacuShield® has been widely used and is known to be safe. Regardless of this, if you have any cause to complain about any aspect of the way you have been approached or treated during the course of this research study, the normal Health Service complaints mechanisms are available to you.

CONFIDENTIALITY – WHO WILL KNOW I AM TAKING PART IN THE RESEARCH STUDY?

All information, which is collected about you during the course of the research will be kept strictly confidential. All information about you, which leaves the hospital, will contain no information as to your identity so that you cannot be recognised from it.

GP NOTIFICATION

Your GP will normally be informed that you are taking part in this study. If this is a problem for you, you should discuss it with your study researcher.

HOSPITAL RESEARCH ETHICS COMMITTEE APPROVAL

This research study has been approved by the Mater Hospital Research Ethics Committee.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results will be published in a peer-reviewed ophthalmology journal. If you wish to receive a copy of the published results, please inform the study researcher.

PROCEDURE TO BE USED IF ASSISTANCE OR ADVICE IS REQUIRED

In the event of a research related injury or any other problems, you can contact Dr. We Fong Siah at 087-6164411. The Principal Investigator for this study is Professor Colm O'Brien and his direct telephone number is 01-8858616.

VOLUNTARY PARTICIPATION

It is up to you to decide whether to take part or not. Even if you do decide to take part, you are free to withdraw at any time and without giving a reason. This will not affect the standard of care you will receive. Your doctor will not be upset if you decide not to take part.

If you have already been involved in a drug trial within the past 3 months, you are not eligible to take part in this research study. If you are a woman of child-bearing potential, you must be on contraception to be eligible to participate in this study. Glaucoma is a disease that is more prevalent in the elderly therefore we will expect to have research participants aged > 65 years old.

We thank you for taking time to read this information leaflet and sincerely appreciate your contribution in this research study. We need your participation as the more people we recruit into the study, the more information we can gather.

If you require any further information or have any query regarding this study, please do not hesitate to ask. You may contact Dr. We Fong Siah at 087-6164411.

Appendix B

PATIENT CONSENT FORM

Reference Number:	**Protocol Number**: MP/I/1112
Title of Research Study: Study of Macular Pigments in Glaucoma	
Patient Name:	
Name of Doctor & Telephone Number:	

1. I confirm that I have read and understood the information leaflet dated…….for the above research study and received an explanation of the nature, purpose, duration, and foreseeable effects and risks of the research study and what my involvement will be □

2. I have had time to consider whether to take part in this research study. My questions have been answered satisfactorily and I have received a copy of the Patient Information Leaflet. □

3. I understand that my participation is voluntary (my choice) and that I am free to withdraw at any time without my medical care or legal rights being affected. □

4. I have to the best of my knowledge informed the investigator of my previous or present illnesses and medication and of any consultation that I have had with a doctor for the last four months. I have not participated in any other clinical trial in the past three months. □

5. I understand that my General Practitioner Dr…… will be informed by Dr …… that I am taking part in this research study. □

6. I will contact the research investigator immediately if I suffer any unexpected or unusual symptoms during the research study. □

7. I am willing to allow access to my medical records by representatives of the sponsor, research ethics committee or local or foreign regulatory authorities but understand that strict confidentiality will be maintained. The purpose of this is to check that the research study is being carried out correctly. □

8. I agree to take part in the above research study. □

……	……...……	……..……..
Name of Patient (in block letters)	Date	Signature

……	……...……	……..……..
Doctor/Researcher	Date	Signature

1 copy for patient, 1 copy for researcher, 1 copy to be inserted in hospital notes

Appendix C

LZQ™: How often do you eat the following foods?

Food Categories	Food items and a prompt for clarification			Times Per Day	Times Per Week	Times Per Month
BREADS	Cornmeal, yellow	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Cornmeal, white	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Cornbread Muffin	1	each	___ to ___	___ to ___	___ to ___
	Corn Tortilla, 6"	1	each	___ to ___	___ to ___	___ to ___
	Bread, roll, bun, bagel	1	slice	___ to ___	___ to ___	___ to ___
CEREALS	Apple Jacks™	1	cup	___ to ___	___ to ___	___ to ___
	Cap'n Crunch ™	1	cup	___ to ___	___ to ___	___ to ___
	Corn Chex ™	1	cup	___ to ___	___ to ___	___ to ___
	Corn Flakes	1	cup	___ to ___	___ to ___	___ to ___
	Corn Pops	1	cup	___ to ___	___ to ___	___ to ___
	Crispix ™	1	cup	___ to ___	___ to ___	___ to ___
	Froot Loops ™	1	cup	___ to ___	___ to ___	___ to ___
	Frosted Flakes	1	cup	___ to ___	___ to ___	___ to ___
	Life™	1	cup	___ to ___	___ to ___	___ to ___
	Reese's Puffs ™	1	cup	___ to ___	___ to ___	___ to ___
CONDIMENTS	Fat Free Mayonnaise	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Regular Mayonnaise	1	Tbsp.	___ to ___	___ to ___	___ to ___
SAUCES	Sauce, Ready-To-Serve, Pepper or Hot	1	Tsp.	___ to ___	___ to ___	___ to ___
	Sauce, Salsa, Ready-To-Serve	0.5	cup	___ to ___	___ to ___	___ to ___
FRUITS	Apple (with skin), medium	1	each	___ to ___	___ to ___	___ to ___
	Apricots see prompt	0.3	cup	___ to ___	___ to ___	___ to ___
	Cantaloupe	1	cup	___ to ___	___ to ___	___ to ___
	Red Grapes	1	cup	___ to ___	___ to ___	___ to ___
	Green Grapes	1	cup	___ to ___	___ to ___	___ to ___
	Kiwi	1	each	___ to ___	___ to ___	___ to ___
	Mango, medium	1	each	___ to ___	___ to ___	___ to ___
	Honeydew	1	cup	___ to ___	___ to ___	___ to ___
	Nectarine, medium	1	each	___ to ___	___ to ___	___ to ___
	Orange Juice	1	cup	___ to ___	___ to ___	___ to ___
	Peaches (canned)	1	cup	___ to ___	___ to ___	___ to ___
	Peach (fresh), medium	1	each	___ to ___	___ to ___	___ to ___
	Watermelon	1	cup	___ to ___	___ to ___	___ to ___
NUTS	Pistachios	1	oz.	___ to ___	___ to ___	___ to ___
PASTA	Macaroni and Cheese	1	cup	___ to ___	___ to ___	___ to ___
	Egg Noodles	1	cup	___ to ___	___ to ___	___ to ___
	Spinach Egg Noodles	1	cup	___ to ___	___ to ___	___ to ___
EGGS	Egg, including yolk, large	1	each	___ to ___	___ to ___	___ to ___
SNACKS	Chex Mix™	1	cup	___ to ___	___ to ___	___ to ___
	Cheetos™	1	oz.	___ to ___	___ to ___	___ to ___
	Fritos™	1	oz.	___ to ___	___ to ___	___ to ___
	Popcorn	1	oz.	___ to ___	___ to ___	___ to ___
	Tortilla Chips	1	oz.	___ to ___	___ to ___	___ to ___
VEGETABLES	Artichoke Quarters (bottled or canned)	1	cup	___ to ___	___ to ___	___ to ___
	Asparagus (cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Green Beans (cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Broccoli (raw or cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Brussels Sprouts (cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Red Cabbage (cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Cilantro (raw),	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Yellow corn	1	cup	___ to ___	___ to ___	___ to ___
	Cucumber (with skin), medium	1	each	___ to ___	___ to ___	___ to ___
	Endive (raw)	1	cup	___ to ___	___ to ___	___ to ___
	Kale	1	cup	___ to ___	___ to ___	___ to ___
	Iceberg Lettuce	1	cup	___ to ___	___ to ___	___ to ___
	Avocado (California)	1	each	___ to ___	___ to ___	___ to ___
	Romaine Lettuce	1	cup	___ to ___	___ to ___	___ to ___
	Lima Beans	1	cup	___ to ___	___ to ___	___ to ___
	Green Olives	6	olives	___ to ___	___ to ___	___ to ___
	Spring Onions, Scallions (raw)	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Spring Onions, Scallions (cooked in oil)	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Parsley (raw)	1	Tbsp.	___ to ___	___ to ___	___ to ___
	Green Peppers	1	cup	___ to ___	___ to ___	___ to ___
	Orange Peppers	1	cup	___ to ___	___ to ___	___ to ___
	Red Peppers	1	cup	___ to ___	___ to ___	___ to ___
	Yellow Peppers	1	cup	___ to ___	___ to ___	___ to ___
	Spinach (cooked)	1	cup	___ to ___	___ to ___	___ to ___
	Spinach (raw)	1	cup	___ to ___	___ to ___	___ to ___
	Acorn Squash	1	cup	___ to ___	___ to ___	___ to ___
	Butternut Squash	1	cup	___ to ___	___ to ___	___ to ___
	Yellow Squash	1	cup	___ to ___	___ to ___	___ to ___
	Zucchini	1	cup	___ to ___	___ to ___	___ to ___

Appendix D

Glaucoma Activity Limitation (GAL-9) Questionnaire

"Does your vision give you any difficulty, even with glasses, with the following activities?"

	No difficulty	A little bit of difficulty	Some difficulty	Quite a lot of difficulty	Severe difficulty
Walking after dark	1	2	3	4	5
Seeing at night	1	2	3	4	5
Walking on uneven ground	1	2	3	4	5
Adjusting to dim lights	1	2	3	4	5
Going from light to dark room and vice versa	1	2	3	4	5
Seeing objects coming from the side	1	2	3	4	5
Walking on steps/stairs	1	2	3	4	5
Judging distance of foot to step/curb	1	2	3	4	5
Finding dropped objects	1	2	3	4	5

Appendix E

TyPE Spec Questionnaire

A. How would you rate your vision?

How well do you see?
Poor = 1
Fair = 2
Good = 3
Very good = 4
Excellent = 5

B. How much does your vision hinder, limit, or disable you in each of the following activities?

	Not at all	A little bit	Some	Quite a lot	Totally disabled	Don't do for other reasons
Your usual activities	1	2	3	4	5	0
Recognising people or objects across the street..	1	2	3	4	5	0
Reading price labels in shops and supermarkets..	1	2	3	4	5	0
Reading a magazine, newspaper or book	1	2	3	4	5	0
Knitting or sewing....	1	2	3	4	5	0
Watching television...	1	2	3	4	5	0
Daytime driving..	1	2	3	4	5	0
Night-time driving...	1	2	3	4	5	0

C. How much are you hindered, limited or disabled by glare (dazzling light) in each of the following activities?

	Not at all	A little bit	Some	Quite a lot	Totally disabled	Don't do for other reasons
Your usual activities	1	2	3	4	5	0
Reading shiny paper (such as a magazine)	1	2	3	4	5	0
Driving towards the sun or oncoming headlights	1	2	3	4	5	0
Walking outside on a sunny day	1	2	3	4	5	0

D. Who filled in this form?

I filled it out with no help = 1
I filled it out with help from family and friends = 2
I filled it out with help form a nurse or doctor = 3
Family and friends filled it out = 4
A nurse or doctor filled it out = 5

E. Have you had a recent illness, injury, or emotional upset that has affected how you answer these questions?

Yes =1
No =2

Appendix F

Macular Pigment & Glaucoma Study

CRF
Case Report Form

Investigator Parties:

1. Mater Misericordiae University Hospital
2. Optometry Department
   Dublin Institute of Technology

Baseline visit

Date: _____
(DD/MM/YYYY)

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

Forename: _____ Surname: _____

Address: _____

_____

_____

Contact No(s): _____

Email: _____

Date of birth: _____ Age: _____(years)

(DD/MM/YYYY)

GP: _____

Address: _____

_____

_____

Please circle the relevant corresponding answer(s)

1. Sex Male / Female

2. Race White / Black / Asian / Spanish or Hispanic / Mixed race

3. Marital status Married (or cohabiting) / Widowed / Single / Divorced or separated

4. Medical History
Have you any of the following medical conditions?

Diabetes / High blood pressure / High cholesterol / Angina / Stroke

If yes for any of the above please give details in the space provided below (e.g. year it occurred, treatment, medication etc.)

---

5. Ocular history

Do you suffer from glare? Yes / No

How long have you been suffering from glare? _____

Do you currently wear spectacles and/or contact lenses? Yes / No

Type of glaucoma: _____ Status: Progressing / Stable

Year Diagnosed: _____ Last IOP: _____

Current treatment Xalatan / Travatan / Saflutan / Lumigan / generic LATANOPROST
Azopt / Trusopt (PF) / Azarga / Cosopt (PF)
Xalacom / Duotrav / Ganfort
Timolol / Betagan (PF) / Alphagan / Iopidine / Pilocarpine / Diamox

Past ocular surgery / laser treatment for glaucoma / refractive laser (if any):

_____

Family history of ocular disease (if any):

_____

6. Smoking

a) Which best describes your smoking habits (whether cigarette, cigar, pipe etc.)?

Never smoker (smoked < 100 cigs in life-time)…….. 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) What is the average number of cigarettes you smoke (or smoked) on a daily basis? ____

c) For how many years have you smoked (or did you smoke)? _____

7. Body Mass Index (BMI)

Weight…… ☐ **Kg**

Height…… ☐ **M**

BMI…… ☐ **Kg/M^2**

B. Diet Questionnaire Yes / No

C. OCT Scan Yes / No

D. Visual Field Plot 24-2 printout Yes / No

10-2 Printout Yes / No

E. GAL-9 Questionnaire Yes / No

F. Glare Questionnaire Yes / No
(Cataract TyPE Spec)

G. Visual acuity and refractive error

1: **Visual Acuity (LogMAR)**

Unaided VA…… ☐ ☐

Habitual VA (own Rx)…… ☐ ☐

2: **Study Eye**

Please indicate which eye will be used for the current study:

R ☐ **L** ☐

H. Contrast Sensitivity / Glare ☐ **U** ☐

1: Functional Acuity Contrast Test (FACT) – Functional vision Analyser

Mesopic and Photopic Contrast Sensitivity With/Without Glare

Mesopic (no glare) **Mesopic (glare 1)**

Spatial Frequency	**Contrast Sensitivity**	**Spatial Frequency**	**Contrast Sensitivity**
1.5 cpd	_____	**1.5cpd**	_____
3.0 cpd	_____	**3.0 cpd**	_____
6.0 cpd	_____	**6.0 cpd**	_____

12.0 cpd	_____	**12.0 cpd**	_____
18.0 cpd	_____	**18.0 cpd**	_____

Photopic (no glare)		**Photopic (glare 2)**	
Spatial Frequency	**Contrast Sensitivity**	**Spatial Frequency**	**Contrast Sensitivity**
1.5 cpd	_____	**1.5cpd**	_____
3.0 cpd	_____	**3.0 cpd**	_____
6.0 cpd	_____	**6.0 cpd**	_____
12.0 cpd	_____	**12.0 cpd**	_____
18.0 cpd	_____	**18.0 cpd**	_____

2: Photo-stress recovery

Photo-stress Recovery Time _____ Secs

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**
0.25^0	CFF-8	

0.5^0	CFF-7	
1^0	CFF-7	
1.75^0	CFF-7	
3^0	CFF-9	
7^0	CFF-14	

	Predicted CFF		Radiance		
		0.25 deg			
Predicted CFF					
Actual CFF					MPOD =
		0.5 deg			
Predicted CFF					
Actual CFF					MPOD =
		1 deg			
Predicted CFF					
Actual CFF					MPOD =
		1.75 deg			
Predicted CFF					
Actual CFF					MPOD =
		3 deg			
Predicted CFF					
Actual CFF					MPOD =
		7 deg			
Predicted CFF					
Actual CFF					MPOD =

Final visit **Date: _____**

(DD/MM/YYYY)

A. Body Mass Index (BMI)

Weight…… ☐ **Kg**

Height…… ☐ **M**

BMI…… ☐ **Kg/M^2**

B. OCT Scan Yes / No

C. Visual Field Plot 24-2 printout Yes / No

10-2 Printout Yes / No

D. GQL Questionnaire Yes / No

E. Glare Questionnaire (Cataract TyPE Spec) Yes / No

F. Visual acuity and refractive error

1: **Visual Acuity (LogMAR)**

Unaided VA…… ☐ ☐

Habitual VA (own Rx)…… ☐ ☐

2: **Study Eye**

Please indicate which eye will be used for the current study:

R ☐ **L** ☐

G. Contrast Sensitivity / Glare ☐ **U** ☐

1: Functional Acuity Contrast Test (FACT) – Functional vision Analyser

Mesopic and Photopic Contrast Sensitivity With/Without Glare

Mesopic (no glare) **Mesopic (glare 1)**

Spatial Frequency Sensitivity	Contrast Sensitivity	Spatial Frequency	Contrast
1.5 cpd	_____	**1.5cpd**	_____
3.0 cpd	_____	**3.0 cpd**	_____
6.0 cpd	_____	**6.0 cpd**	_____
12.0 cpd	_____	**12.0 cpd**	_____
18.0 cpd	_____	**18.0 cpd**	_____

Photopic (no glare) **Photopic (glare 2)**

Spatial Frequency Sensitivity	Contrast Sensitivity	Spatial Frequency	Contrast
1.5 cpd	_____	**1.5cpd**	_____
3.0 cpd	_____	**3.0 cpd**	_____
6.0 cpd	_____	**6.0 cpd**	_____
12.0 cpd	_____	**12.0 cpd**	_____
18.0 cpd	_____	**18.0 cpd**	_____

2: Photo-stress recovery

Photo-stress Recovery Time _____ Secs

H. 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
0.25^0	CFF-8	
0.5^0	CFF-7	
1^0	CFF-7	
1.75^0	CFF-7	
3^0	CFF-9	
7^0	CFF-14	

	Predicted CFF		Radiance		
		0.25 deg			
Predicted CFF					
Actual CFF					MPOD =
		0.5 deg			
Predicted CFF					
Actual CFF					MPOD =
		1 deg			
Predicted CFF					
Actual CFF					MPOD =
		1.75 deg			
Predicted CFF					
Actual CFF					MPOD =
		3 deg			
Predicted CFF					
Actual CFF					MPOD =
		7 deg			
Predicted CFF					
Actual CFF					
					MPOD =

Appendix G

Functional Acuity Contrast Test